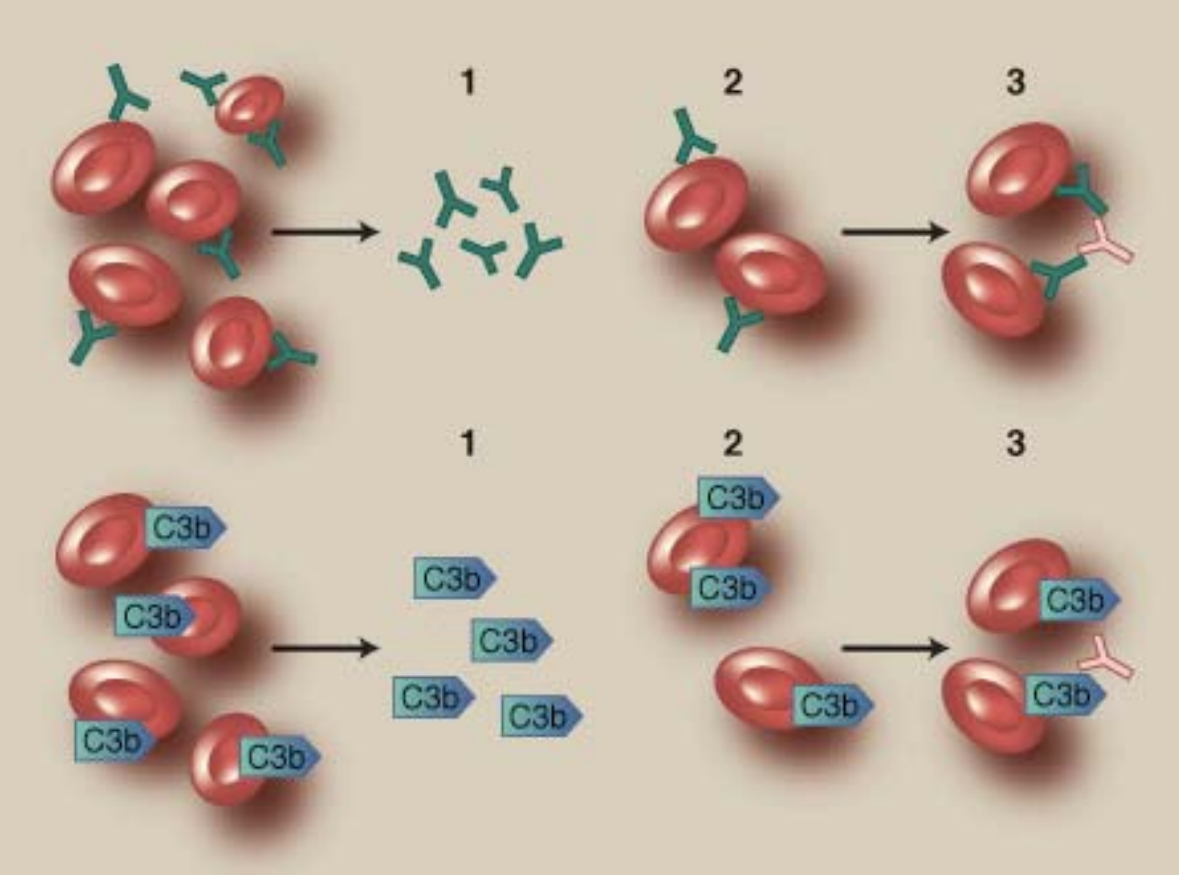
Imperial College London

School of Medicine

Year 2 – 2011-2012

Molecules Cells and Disease

Spring Term Course Guide



The Coombs' test
Tefferi A, Li C. Atlas of Clinical Hematology: Clinical Hematology

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Molecules Cells and Disease

Year 2 – Spring Course Guide

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SOLE FEEDBACK – *Molecules Cells and Disease*

The following pages provide you with templates on which you can record your thoughts as the course proceeds. At the end of the course you can enter your views onto SOLE.

Please answer all questions by selecting the response which best reflects your view.

	Very Good	Good	Satisfac tory	Poor	No Response
The support materials available for this module (e.g. handouts, web pages, problem sheets and/or notes on the board).	☐	☐	☐	☐	☐
The organisation of the module.	☐	☐	☐	☐	☐
	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
Feedback on my work has been prompt (this refers to your work being commented upon within a specified time).	☐	☐	☐	☐	☐
Feedback on my work has helped me clarify things I did not understand.	☐	☐	☐	☐	☐

Please use this box for constructive feedback and suggestions for improvement.

SOLE FEEDBACK - INDIVIDUAL LECTURERS

Please note that for SOLE, a Lecturer's name will only appear once. This template gives you the opportunity to record your comments about each lecture in the order of delivery.

On the following section, you have an opportunity to record any comments and constructive feedback you have for each lecturer.

	The structure and delivery of the lectures.					**The explanation of concepts given by the lecturer.**					**The approachability of the lecturer.**					**The interest and enthusiasm generated by the lecturer.**				
Lecturer and Lecture Title	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor
Paolo Vineis: Epidemiology of cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Nigel Gooderham: Oncogenes and tumour suppressors	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Marjorie Walker: Cellular pathology of cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tony Magee: Apoptosis	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vania Braga: The cell cycle and its regulation	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tony Magee: Signalling mechanisms in growth and division	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Nigel Gooderham: DNA damage and repair	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	The structure and delivery of the lectures.					The explanation of concepts given by the lecturer.					The approachability of the lecturer.					The interest and enthusiasm generated by the lecturer.				
Lecturer and Lecture Title	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor
Peter Clark: External factors controlling division and behavior of normal and cancerous cells	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mike Ferenczi: The cytoskeleton	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vania Braga: Invasion: regulation of cell migration	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mike Osborn: Colorectal cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Henning Walczak: Tumour immunology	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Justin Stebbing: Biological basis of cancer therapy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Keith Gould: Tolerance and autoimmunity	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tony Chu: Skin cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	The structure and delivery of the lectures.					The explanation of concepts given by the lecturer.					The approachability of the lecturer.					The interest and enthusiasm generated by the lecturer.				
Lecturer and Lecture Title	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor	Very Good	Good	Satisfactory	Poor	Very Poor
Laki Buluwela: Breast cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sebastian Johnston: Hypersensitivity and allergy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Candice Roufosse: Transplantation	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Barbara Bain: Leukaemia	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Angelika Gründling: Community infections	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Angelika Gründling: Hospital infections	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Elaine Bignell: Immunity to fungal infections	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wendy Barclay: Viral evasion of host immunity	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ingrid Müller: Parasitic infections 1	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ingrid Müller: Parasitic infections 2	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Lecturer and Lecture Title	Please use this box for additional constructive feedback.
Prof Paolo Vineis **Cancer 1:** Epidemiology of cancer	
Prof Nigel Gooderham **Cancer 2:** Oncogenes and tumour suppressors	
Dr Marjorie Walker **Cancer 3:** Cellular pathology of cancer	
Prof Tony Magee **Cancer 4:** Apoptosis	
Dr Vania Braga **Cancer 5:** The cell cycle and its regulation	
Prof Tony Magee **Cancer 6:** Signalling mechanisms in growth and division	
Prof Nigel Gooderham **Cancer 7:** DNA damage and repair	
Dr Peter Clark **Cancer 8:** External factors controlling division and behavior of normal and cancerous cells	
Prof Mike Ferenczi **Cancer 9:**The cytoskeleton	
Dr Vania Braga **Cancer 10:** Invasion: regulation of cell migration	
Dr Mike Osborn **Cancer 11:** Cancer as a disease – Colorectal cancer	
Prof Henning Walczak **Immunology 1:** Tumour immunology	
Prof Justin Stebbing **Cancer 12:** Biological basis of cancer therapy	

Lecturer and Lecture Title	Please use this box for additional constructive feedback.
Dr Keith Gould Immunology 2: Tolerance and autoimmunity	
Dr Tony Chu Cancer 13: Cancer as a disease – Skin cancer	
Dr Laki Buluwela Cancer 14: Cancer as a disease - Breast cancer	
Prof Sebastian Johnston Immunology 3: Hypersensitivity and allergy	
Dr Candice Roufosse Immunology 4 Transplantation	
Prof Barbara Bain Cancer 15: Cancer as a disease – Leukaemia	
Dr Angelika Gründling Microbiology 1: Community infections	
Dr Angelika Gründling Microbiology 2: Hospital infections	
Dr Elaine Bignell Microbiology 3: Immunity to fungal infections	
Prof Wendy Barclay Microbiology 4: Viral evasion of host immunity	
Dr Ingrid Müller Microbiology 5: Parasitic infections 1	
Dr Ingrid Müller Microbiology 6: Parasitic infections 2	

Molecules Cells and Disease

INTRODUCTION

The Molecules Cells and Disease theme is spread across two terms in Year 2.
The **courses** that comprise MCD in Year 2 and the **course leaders** are:

Haematology	Dr Nina Salooja
Diagnostics	Dr Mike Barrett
Cancer	Dr Laki Buluwela & Dr Marjorie Walker
Immunology	Dr Keith Gould
Microbiology	Dr Angelika Gründling

In the spring term the MCD Theme will concentrate on Cancer (Cellular and Molecular Biology, Cell cycle, Cancer as a disease, Carcinogenesis), but will also include Immunology and Microbiology, together with some revision of Haematology.

Teaching will consist of tutorials and practicals as well as lectures, along the lines of MCD in Year 1.

Come prepared. In advance of each session look at this handbook to check what you will be doing, and read through the objectives relating to lectures.

Student's responsibilities:

We shall endeavour to make sure that the Theme material is delivered to you in the most interesting and fruitful way. In return, we expect that you will make an effort to make the most of what is made available to you, and not to spoil your colleagues' experience.

Practically, this means that:

You are expected to arrive on time, at the right place. Your timetable for this term is electronic, viewable on the Intranet at:
https://education.med.imperial.ac.uk/Years/2-1112/Springrot/index.html
Make sure you know your tutorial group, and check the timetable regularly for any changes. Some sessions involve the Brian Drewe Lecture theatre, Reynolds Building, at the Charing Cross (CX) campus, others involve the SAFB lecture theatre on the South Kensington (SK) Campus. All teaching sessions are compulsory, and you should report any absence due to illness to the FEO using the online reporting system.

Come prepared. You are expected to have read the relevant material in the Study Guide in advance of tutorials. This makes sure that the learning experience is more beneficial and enjoyable. Much material, including lecture material, is available on the intranet. You are encouraged to make yourself familiar with this material.

Come equipped. This Study Guide and the Lecture Notes are needed daily. Of course you need writing material, a ruler and possibly pens/pencils of different colours, a highlighter and a calculator. The Student Union shop has a good supply of these, on site, were you to forget or lose the essentials.

Be safe. Safety should be foremost in your mind as you embark on a medical career. Follow safety advice scrupulously. Breaches of safety can result in accidents and/or disciplinary procedures.

The Intranet

You will find MCD teaching material on the intranet for the School of Medicine: https://education.med.imperial.ac.uk. You will have to provide your Imperial College username and password to gain access to it. In addition to the Study Guide, timetable etc., the Intranet provides the lecture slide material itself. Various formats are used: PDF files, Word documents and PowerPoint presentations.

You are also encouraged to use the Discussion Boards inside Blackboard which you can access at **http://learn.imperial.ac.uk**

Look at the intranet daily: the intranet is regularly updated, this Course Guide is not!

Dr Keith Gould
Theme Leader

Dr James Pease
Deputy Theme leader

January 2012

Computer-assisted learning (CAL - Intranet)

- Bain BJ, Interactive Haematology Imagebank, Blackwell Science, Oxford, 1999.

This web-based collection is on the Intranet – navigate via links on the Year 2 MCD page or go straight to the Intranet CAL at https://education.med.imperial.ac.uk/CAL/index.htm

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ASSESSMENT

The MCD courses delivered in the Autumn term and those delivered in the Spring term will be examined in a single examination **on Friday 4th May 2012 at 10:00 am,** covering all courses: - **Molecules, Cells and Disease II — Paper 2 (2 hr)**

The questions will be in SAQ and SBA formats. Further details about examinations are provided on the Intranet.

LEARNING OBJECTIVES

Epidemiology of cancer (Professor Paolo Vineis)

- Define incidence and mortality rates of the major adult tumours as well as their spatial distribution and temporal trends
- List the sites of the most common cancers occurring in children
- Discuss the epidemiological evidence for cancer causation

Oncogenes and tumour suppressors (Professor Nigel Gooderham)

- Define proto-oncogene, oncogene and tumour suppressor gene
- Explain how a proto-oncogene can be activated to an oncogene
- Explain how activating an oncogene can disrupt tightly controlled pathways in the cell
- Describe how rare heritable cancers have led to an understanding of tumour suppressor genes
- Summarise the role of the tumour suppressor gene p53 in cellular decision making
- Describe the way in which successive gene mutations are thought to lead to clinical cancer

Cellular pathology of cancer (Dr Marjorie Walker)

- Define the words metaplasia, dysplasia, neoplasia, tumour, malignancy, hamartoma, carcinoma, sarcoma, teratoma, lymphoma, leukaemia, carcinogen, metastasis
- Explain the principles underlying the nomenclature of tumours
- List four features which distinguish benign from malignant tumours and explain how they are of use in making that distinction
- Describe five morphological features which allow assessment of the differentiation of a tumour

Apoptosis (Professor Tony Magee)

- Explain the difference between necrosis and apoptosis and describe how they may be differentiated
- Discuss whether necrosis and apoptosis are the only forms of cell death
- Describe the proteolytic caspase cascades which execute the apoptotic response
- Discuss how apoptosis may be mediated through death receptors and/or the mitochondria
- Discuss how Bcl-2 family proteins may modulate apoptosis

The cell cycle and its regulation (Dr Vania Braga)

- Describe the cell cycle in terms of the named phases (G0, G1, G2, S, M) and explain what these mean in terms of protein and DNA synthesis
- Identify (or sketch or describe) the named stages of mitosis
- Explain the importance of checkpoints in controlling progression through the cell cycle, and give examples of external factors which provide signals allowing cells to pass these checkpoints and enter cell division
- Describe the way the cell cycle allows decision making about whether a cell divides, differentiates or undergoes programmed cell death (apoptosis)
- Introduce the principle of the molecular timing process which regulates the cell cycle through oscillating amounts or activities of cyclins and their kinases

Signaling mechanisms in growth and division (Professor Tony Magee)

- Explain how ligands which activate tyrosine kinase receptors signal through the small G protein, Ras, to activate the extracellular signal-regulated kinase (ERK) cascade
- Describe how the ERK cascade pathway regulates gene expression and leads to progression through G1 of the cell cycle
- Outline the principle of the molecular timing process which regulates the cell cycle through oscillating amounts or activities of cyclins, their kinases and inhibitor proteins

DNA damage and repair (Professor Nigel Gooderham)

- Describe how DNA can be damaged by radiation or chemicals (carcinogens) and the role metabolism can play in these reactions
- Outline in general terms the role of p53 in the detection of, and response to, DNA damage
- Summarise the natural repair mechanisms for damaged DNA
- Explain how unrepaired or misrepaired DNA damage can become “fixed” as a mutation
- Summarise how the potential of a chemical/agent to damage DNA can be assessed

External factors controlling division and behaviour of normal and cancerous cells (Dr Peter Clark)

- Describe using examples the role of external growth factors in controlling the decision of a cell to divide
- Describe using examples how mutation of a proto-oncogene can perturb the normal controls on cell division
- Explain why signalling pathways involving growth factors are often implicated in the uncontrolled division of cancerous cells
- Explain the role of contact inhibition and anchorage dependence in limiting the division of normal cells within their tissues
- Discuss the factors which restrain cells within their normal tissue boundaries

The cytoskeleton (Professor Michael Ferenczi)

- Review previous outline knowledge of the cytoskeleton and the three filament systems which make it up
- Describe the assembly and organisation of subunits in intermediate filaments
- Explain why these filaments do not show a polarity and how the same class of filament can be made from different subunit molecules in different cell types
- Define the role of intermediate filaments, their size and localisation
- Describe how the structure of microtubules results from polymerisation of subunits consisting of α,β-tubulin dimers
- Describe the polymerisation dynamics of microtubules in terms of preferred (+) and non-preferred (-) ends and GTP hydrolysis

Invasion: regulation of cell migration (Dr Vania Braga)

- Describe the cytoskeletal processes occurring during cell locomotion
- Explain the different actin binding proteins and the activities in which they participate to remodel actin filaments
- Describe the role of phosphorylation and other second messengers as control mechanisms for cytoskeletal components
- Describe the role of small G proteins of the Rho family in controlling organisation of the actin cytoskeleton
- Explain why the onset of metastasis is a critical stage in the development of a cancer, and give examples of the changes at the cellular and molecular levels which are necessary for it to occur

Colorectal cancer (Dr Michael Osborn)

- Describe the different modes of clinical presentation of colorectal carcinoma
- List the principles of the adenoma-carcinoma sequence
- Define the Duke’s and TNM staging systems
- Develop an understanding of molecular pathogenesis of colorectal carcinoma
- Describe the major pathological features which are associated with aggressive malignant behaviour of colorectal carcinoma

Tumour immunology (Professor Henning Walczak)

- Outline evidence for the importance of tumour surveillance by the immune system
- Understand that immune responses to tumours have some similarities with those to virus infected cells
- Explain the concepts of tumour-associated antigens and cancer immunoediting
- Outline approaches being developed for tumour immunotherapy

Biological basis of cancer therapy (Professor Justin Stebbing)

- Describe the main chemotherapeutic and radiotherapeutic approaches to treating cancer
- Explain why many cancer treatments cause side effects such as nausea, hair loss, anaemia and immunosuppression, and indicate the approaches which have been tried to minimise these
- Explain the rationale for the newer drugs in cancer therapy
- Discuss the prospects for new therapies based on the biology of cancer development

Tolerance and autoimmunity (Dr Keith Gould)

- To understand the concept of immunological tolerance
- To understand the mechanisms underlying immunological tolerance
- To understand how defects in tolerance lead to autoimmune diseases and know examples of these

Skin cancer (Dr Tony Chu)

- Summarise the epidemiology of skin cancer
- Explain the pivotal role of ultraviolet light in the pathogenesis of skin cancer
- Describe the effect of ultraviolet light on DNA and on immune function in the skin
- Explain the role of the tumour suppresser gene p53 in relation to UV-induced mutations
- Describe the role of oncogenic human papilloma virus in the pathogenesis of squamous cell carcinoma (genetic model of epidermodysplasia verruciformis and renal transplant recipients)

Breast cancer (Dr Laki Buluwela)

- Summarise information on the incidence of breast cancer
- Describe the histology and clinical features of breast cancer
- Describe breast cancer as a steroid hormone regulated disease
- Explain the nature of endocrine therapies in breast cancer
- Summarise what is known about the genetics of familial breast cancer
- Briefly describe the consequences of metastasis of breast tumours

Hypersensitivity and allergy (Professor Sebastian Johnston)

- Outline the mechanisms by which IgE, antibodies, immune complexes and T cells can cause tissue damage and inflammation (the four types of hypersensitivity), giving examples of the clinical syndromes associated with each
- Outline the factors underlying the development of atopic/allergic diseases
- Describe the important clinical features of asthma, hay fever, allergic eczema and anaphylaxis
- Briefly describe the approach to investigation and management of patients with these disorders

Transplantation (Dr Candice Roufosse)

- To understand which organs can be transplanted, why, and where the transplanted organs come from
- To understand some of the ethical and structural/organisational issues surrounding organ transplantation

- To understand the immunological issues in transplantation and their impact on organ allocation and rejection after transplantation (including the main types of transplant rejection)
- To understand the risks and complications associated with transplantation and transplant immunosuppression

Leukaemia (Professor Barbara Bain)
- Explain what “leukaemia” is
- Explain the difference between lymphoid and myeloid leukaemias and between acute and chronic leukaemias
- Outline the clinical and haematological features and representative cytogenetic and molecular genetic abnormalities of acute lymphoblastic leukaemia

Community infections (Dr Angelika Gründling)
- Define an outbreak of infectious disease
- Describe how outbreaks are identified
- Name bacterial pathogens that cause community acquired infection
- Describe the routes and types of infection caused by these bacterial pathogens
- Explain the molecular bases for the virulence of these bacterial pathogens

Hospital infections (Dr Angelika Gründling)
- Explain why patients in hospital are particularly at risk from acquiring new infections
- Give examples of common hospital infections caused by bacterial pathogens
- Describe the routes and types of infection caused by these bacterial pathogens
- Explain the molecular bases for the virulence of these bacterial pathogens
- Explain why prudent antibiotic use is important in limiting hospital acquired infections
- Explain the problem of antibiotic resistance

Immunity to fungal infections (Dr Elaine Bignell)
- Name the major antifungal immune effector cells
- Describe 2 mechanisms of cell-mediated antifungal defence
- Summarise the sequence of events leading to effective clearance of infectious fungal cells
- Understand how immune status determines risk of fungal infection

Viral evasion of host immunity (Professor Wendy Barclay)
- Outline, using examples of named viruses, how viruses escape innate immunity by subversion of host innate immune responses
- Describe, with named examples of viruses, how antigenic variation may lead to viral evasion of host immunity
- Describe with named examples how viruses escape host cellular immune responses

Parasitic infections (Dr Ingrid Muller)
- Distinguish infection from disease
- Understand the word ‘parasite’
- Understand ‘Linnaean nomenclature’ especially in terms of Genus and species
- Describe the main features of the malaria life cycle which have clinical relevance
- Understand what is meant by a vector and give 2 examples
- List a few common causes of helminthic infections
- Understand the role of eosinophils in helminthic infections
- Distinguish protozoal from helminthic infections

ROTATION 1

Tutorial 1 – The cell cycle and cancer

- Understand how signals trigger cell cycle entry from Go in mammalian cells
- Understand how cell cycle inhibitors control Cdk activity
- Understand how cells check for DNA damage
- Give examples of proteins controlling the cell cycle that are commonly mutated in cancers

Practical 1 – The cellular pathology of cancer

Understand the value of histology to determine **dysplasia**, low grade and high grade (in situ cancer), invasive **cancer** (of different types and grade) – adenocarcinoma, squamous carcinoma and to demonstrate cancer **staging** – lymph node **metastases,** using examples of cancer discussed in the cancer lectures

ROTATION 2

Tutorial 2 – Exploitation of the actin cytoskeleton by *Listeria*

- Describe the formation of actin filaments (F-actin) by polymerisation of monomeric units(G-actin) of actin, using the concepts of nucleation and elongation
- Explain what is meant by the preferred (or + or barbed) end of the filament for polymerisation, and the non-preferred (–, pointed) end
- Explain why polymerisation of actin can be a force-producing process
- Describe the way *Listeria* uses cytoplasmic actin and associated proteins to achieve intracellular motility.
- Summarise the role of this property in the life cycle of *Listeria*, and explain its relationship to the risk factors for infection.

Practical 2 – Diagnosis of bacterial infections using the polymerase chain reaction (PCR)

This practical introduces the molecular technique PCR and its application in clinical settings. Upon completion of this practical you should understand and know more about:

- How the PCR technique works in practice
- How to analyse a PCR product by agarose gel electrophoresis and how to interpret the results
- Different applications of PCR in clinical settings
- Different applications of PCR in research settings
- The pro's and con's of using PCR as a tool in clinical applications

ROTATION 3

Tutorial 3 – Prostate cancer

- Discuss the strengths and limitations of current diagnostic tests for prostate cancer
- Explain the use of hormone therapy for breast and prostate cancers, and the possible causes of its failure
- Discuss whether screening or treatment for cancer is always desirable

Practical/CAL/Seminar – Haematology (15 March only, all day)

- Recognise, on microscope slides or images, common important abnormalities in blood
- cells
- Explain the meaning of the words used in laboratory reports to describe blood cell
- abnormalities
- Interpret reports of blood counts and other common haematology tests in the light of the clinical features, and propose what action should be taken next and why

Recommended reading

Alberts, Bray, Hopkin, Johnson, Lewis, Raff, Roberts and Walter, *Essential cell biology,* 3rd Edition (2009) Garland Press Publishing, ISBN: 978-0-8153-4130-7. 860 pages.

For a much briefer overview of cell biology and as an aid for revision try:
Norman and Lodwick, *Flesh and bones of medical cell biology,* (2007) Mosby/Elsevier, ISBN 978-0-7234-3367-5. 124 pages.
These two books are relevant for the MCD Courses: Introduction to cells, Nucleic acids and gene expression, Metabolism, Cellular organisation of Tissues, and Cancer.

Nairn and Helbert, *Immunology for medical students*, 2nd Edition (2007) Mosby/Elsevier, ISBN: 978-0-3230-4331-1. 308 pages. Recommended for the MCD Immunology Course.

Gillespie and Bamford, *Medical microbiology and infection at a glance,* 3rd Edition (2007) Blackwell Publishing, ISBN: 978-1-4051-5255-6. 128 pages.
A useful book at a suitable level which integrates microbiology with immunology is:
Playfair and Bancroft, *Infection and immunity,* 3rd Edition (2008) Oxford University Press, ISBN: 978-0-19-9206735. 341 pages.

Bateman and Carr, *Flesh and bones of pathology,* (2009) Mosby/Elsevier, ISBN: 978-0-7234-3396-5. 145 pages.
This is at a basic level appropriate for years 1 and 2, and is recommended for the MCD Cell Pathology Course. It is also relevant for the Cancer Course.

Comprehensive textbooks covering cellular pathology and cancer, also suitable for the whole medical course are either of:

Kumar, Abbas, Fausto and Mitchell, *Robbins basic pathology,* 8th Edition (2007) Saunders/Elsevier, ISBN: 978-1-4160-2973-1. 960 pages.
Underwood and Cross, *General and systematic pathology*, 5th Edition (2009) Churchill Livingstone/Elsevier, ISBN: 978-0-4430-6888-1. 872 pages.
For background and additional reading to support the MCD Cancer Course an excellent book is:
Weinberg, *The biology of cancer,* (2006) Garland Science, ISBN: 978-0815340768. 850 pages.

Recommended Haematology text book

Bain BJ, *Haematology: a core curriculum,* (2010) Imperial College Press, ISBN: 978-1-84816-499-4. 336 pages.

Other haematology books you may find useful are:

- Mehta A and Hoffbrand AV, *Haematology at a glance*, 3rd Edition (2009) Wiley-Blackwell, ISBN: 978-1-4051-7970-6. 128 pages.
- Hoffbrand AV and Moss PAH, *Essential haematology*, 6th Edition (2011) Wiley-Blackwell, ISBN: 978-1-4051-9890-5. 468 pages.
- Howard MR and Hamilton PJ*, Haematology: an illustrated colour text*, 3rd Edition (2007) Churchill Livingstone/Elsevier, ISBN: 978-0-4431-0362-9. 124 pages.

Supplementary Haematology reading and reference

- Hughes-Jones N, Wickramasinghe SN, and Hatton C, *Lecture notes on Haematology*, 8th ed, Wiley-Blackwell, Oxford (2008) ISBN: 978-1405180504. 216 pages.
- Bain BJ, *A Beginner's Guide to Blood Cells*, 2nd Edition (2004), Blackwell Science, ISBN: 978-1405121750. 136 pages.
- Contreras M, *ABC of Transfusion*, 4th Edition (2009) Wiley-Blackwell, ISBN: 978-1405156462. 128 pages.
- McClelland DBL (ed), *Handbook of Transfusion Medicine*, 4th Edition (2007) HMSO Publications, ISBN: 978-0113226771. 92 pages. Can be downloaded from:

  http://www.transfusionguidelines.org.uk/docs/pdfs/htm_edition-4_all-pages.pdf

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and on Blackboard for Discussion boards

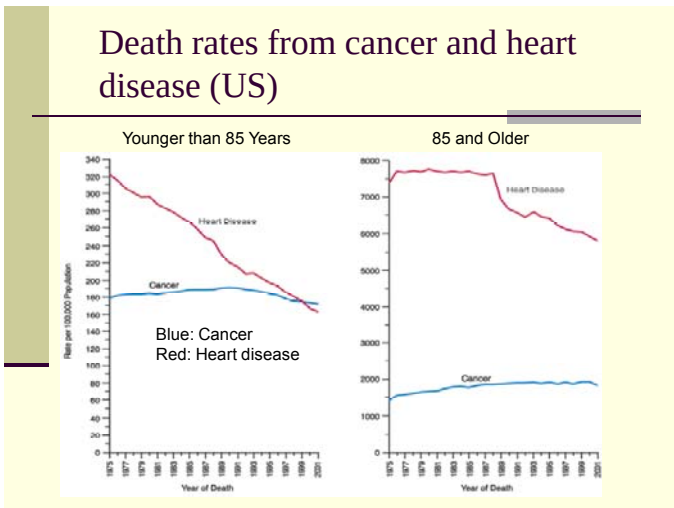
CANCER 1

EPIDEMIOLOGY OF CANCER
Professor Paolo Vineis

Leading causes of death in 2003: US

Cause of death	Number	Percent
1 Heart disease	685,089	28.0
2 Malignant neoplasm	556,902	22.7
3 Cerebrovascular disease	157,689	6.4
4 Chronic lower resp. tract dis.	126,382	5.2
5 Accidents	109,227	4.5
6 Diabetes mellitus	74,219	3.0
7 Influenza and pneumonia	65,163	2.7
8 Alzheimer's disease	63,457	2.6
9 Nephritis, nephrotic syndrome	42,453	1.7
10 Septicemia	34,069	1.4
Total	2,448,288	100

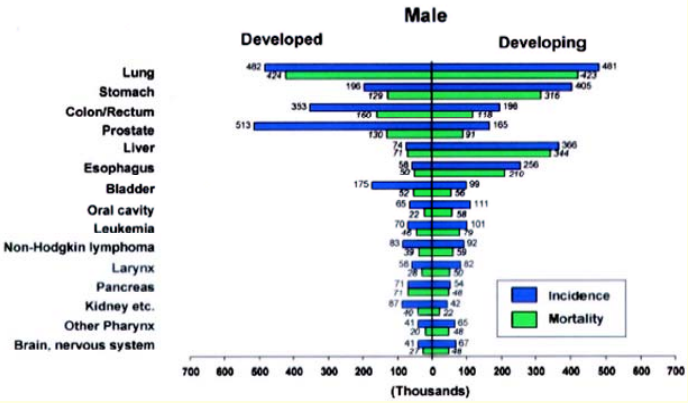
(CDC/NCHS, 2005)



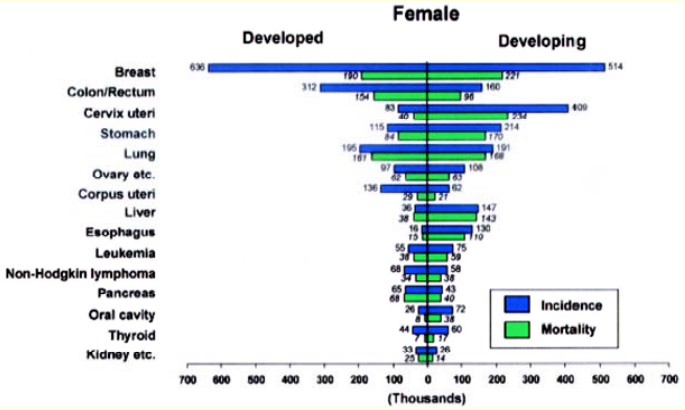
Global burden of cancer (2002)

- 10.9 million new cases of cancer worldwide (excluding non-melanoma skin cancer)
- 6.7 million deaths
- 24.6 million persons alive with cancer (within 5 years of diagnosis)
- Cancer mortality
  - 1985 50/50 (%) developing/developed countries
  - 2002 61/39 (%) developing/developed countries

Worldwide estimated number of new cases and deaths, in men (2002)



Worldwide estimated number of new cases and deaths, in women (2002)



Five leading cancer types for incident cases (in thousands), Worldwide (2002) and UK (2005)

Males

Worldwide	UK
Lung (965)	Prostate (34)
Prostate (679)	Lung (22)
Stomach (603)	Colorectal (20)
Colorectal (550)	Bladder (7)
Liver (442)	Non-Hodgkin Lymphoma (5)

Females

Worldwide	UK
Breast (1152)	Breast (46)
Cervix uteri (494)	Colorectal (17)
Colorectal (473)	Lung (16)
Lung (387)	Corpus uteri (7)
Stomach (330)	Ovary (7)

Five leading cancer types for deceased cases (in thousands), Worldwide (2002) and UK (2006)

Males

Worldwide	UK
Lung (848)	Lung (20)
Stomach (446)	Prostate (10)
Liver (417)	Colorectal (8)
Colorectal (278)	Oesphageal (5)
Oesophageal (261)	Pancreas (4)

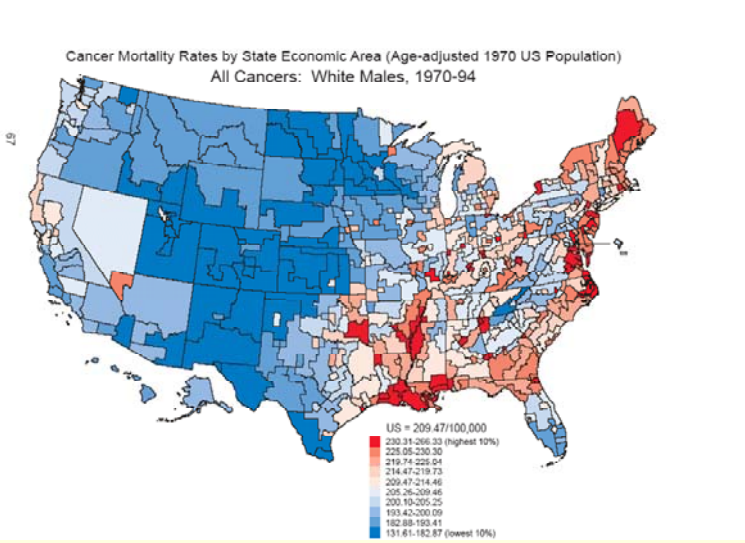
Females

Worldwide	UK
Breast (410)	Lung (15)
Lung (330)	Breast (12)
Cervix uteri (273)	Colorectal (7)
Stomach (254)	Ovary (4)
Colorectal (250)	Pancreas (4)

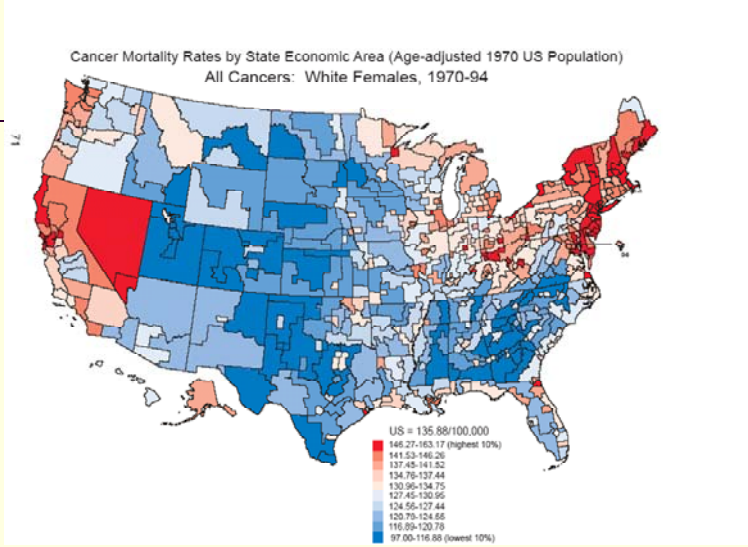
International geographic variation in cancer incidence (rates per 100,000), 2002

Cancer	High-incidence area	Low-incidence area	Ratio
Melanoma	Australia/NZ (M 38)	China (M 0.2)	189
Prostate	N. America (120)	China (1.6)	75
Lung	E. Europe (M 66)	W. Africa (M 2.4)	27
Colorectal	Japan (M 49)	Middle Africa (M 2.3)	21
Esophageal	China (M 27)	W.Africa (M 1.3)	21
Liver	China (M 38)	South Central Asia (2.6)	15
Bladder	S. Europe (M 27)	Melanesia (M 1.8)	15
Breast	N. America (99)	Middle Africa (16.5)	6
Non-Hodgkin	N. America (M 17)	China (M 3.0)	6

M= Males

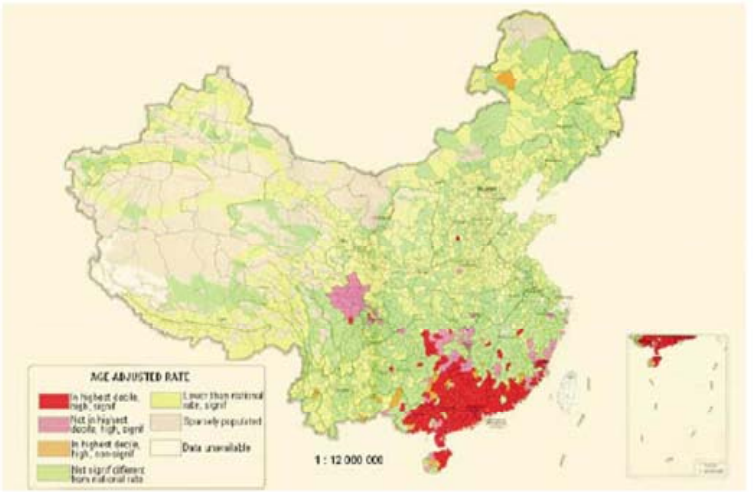


http://www3.cancer.gov/atlasplus/



http://www3.cancer.gov/atlasplus/

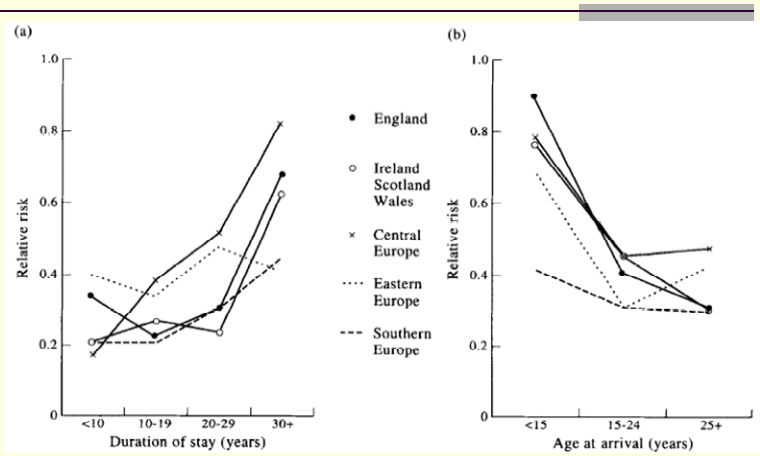
Mortality from cancer of nasopharynx (males) in China, 1973-1975



MIGRANT STUDIES

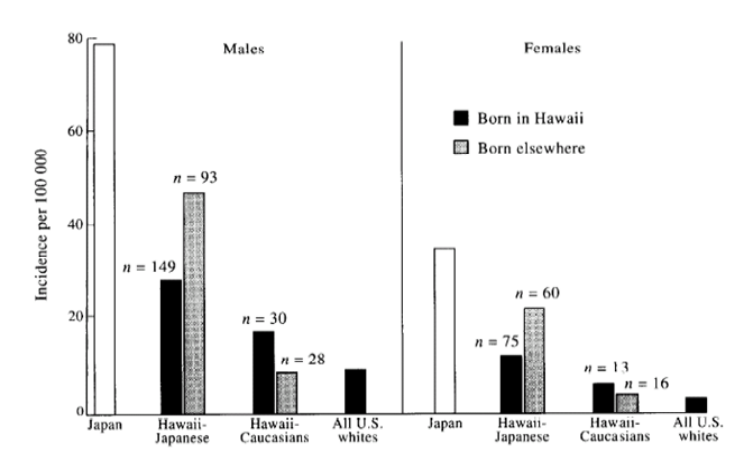
- Offer insights into the relative importance of environment and genetic make-up in cancer etiology
- **Strengths**
  - Data from cancer registry or death certificates is easily accessible and convenient
  - Large numbers, population-based
  - Migrant status defined by birth place (high accuracy)
- **Weaknesses**
  - Useful only if genetically different populations
  - Bias can exist in many forms: confounding, data quality (e.g. ‘overshoot’), selection bias

Melanoma in male migrants to Australia



Risk of death for six migrant populations compared to Australia-born

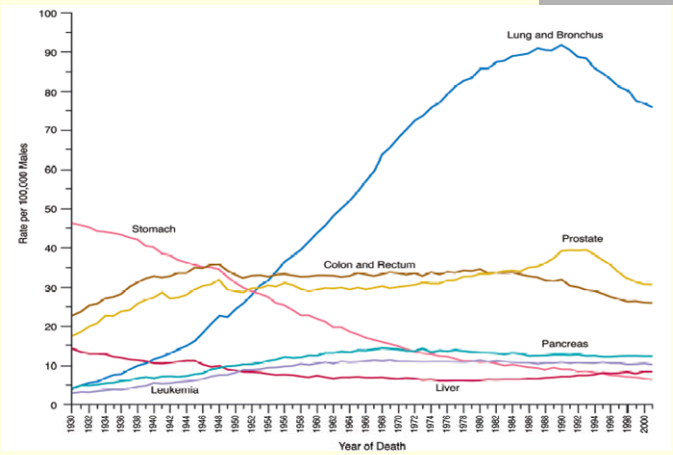
Stomach cancer incidence in Hawaii Japanese and Caucasians by place of birth, 1973-77



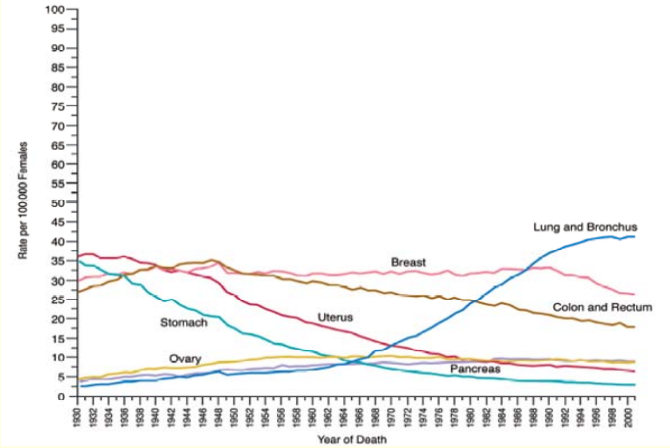
Common reasons for secular trends in cancer rates

- Changes in completeness of the sources of data
- Changes in diagnostic abilities
- Changes of practice in data classification
- Demographic changes in the population in which the trend is being observed
- Changes in screening practices
- Changes in treatment practices
- Change in risk factor distribution

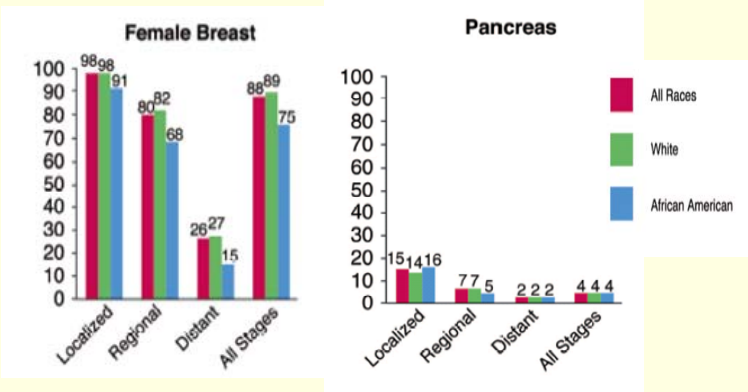
Cancer death rates for selected cancer types among males, US, 1930-2001



Cancer death rates for selected cancer types among females, US, 1930-2001



5-year survival rates by race and stage at diagnosis, 1995-2000



Sources of cancer data

- Cancer Research UK
  http://info.cancerresearchuk.org/cancerstats/types/

- **National Statistics (deaths)**
  http://www.statistics.gov.uk/CCI/nscl.asp?ID=6444

Cancer Etiology
- What do we know?
- What can we do to prevent cancer?
- What remains unexplained?

What are the main risk factors for cancer?
The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. Doll R, Peto R. J Natl Cancer Inst. 1981; 66(6):1191-308

Population attributable risks percent:

Smoking	29-31
Diet	20-50
Alcohol	4-6
Infection	10-20
Occupation	2-4
Reproductive hormone	10-20

These estimates are probably not too far from reality but have several limitations.
- published in 1981

- for US only

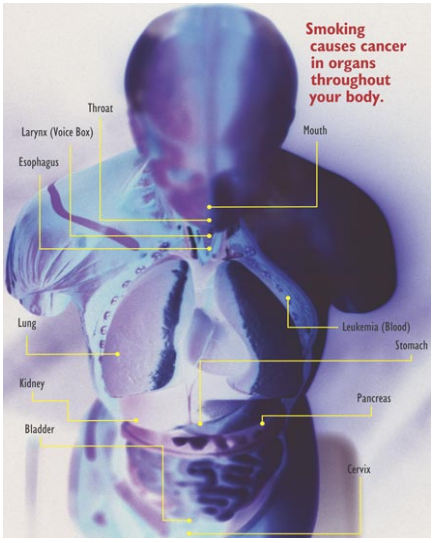
- based essentially on case-control studies

- ignore gene-environment interactions

- ignore molecular and biochemical evidence

SMOKING
Smoking accounts for at least 30% of all cancer deaths

- Smoking is associated with increased risk for at least 15 types of cancers
- Smoking causes 90% of lung cancer deaths in men and
  80% in women



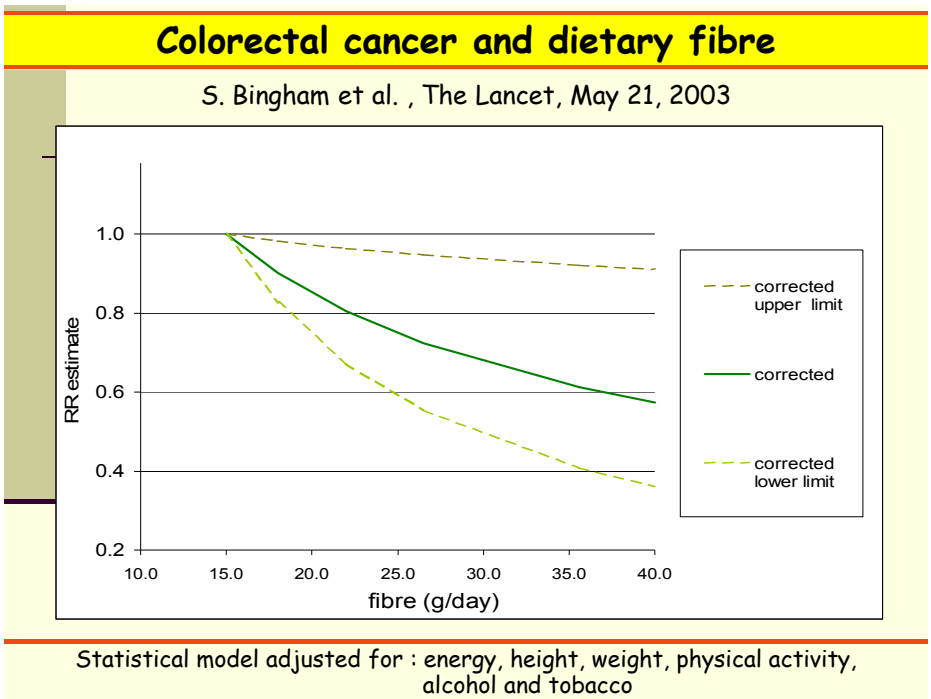
Association between conclusions of the papers on ETS and cancer vs. relationship of the authors with the tobacco industry

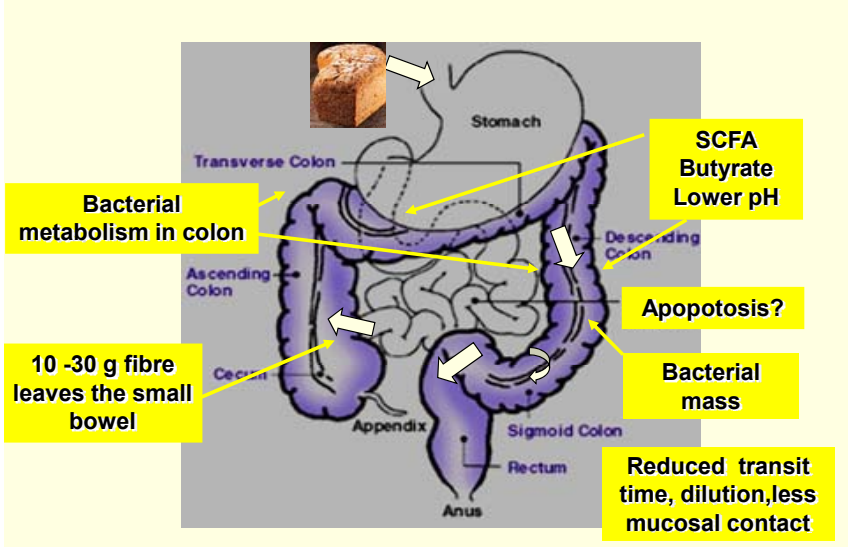
		Relationship with the tobacco industry	
		yes	**no**
Association	**yes**	2	65
	no	29	10

odds ratio 88.4; 95% confidence interval 16.4-476.5; P<.001

Barnes & Bero, JAMA 1998; 279: 1566-70

DIET



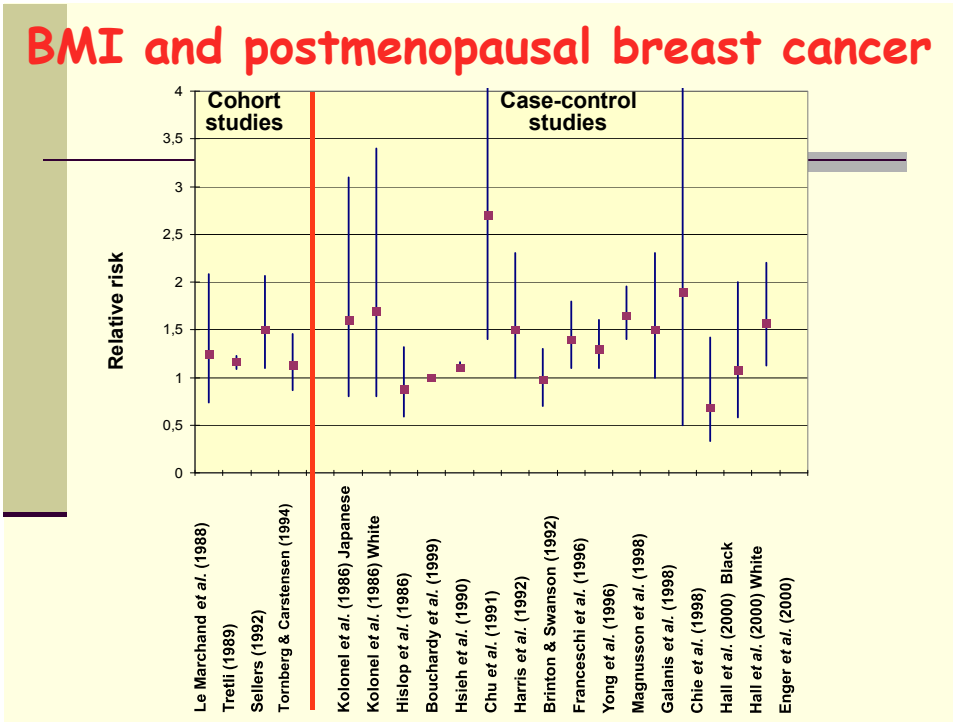


Mechanism of action of dietary fibre (and resistant starch)

ALCOHOL

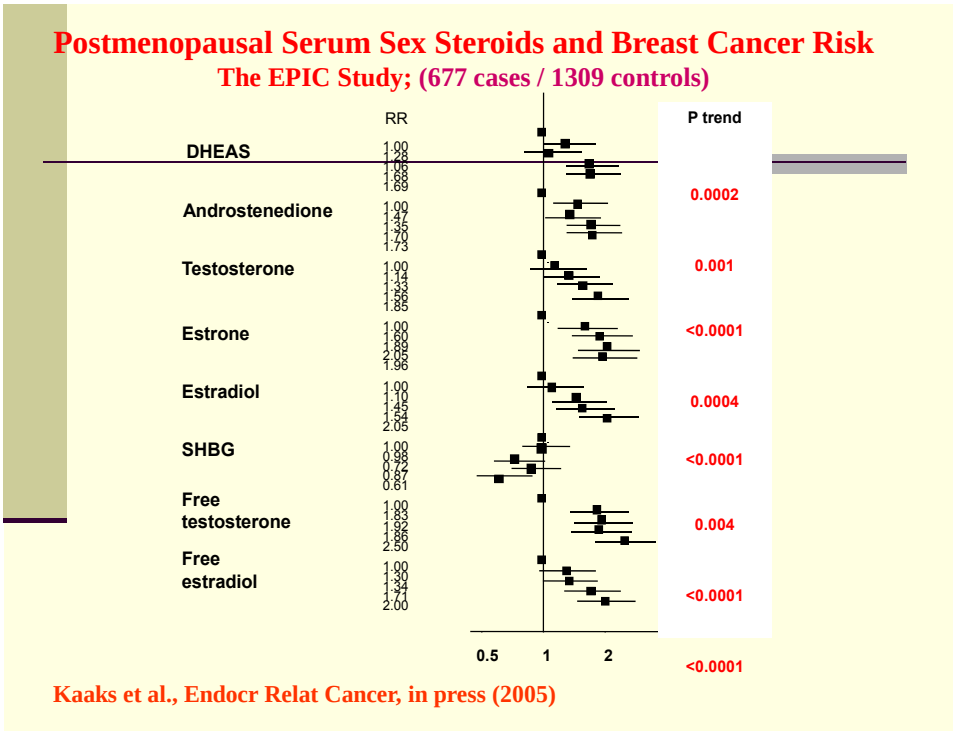
- Oral cavity, pharynx, larynx, oesophagus, liver
- all types of alcohol
- mechanisms poorly understood
- synergism with tobacco
- balance with preventive effect for CHD

ANTHROPOMETRY



BREAST CANCER:

Exogenous & Endogenous Hormones



WESTERNIZATION OF LIFESTYLE AND CANCER

Western Lifestyle:

- Energy dense diet, rich in
  - fat,
  - refined carbohydrates
  - animal protein

- Low physical activity
- Smoking and drinking

Consequences:

- Greater adult body height
- Early menarche
- Obesity
- Diabetes
- Cardiovascular disease
- Hypertension

…and Cancer

H pylori and gastric cancer

- In a pooled analysis of the three cohort studies, the relative risk was 3.8, which was significant. In these cohort studies, potential confounding by dietary and other factors that have previously been associated with gastric cancer was not assessed.
- Nine retrospective case-control studies have addressed the association between sero-prevalence for *H. pylori* infection and incidence of gastric cancer. The estimated relative risks for gastric cancer were elevated in six studies, ranging from 1.2 to 4.2, and were significant in three studies. In a number of studies, the control series may not have been representative of the population that gave rise to the cases.

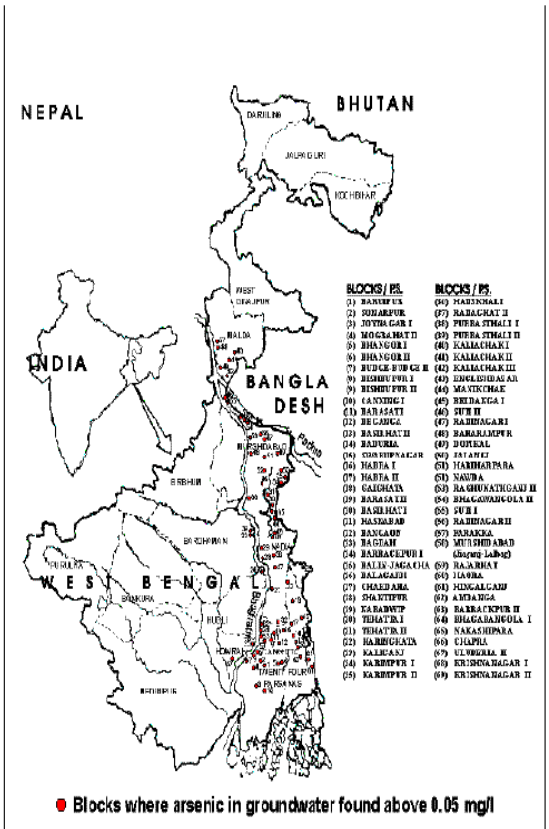


Figure-1: Arsenic affected 9 districts and 67 blocks of West Bengal-India

Arsenic-associated cancer epidemic in West Bengal

- West Bengal:

978 villages/wards in 67 blocks are affected in 9 districts (including southern part of Calcutta)

- Total population in these 9 districts is 42.8 million
- Those having severe keratosis will get cancer in the long run

Arsenic-associated cancer epidemic in Bangladesh

Recent field survey in Bangladesh:

- people suffering from arsenical skin lesions were identified in 75 of 91 villages surveyed
- 2000 water samples were collected
- 60% had increased levels of arsenic above 50 microgram/l

CANCER 2
ONCOGENES and TUMOUR SUPPRESSORS

Professor Nigel Gooderham

Learning Objectives

- Define proto-oncogene, oncogene and tumour suppressor gene.
- Explain how a proto-oncogene can be activated to an oncogene.
- Explain how activating an oncogene can disrupt tightly controlled pathways in the cell.
- Describe how rare heritable cancers have led to an understanding of tumour suppressor genes.
- Summarise the role of the tumour suppressor gene p53 in cellular decision making.
- Describe the way in which successive gene mutations are thought to lead to clinical cancer.

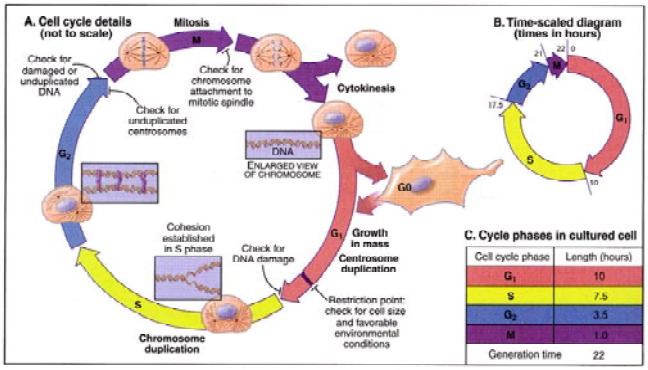
Hallmarks of Cancer

The Cancer Cell Phenotype

- Disregard of signals to stop proliferating.
- Disregard of signals to differentiate.
- Capacity for sustained proliferation.
- Evasion of apoptosis.
- Ability to invade.
- Ability to promote angiogenesis.

Hanahan and Weinberg (2000)

Cell cycle – The key to life, death and cancer



- Cycle checkpoints (growth arrest ensures genetic fidelity).
- Specific proteins accumulate/ are destroyed during the cycle.
  - Cyclins, cycle dependent kinases, cycle dependent kinase inhibitors
- Permanent activation of a cyclin can drive a cell through a checkpoint.

Critical gene targets – Proto-oncogenes

- **Proto-oncogenes** code for essential proteins involved in maintenance of cell growth, division and differentiation.
- Mutation converts a proto-oncogene to an **oncogene**, whose protein product no longer responds to control influences.
- Oncogenes can be aberrantly expressed, over-expressed or aberrantly active.
  - E.g. *MYC, RAS, ERB, SIS*
- Proto-oncogenes can be converted to an oncogene by a single mutation.

Oncogene activation

Normal proto-oncogene

Mutation in the coding sequence
(point mutation of deletion)

Aberrantly active protein

Gene amplification
(Multiple gene copies)

Overproduction of normal protein

Chromosomal translocation
(Chimaeric genes)

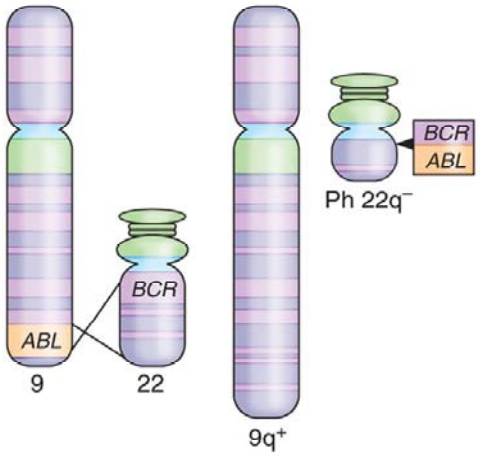
Strong enhancer increases normal protein levels
e.g. Burkitt's lymphoma

or

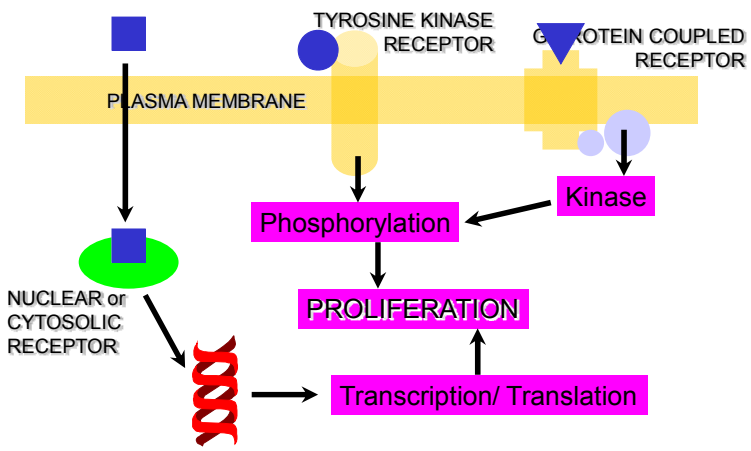
Insertional mutagenesis
(e.g. viral infection)

Fusion to actively transcribed gene overproduces protein or fusion protein is hyperactive.
e.g. Philadelphia chromosome

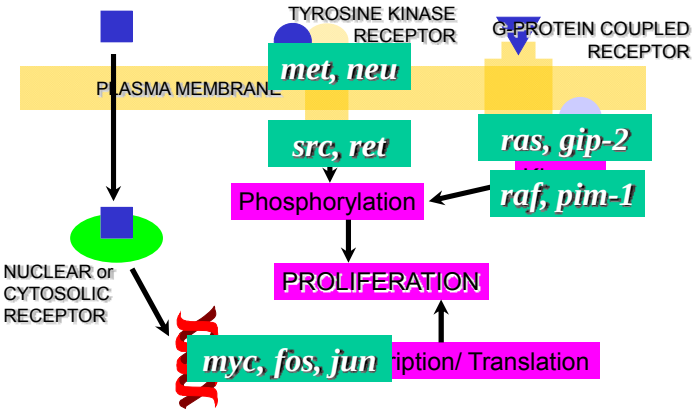
Philadelphia chromosome



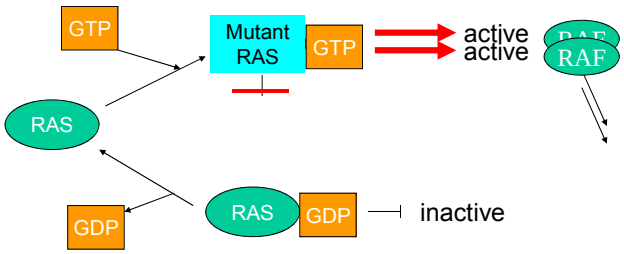
Proteins involved in signal transduction are potential critical gene targets (proto-oncogenes)



Activation of proto-oncogenes to oncogenes disrupts normal activity



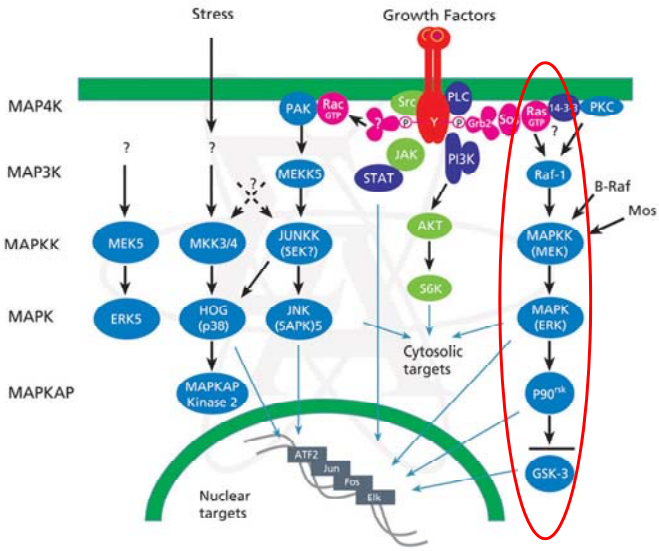
Mutant RAS has aberrant activity



•Upon binding GTP, RAS becomes active.
•Dephosphorylation of the GTP to GDP switches RAS off.
•Mutant RAS fails to dephosphorylate GTP and remains active.

The Mitogen-activated Protein Kinase (MAPK) Cascades

- *RAS* genes (*Ki-*, *H-*, and *N-RAS*) are highly conserved.
- Membrane bound GTPases (GTP bound - active, GDP bound – inactive).
- RAS proteins are central to the stimulation of cell proliferation.



SIGMA-ALDRICH

Oncogenes and Human tumours

Gene	Function	Mechanism of activation	Location	Associated hum cancers
SRC	Tyrosine kinase	Overexpression / C-terminal deletion	Cytoplasmic	Breast, colon, lung
MYC	Transcription factor	Translocation	Nuclear	Burkitt's lymphoma
JUN	Transcription factor	Overexpression / deletion	Nuclear	Lung
Ha-RAS	G protein	Point mutation	Cytoplasmic	Bladder
Ki-RAS	G protein	Point mutation	Cytoplasmic	Colon, lung

Critical gene targets – Tumour suppressor genes

- Typically proteins whose function is to regulate cellular proliferation, maintain cell integrity.
  - *E.g. RB, .*
- Each cell has two copies of each tumour suppressor gene.
- Mutation or deletion of one gene copy is usually insufficient to promote cancer.
- Mutation or lost of both copies means loss of control.

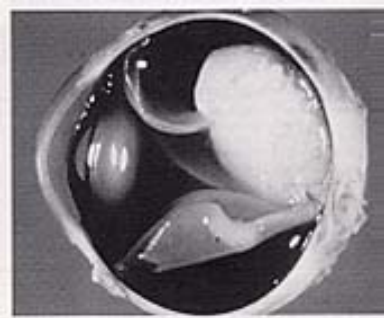
Inherited cancer susceptibility (discovery of tumour suppressor genes)

Features

- Family history of related cancers.
- Unusually early age of onset.
- Bilateral tumours in paired organs.
- Synchronous or successive tumours.
- Tumours in different organ systems in same individual.
- Mutation inherited through the germline.

Retinoblastoma

- Malignant cancer of developing retinal cells.
- Sporadic disease usually involves one eye. Hereditary cases can be unilateral or bilateral and multifocal.
- Due to mutation of the RB1 ***tumour suppressor gene*** on chromosome 13q14.
- RB1 encodes a nuclear protein that is involved in the regulation of the cell cycle.



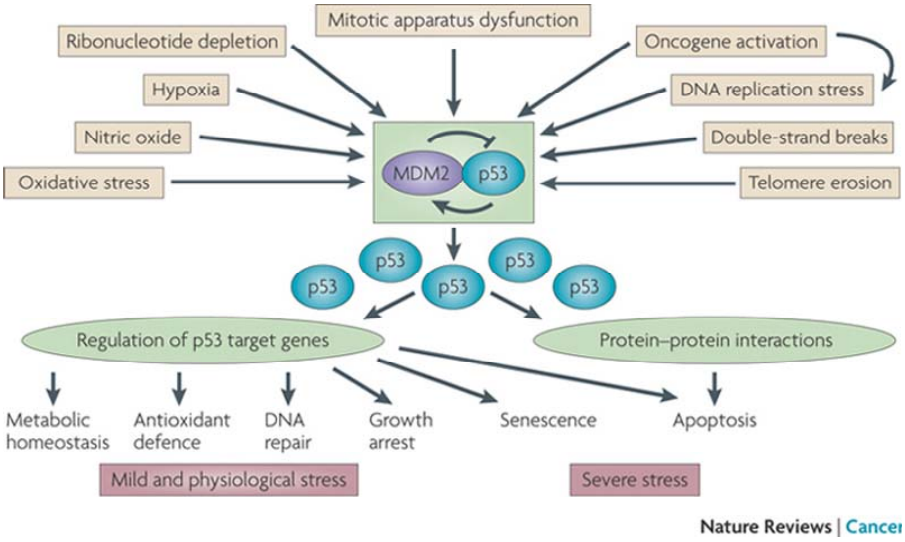
Functional classes of Tumour suppressor genes

- Regulate cell proliferation
- Maintain cellular integrity
- Regulate cell growth
- Regulate the cell cycle
- Nuclear transcription factors
- DNA repair proteins
- Cell adhesion molecules
- Cell death regulators
    - Suppress the neoplastic phenotype

Tumour suppressor genes and human tumours

Gene	Function	Location	Associated human cancer
p53	Cell cycle regulator	Nuclear	Many (colon, breast, bladder, lung etc)
BRCA1	Cell cycle regulator	Nuclear	Breast, ovarian, pros
PTEN	Tyrosine and lipid phosphatase	Cytoplasmic	Prostate, glioblastom
APC	Cell signaling	Cytoplasmic	Colon
p16^{-INK4A}	Cell cycle regulator	Nuclear	Colon and others
MLH1	Mismatch repair	Nuclear	Colon, gastric

P53 – the guardian of the genome



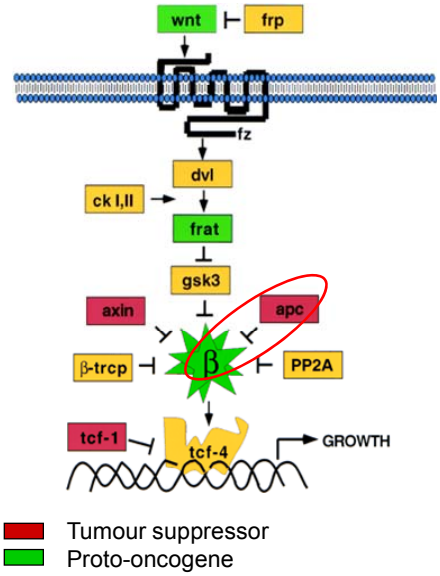
Although p53 is a tumour supressor gene, mutants of p53 act in a dominant manner and **mutation of a single copy** is sufficient to get dysregulation of activity.

APC tumour suppressor gene
(Familial adenomatous polyposis coli)

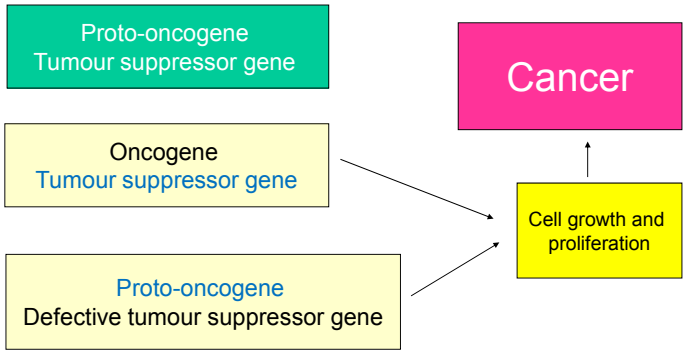
- Due to a deletion in 5q21 resulting in loss of APC gene (***tumour suppressor gene***).
- Involved in cell adhesion and signaling.
- Sufferers develop multiple benign adenomatous polyps of the colon.
- There is a 90% risk of developing colorectal carcinoma.

APC tumour suppressor gene

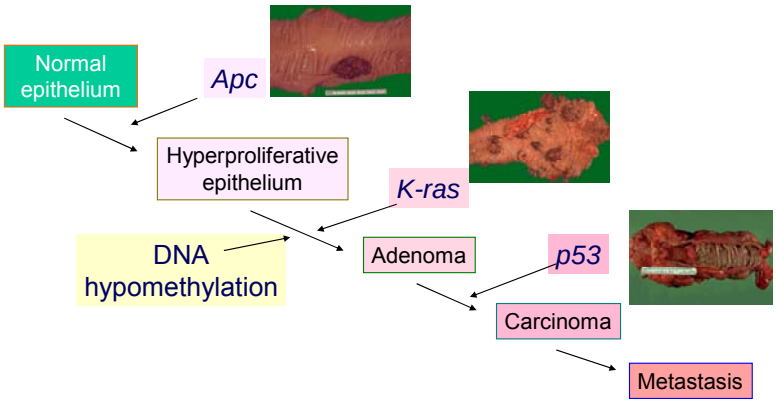
- The tumour suppressor gene *APC* participates in the WNT signalling pathway.
- APC protein helps control the activity of β-catenin and thereby preventing uncontrolled growth.
- Mutation of *APC* is a frequent event in colon cancer.



The route to cancer



The development of colo-rectal Cancer



Oncogenes and tumour suppressor genes

Oncogene	Tumour suppressor gene
Gene active in tumour	Gene inactive in tumour
Specific translocations/point mutations	Deletions or mutations
Mutations rarely hereditory	Mutations can be inherited
Dominant at cell level	Recessive at cell level
Broad tissue specificity	Considerable tumour specificity
Leukaemia and lymphoma	Solid tumours

Summary

- Human cancer involves damage to DNA, or inheritance of aberrant sequences, at critical gene targets.
- These targets, proto-oncogenes and tumour suppressor genes, regulate cell cycle decisions (mitosis, arrest, differentiation, apoptosis).
- The 'guardian of the genome', p53 is a key player in decision making during the cell cycle.
- Studies of rare heritable cancers have led to an understanding of tumour suppressor genes.
- Colon cancer is a model for many of these factors

Reading material

1. The Cancer handbook 2nd Ed (*MR Alison, 2007, Wiley pub*).
2. Cells *(B. Lewin et al. 2007, Pub Jones and Bartlett)*
3. Review articles in journals *Cell* and *Cancer Research.*

CANCER 3

CELLULAR PATHOLOGY OF CANCER

Dr Marjorie Walker

DEFINITIONS AND TERMINOLOGY
Define metaplasia, dysplasia, neoplasia, tumour, malignancy, hamartoma, carcinoma, sarcoma, teratoma, lymphoma, leukaemia, metastasis

METAPLASIA
A reversible change in which one adult cell type (epithelial or connective tissue) is replaced by another adult cell type.

ADAPTIVE response where cells sensitive to the stressful stimulus - reflux of acid, cigarette smoke, etc - and are replaced by cells which can withstand the adverse environment e.g. respiratory columnar ciliated epithelium changes to squamous, squamous oesophageal to columnar/ intestinal

Or reprogramming of stem cells (reserve cells) to differentiate along a different pathway in response to signalling by cytokines, growth factors and extracellular matrix components.

DYSPLASIA
A reversible change in the histological (or cytological) features of epithelial cells including:
Loss of architectural orientation
Loss in uniformity of individual cells
Variability in size and shape
deeply staining nuclei (hyperchromatic), enlarged nuclei and nucleoli
mitotic figures abundant, in places where not usually found
an abnormal pattern of growth in which some of the histological features of malignancy are present, but at a non- or pre-invasive stage (ie premalignant)

DYSPLASIA is common in…

CERVIX - HPV infection
BRONCHUS - Smoking
COLON - Chronic Ulcerative Colitis
LARYNX - Smoking
STOMACH -Pernicious Anaemia
OESOPHAGUS-Barret's metaplasia

NEOPLASIA, TUMOUR, MALIGNANCY
A tumour is an abnormal, autonomous proliferation of cells which are unresponsive to normal control mechanisms governing their growth, and which persists in proliferating even when whatever stimulus started it going has stopped

FEATURES DISTINGUISHING BENIGN FROM MALIGNANT TUMOURS

BENIGN
DO NOT INVADE SURROUNDING TISSUES
DO NOT METASTASISE

ENCAPSULATED
USUALLY WELL DIFFERENTIATED
SLOWLY GROWING
MITOSES NORMAL

Not often fatal unless...

… SOMETHING GOES WRONG, LIKE…

In a dangerous place…	meninges, pituitary
secretes something dangerous…	Insulinoma
gets infected…	bladder
bleeds…	benign gastric muscle tumours
ruptures…	cysts of ovary, liver adenoma
gets torted (twisted)	benign ovarian cyst infarcts

MALIGNANT
INVADE SURROUNDING TISSUES
SPREAD TO DISTANT SITES
NO CAPSULE
WELL TO POORLY DIFFERENTIATED
RAPIDLY GROWING
ABNORMAL MITOSES

METASTASIS
A metastasis is a discontinuous growing colony of tumour cells, at some distance from the primary cancer, most having got there by invasion of lymphatics or blood vessels
There are common patterns of metastatic spread
These depend on the lymphatic and vascular drainage of the primary site
Lymph nodal involvement has a worse prognosis
Dukes A colon - 90%, Dukes C - 30%

NOMENCLATURE OF TUMOURS

CARCINOMA
A malignant tumour derived from epithelium

SARCOMA
A malignant tumour derived from connective tissue (mesenchyme)

LEUKAEMIA & LYMPHOMA
Tumours of white cells, but Leukaemia are malignant tumour of primitive bone marrow-derived cells which circulate in blood stream.

Lymphoma a malignant tumour of lymphocytes proliferating (usually) within lymph nodes

TERATOMA
A teratoma is a tumour derived from germ cells, which has the potential to develop into tumours of all three germ cell layers - ectoderm, mesoderm, endoderm
They are common in the gonads, but occur in midline situations ouside the gonads (Pituitary, pineal, mediastinum, sacrococcygeal areas)
In gonadal teratomas in males, all malignant
In gonadal teratomas in females, most benign

HAMARTOMA
An excessive but localised OVERGROWTH of cells and tissues native to the organ they are in. Cells are MATURE but ARCHITECTURALLY a jumbled-up version of what normally is there. Common in children, and should stop growing when they do, or may involute later in life. Common ones are haemangiomas, bronchial hamartomas, Peutz-Jegher polyps in the gut.

BENIGN EPITHELIAL TUMOURS
Of surface epithelium = PAPILLOMA skin, bladder, colon, etc
Of glandular epithelium = ADENOMA glands or are secretory - mucin, thyroid colloid, bile, hormones occur in stomach, thyroid, breast, colon, kidney, pituitary, pancreas, parathyroid

CARCINOMAs
Squamous, adenocarcinoma, transitional cell carcinoma, basal cell carcinoma, and various qualifying names

SARCOMAS
A malignant tumour derived from CONNECTIVE TISSUE or mesenchymal cells
Prefix indicates line of origin, e.g.
Fat = LipoSARCOMA
Bone = OsteoSARCOMA
Cartilage = ChondroSARCOMA
Muscle = RhabdomyoSARCOMA, LeiomyoSARCOMA
Nerve sheath = Malignant Peripheral Nerve Sheath Tumour

DIFFERENTIATION OF TUMOURS
CRITERIA FOR ASSESSING DIFFERENTIATION OF A MALIGNANT TUMOUR

- If evidence of normal function still present - production of keratin, bile, mucin, hormones, etc., unlikely to be high grade
- If no evidence of this, could be high grade or even anaplastic
- Mitoses important, particularly when abnormal
- Some tumours require a standard mitotic count, so that a tumour with 15 mitoses/mm^2 will behave more aggressively than one with 6
- Various grading systems - for Ca. Breast, prostate, colon
- no differentiation, called ANAPLASTIC carcinoma

CANCER 7

APOPTOSIS

Professor Tony Magee

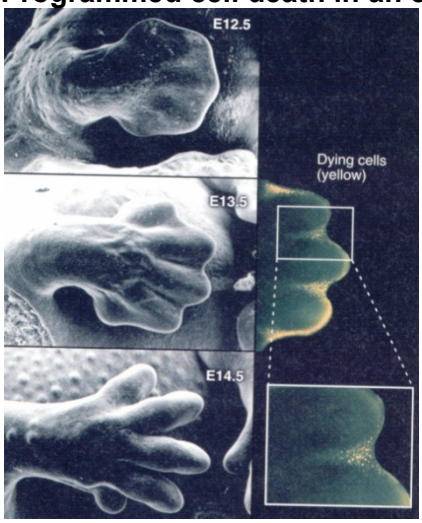
Learning Objectives

1. Explain the difference between necrosis and apoptosis; describe how they may be distinguished.
2. Discuss whether necrosis and apoptosis are the only forms of cell death.
3. Describe the proteolytic caspase cascades which execute the apoptotic response.
4. Discuss how apoptosis may be mediated through death receptors and/or mitochondria.
5. Discuss how Bcl-2 family proteins may modulate apoptosis.

Programmed cell death – why?

1. Harmful cells (e.g. cells with viral infection, DNA damage)
2. Developmentally defective cells (e.g. B lymphocytes expressing antibodies against self antigens)
3. Excess/unnecessary cells:
   (embryonic development – e.g. brain to eliminate
   excess neurons; sculpting of digits and organs)
4. Obsolete cells (e.g. mammary epithelium at the end of lactation)
5. Chemotherapeutic killing of cells

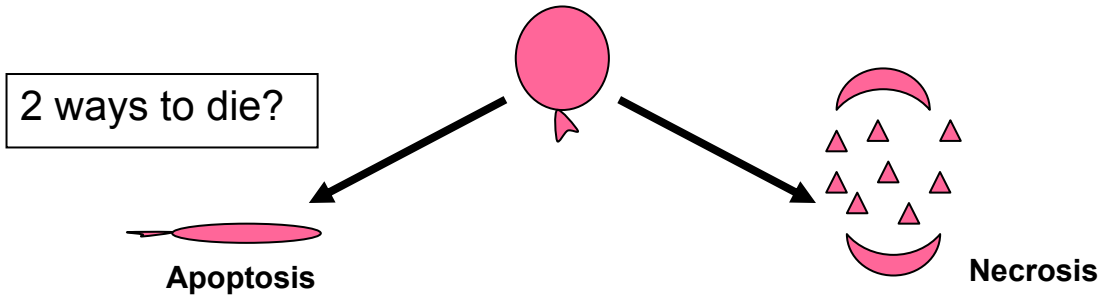
Programmed cell death in an embryonic mouse paw



Cell death: necrosis vs. apoptosis?

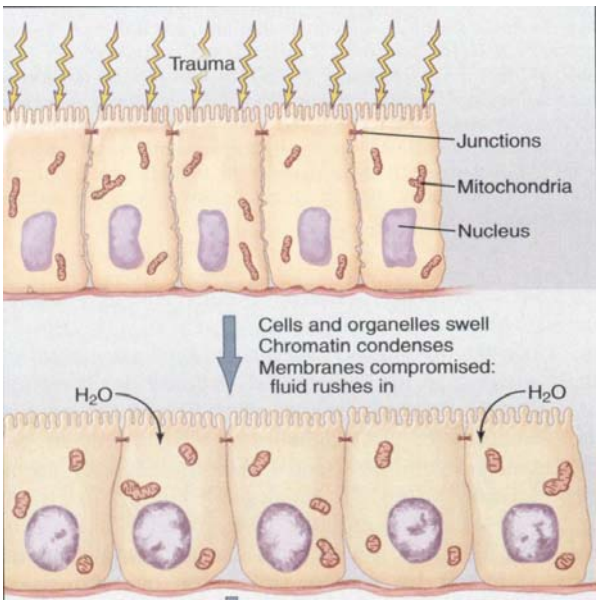
- Necrosis - unregulated cell death associated with cellular disruption and an inflammatory response

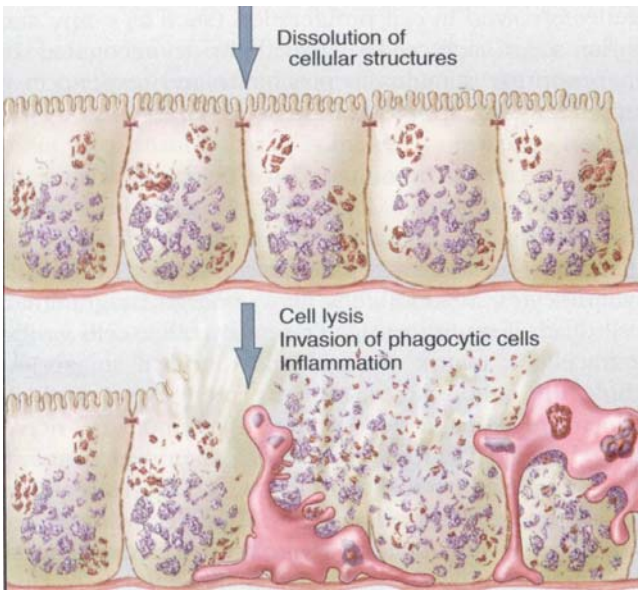
- Apoptosis (programmed cell death) - Regulated cell death; controlled disassembly of cellular contents; no inflammatory response (e.g. haematopoietic cells)



Necrosis

- Plasma membrane becomes permeable
- Cell swelling and rupture of cellular membranes
- Release of proteases leading to autodigestion and dissolution of the cell
- Localized inflammation



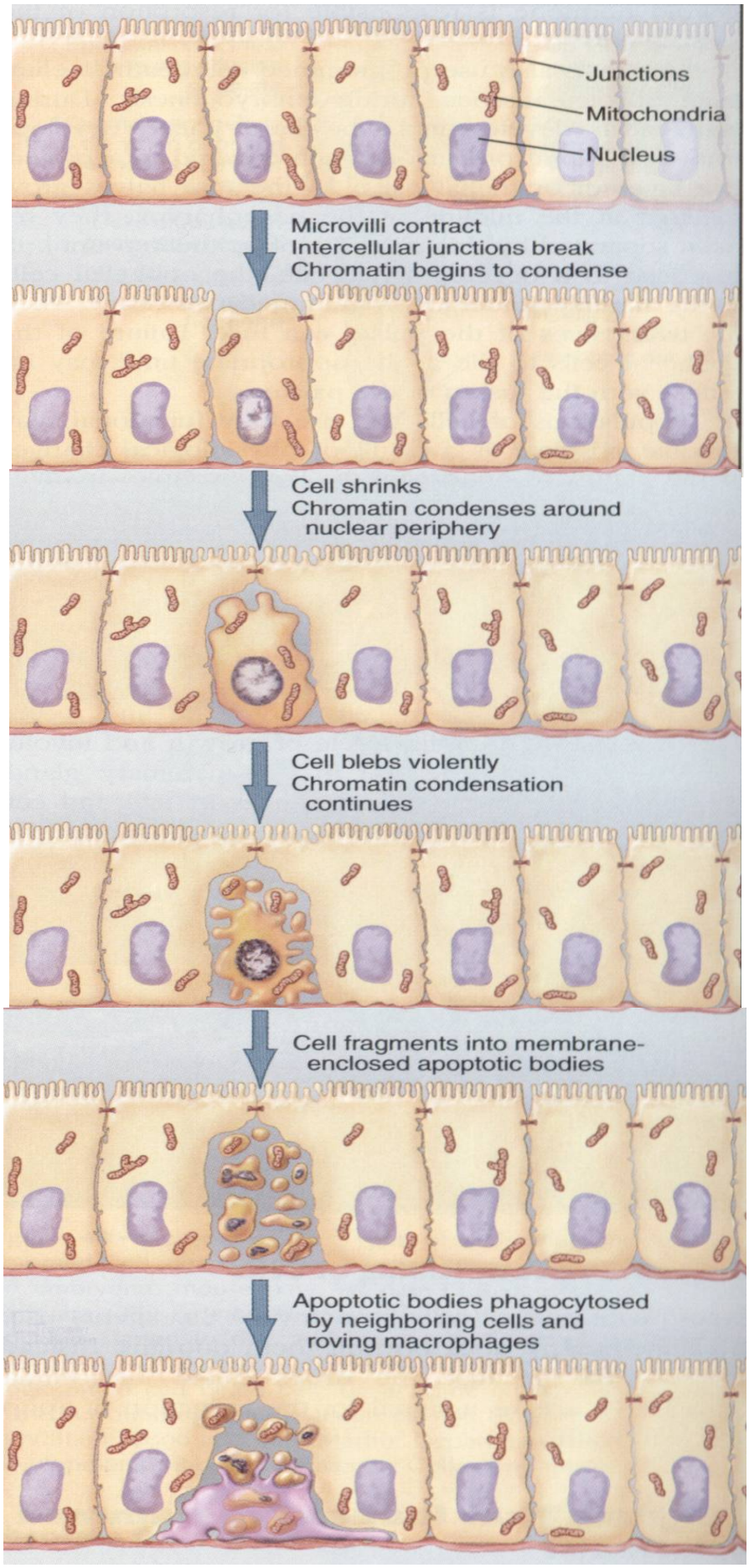


Apoptosis

Latent phase – death pathways are activated, but cells appear morphologically the same

Execution phase –
- Loss of microvilli and intercellular junctions
- Cell shrinkage
- Loss of plasma membrane asymmetry (phosphatidylserine appears in outer membrane)
- Chromatin and nuclear condensation
- DNA fragmentation
- Formation of membrane blebs
- Fragmentation into membrane-enclosed apoptotic bodies

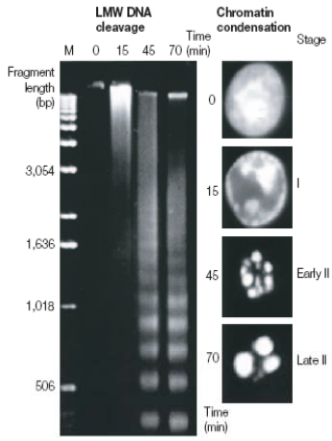
Plasma membrane remains intact

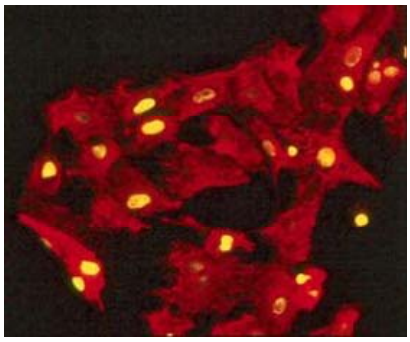


DNA fragmentation in apoptosis

DNA ladders
(agarose gel)

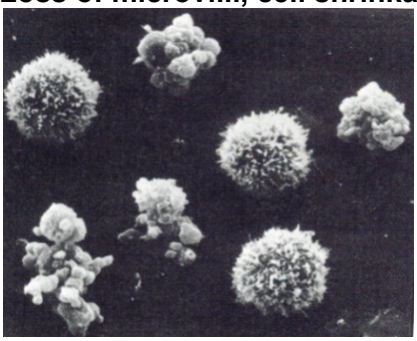
TUNEL assay
(**T**erminal deoxynucleotidyl Transferase
Biotin-d**U**TP **N**ick **E**nd **L**abelling)





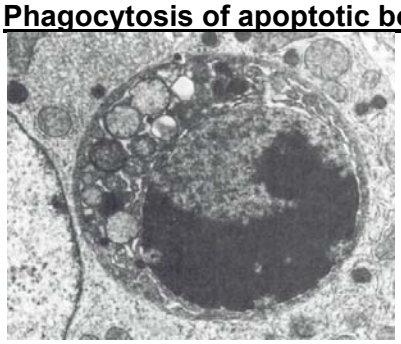
- DNA fragmentation leads to more "ends" which are labelle by transferring a biotinylated U onto the end by TdT
- this is stained with fluorescent avidin that recognises bioti

Loss of microvilli, cell shrinkage and cell blebbing



Normal and apoptotic mouse sarcoma cells

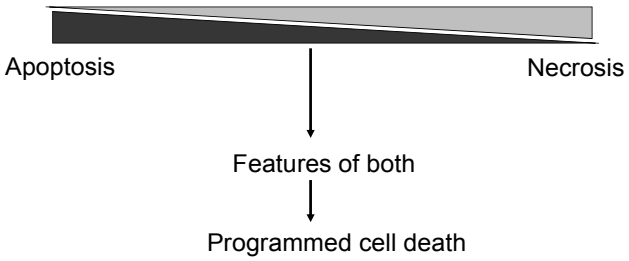
Phagocytosis of apoptotic body by surrounding cells. e.g. macrophages



Cell death: Four deaths and a funeral?
(Nature Reviews: Molecular Cell Biology 2001 2: 1 - 10)

- **Necrosis** - Unregulated cell death associated with cellular disruption and an inflammatory response
- **Apoptosis** (programmed cell death, PCD) - Regulated cell death; controlled disassembly of cellular contents; no inflammatory response (e.g. haematopoietic cells)
- **Apoptosis-like PCD** - some, but not all, features of apoptosis. Display of phagocytic recognition molecules before plasma membrane lysis
- **Necrosis-like PCD** - Variable features of apoptosis before cell lysis; "Aborted apoptosis"

Is there a graded response?



Mechanisms of cell death

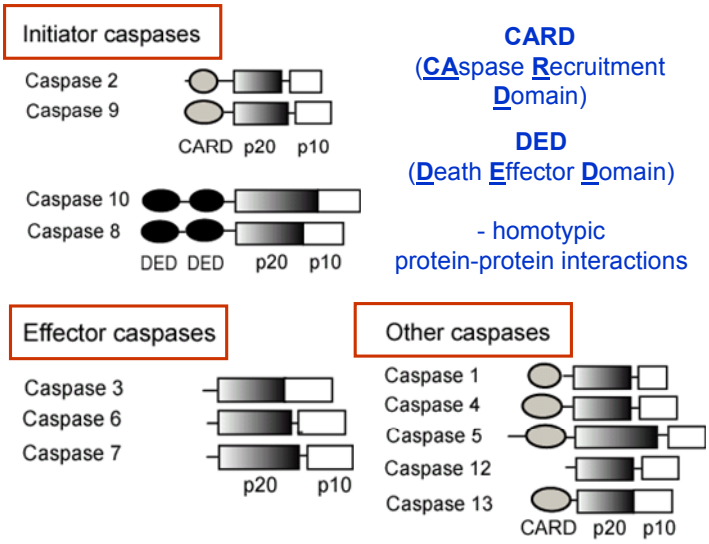
1. The executioners – Caspases
2. Initiating the death programme
   Death receptors
   Mitochondria
3. The Bcl-2 family

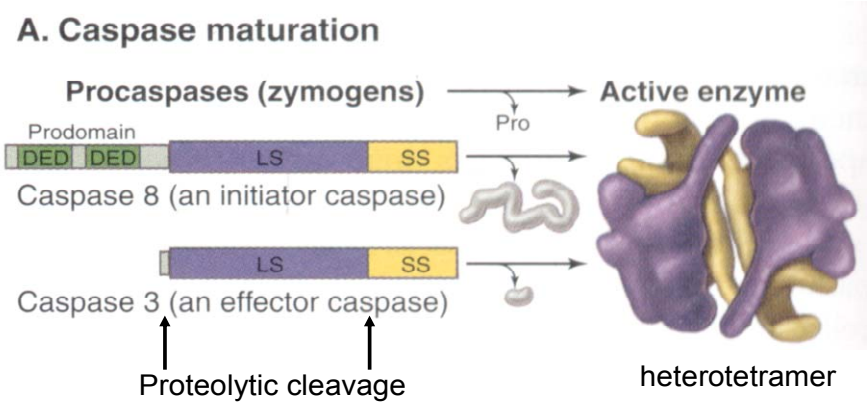
The executioners - Caspases

Cysteine-dependent **asp**artate-directed prote**ases**

- Executioners of apoptosis
- Activated by proteolysis
- Cascade of activation

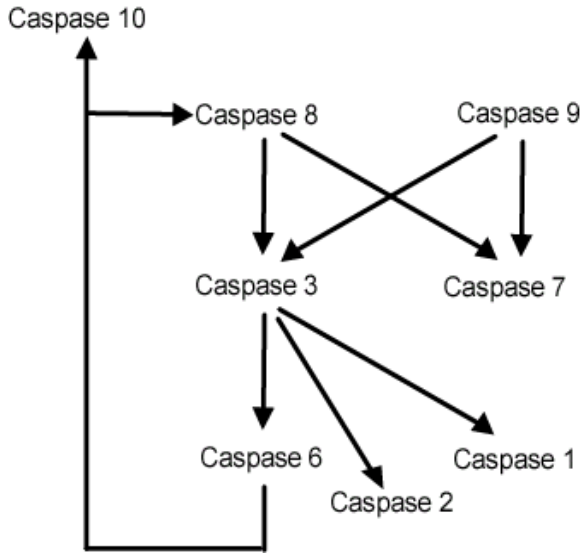
(Other proteases may be involved – calpains, cathepsins)





Caspase cascades

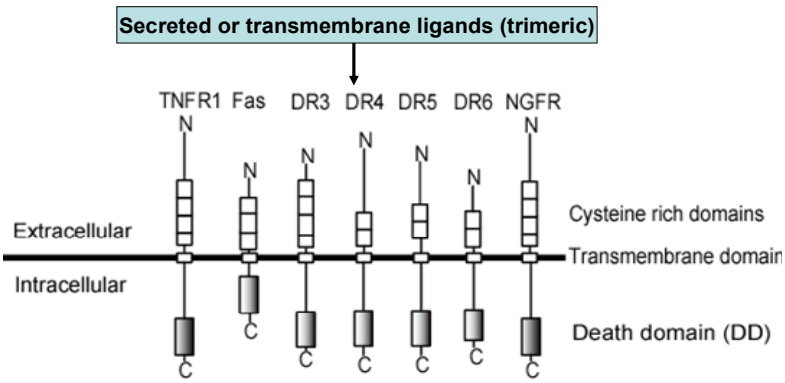
- amplification
- divergent responses
- regulation



Mechanisms of Caspase activation

- Death by design – Receptor-mediated (extrinsic) pathways
- Death by default – Mitochondrial (intrinsic) death pathway

The Death Receptors



TNFR1 – Tumour Necrosis Factor Receptor 1

Adapter proteins in receptor-mediated apoptosis

FADD — DED — DD

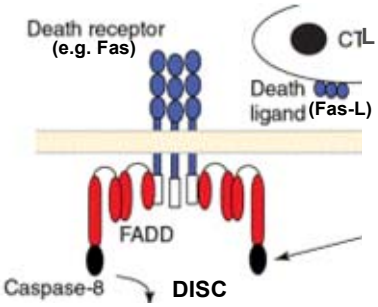
Death **D**omain (DD)

FLIP — DED — DED

Death **E**ffector **D**omain (DED)

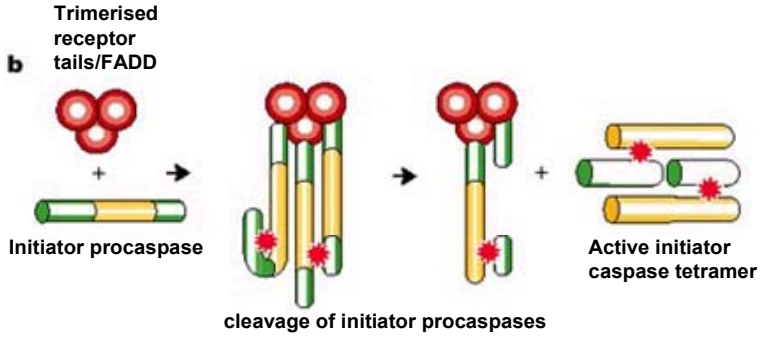
Signalling through Death Receptors, e.g. Fas/Fas-ligand

- Receptor (Fas) trimerization by ligand (Fas-L on lymphocyte)
- Recruitment of adapter protein (FADD) through DD



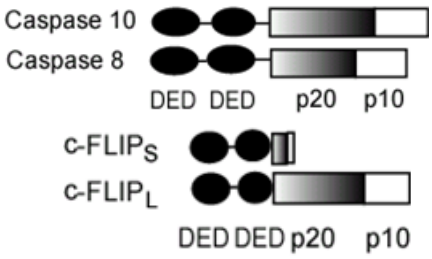
- Recruitment and oligomerization of caspase 8 through DED –> **D**eath-**I**nducing **S**ignalling **C**omplex (DISC)

Initiator caspase oligomerisation results in cleavage and activation



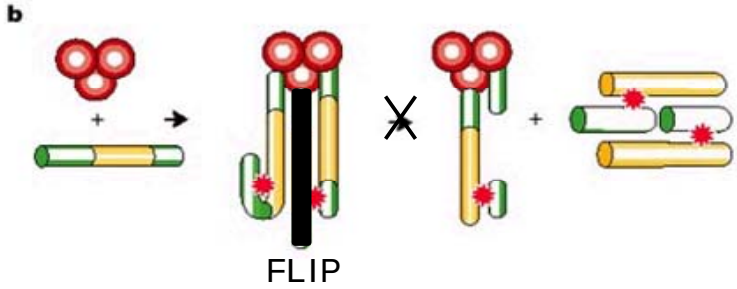
- **Some initiator procaspases have intrinsic low catalytic activity – oligomerisation allows transcleavage**
- **Some procaspases are activated by conformational change on oligomerisation**

Death Receptor activation of caspase 8 is inhibited by FLIP



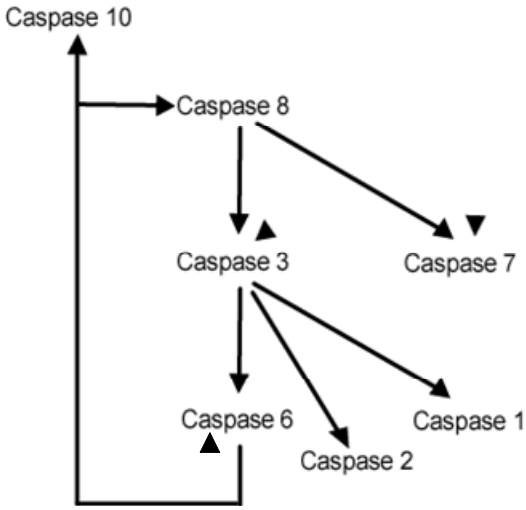
FLIP - caspase 8 homology, but no proteolytic activity

FLIP inhibits caspase 8 activation



- **Competes for binding to receptor tails/FADD**
- **Incorporates into receptor-caspase complexes and interferes with transcleavage**

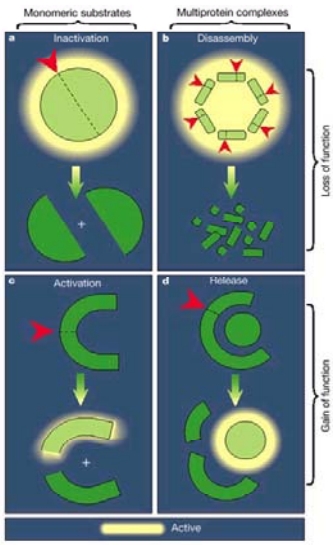
Caspase 8 activates downstream effector caspases



<u>Effector caspases execute the apoptotic programme</u>

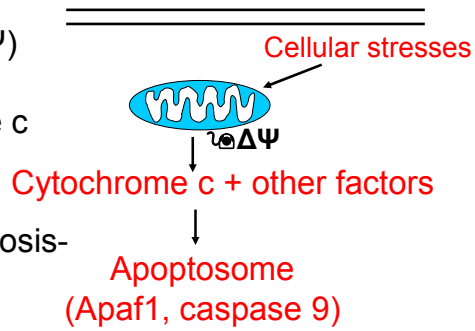
Cleave and inactivate proteins or complexes (e.g. nuclear lamins leading to nuclear breakdown)

Activate enzymes (incl. protein kinases; nucleases, e.g. **C**aspase-**A**ctivated **D**Nase, CAD) by direct cleavage or cleavage of inhibitory molecules



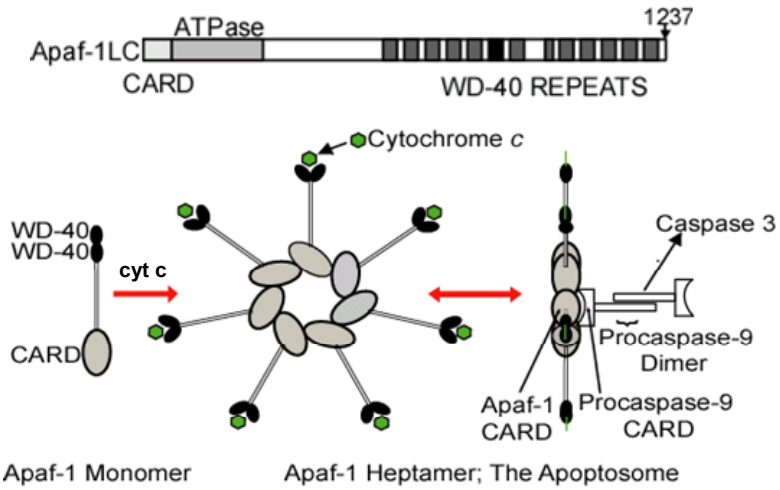
Mitochondrial regulation of apoptosis

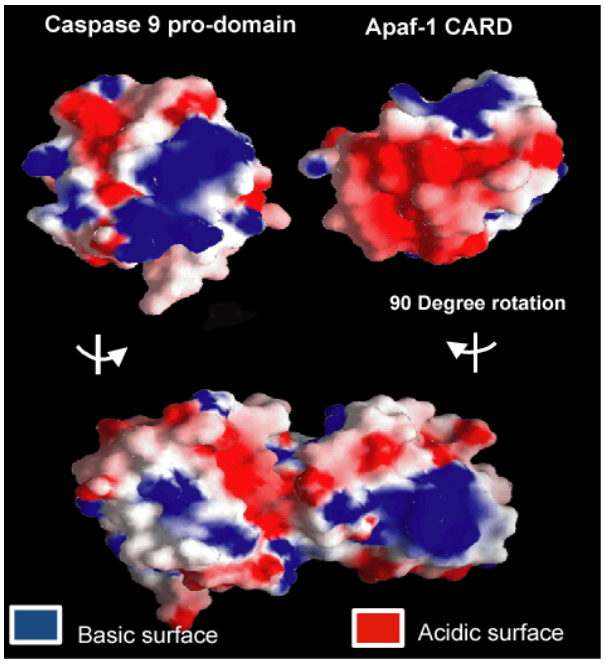
- Loss of mitochondrial membrane potential (ΔΨ)
- Release of cytochrome c
- Release of other apoptosis-inducing factors
- Formation of the apoptosome complex

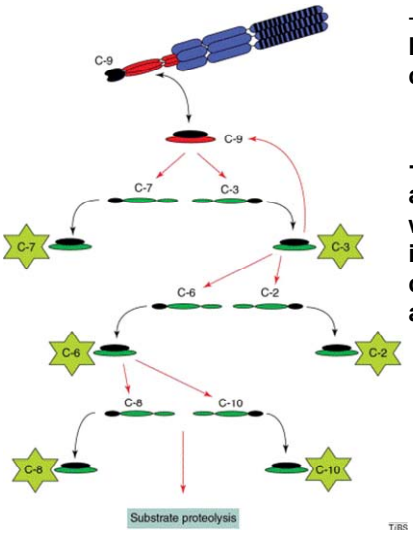


The apoptosome:

Cytochrome c, Apaf-1 (Apoptotic activating factor-1), ATP, caspase 9



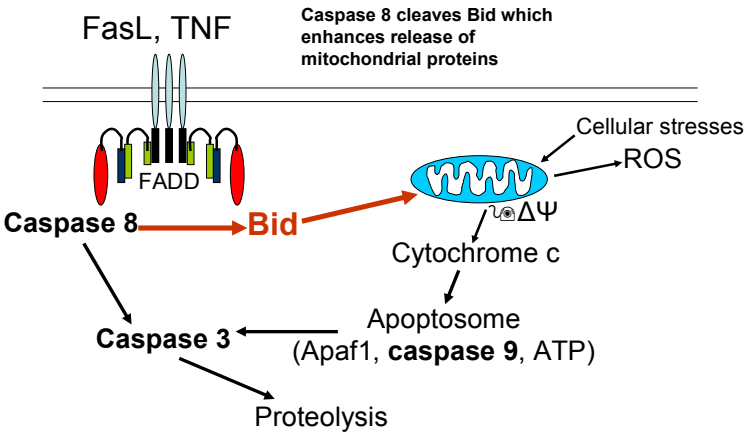




- each Apaf-1 in the heptameric apoptosome can bind a caspase 9

- oligomerization activates caspase 9 which is released and initiates a caspase cascade leading to apoptosis

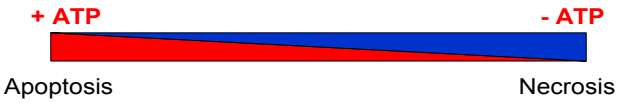
Principal mechanisms of apoptosis: Bid links receptor and mitochondrial death pathways



Apoptosis requires energy

The apoptosome requires ATP

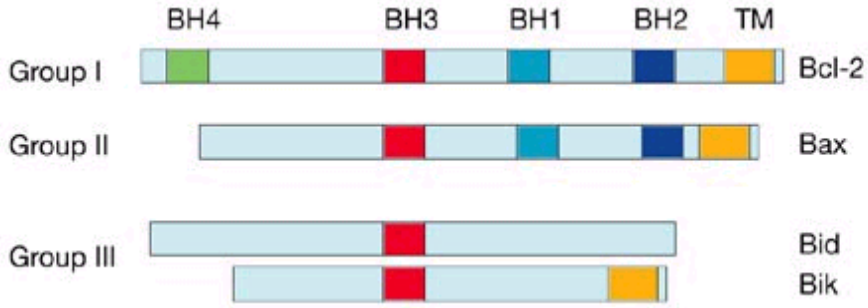
Energy levels in the cell may determine whether death is by necrosis or apoptosis



Inhibitor of Apoptosis Proteins (IAPs) regulate PCD

- Bind to procaspases and prevent activation
- Bind to active caspases and inhibit their activity
- mitochondrial leakage also releases an inhibitor of IAPs (DIABLO) that potentiates cell death

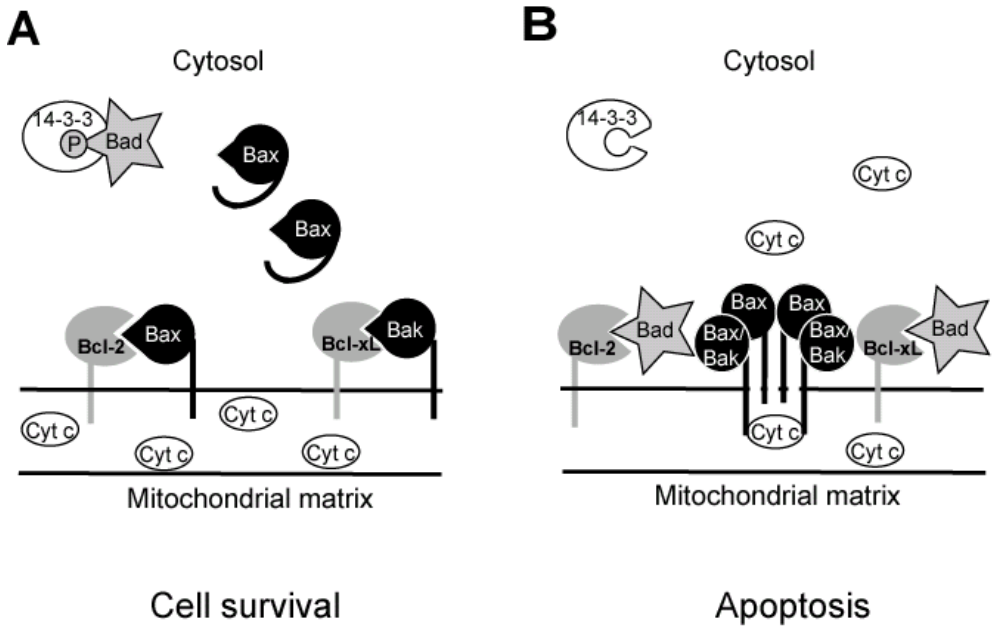
Modulators of apoptosis: Bcl-2 family proteins



Bcl-2 family proteins

Anti-apoptotic	Pro-apoptotic
Bcl-2	Bad
Bcl-xL	Bax
	Bcl-xS
(Mitochondrial)	(Cytosolic/Mitochondrial)

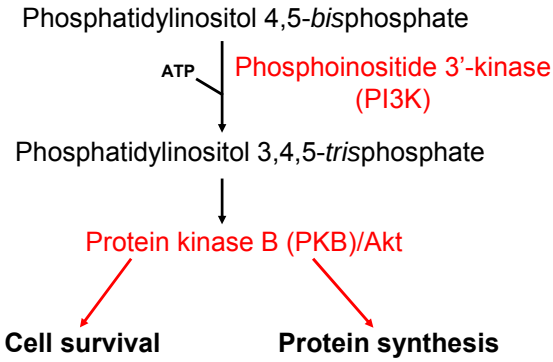
A model for the regulation of apoptosis by Bcl-2 family proteins by heterodimerization



Cytoprotective pathways

- Bcl-2, Bcl-xL: intrinsic pathway
- FLIP, IAPs: extrinsic pathway
- Phosphatidylinositol 3'-kinase and protein kinase B (Akt)

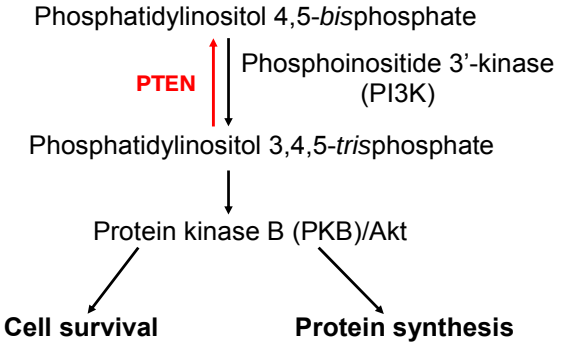
P13K and PKB (Akt)



PKB and cell survival

1. Phosphorylates and inactivates Bad
2. Inactivates FOXO transcription factors (FOXOs promote expression of apoptosis-promoting genes)
3. Phosphorylates and inactivates caspase 9
4. Other?

PTEN (lipid phosphatase) inhibits PI3K signalling



Apoptosis and cancer:
Programmed cell death – why?

1. Harmful cells (e.g. cells with viral infection, DNA damage)
2. Developmentally defective cells (e.g. B lymphocytes expressing antibodies against self antigens)
3. Excess/unnecessary cells:
   (embryonic development – brain to eliminate excess neurons; sculpting of digits and organs)
4. Obsolete cells (e.g. mammary epithelium at the end of lactation)
5. Chemotherapeutic killing of cells, e.g. Dexamethasone

Proto-oncogenes/tumour suppressors associated with apoptosis

Bcl-2
PKB/Akt
PTEN

References

- Alberts *et al*., *Mol. Biol. of the Cell*, 4th edn., Chapter 17
- Leist and Jaattela, 2001, *Nature Reviews: Mol. Cell Biol*. **2**: 1 – 10
- Clerk *et al*., 2003, *Pharmacology and Therapeutics* **97**: 223-261
- Riedl and Shi, 2004, *Nature Reviews: Mol. Cell Biol.* **5**: 897-907

CANCER 5

THE CELL CYCLE AND ITS REGULATION

Dr Vania Braga

Cell division in specific cells

Different cells divide at different rates

1. Embryonic vs adult cells (early frog embryo cells - 30 min)
2. Complexity of system (yeast cells - 1.5 - 3 h)
3. Necessity for renewal
   (intestinal epithelial cells - ~20 h
   hepatocytes - ~1 year)
4. State of differentiation (some cells never divide -
   i.e. neurons and cardiac myocytes)
5. Tumour cells?

Relevance of the appropriate regulation of the cell division

- Premature, aberrant mitosis result in cell death
- In addition to mutations in oncogenes and tumour suppressor genes, most solid tumours are aneuploid (abnormal chromosome number and content).
- Various cancer cell lines show chromosome instability (loose and gain whole chromosomes during cell division)
- Perturbation of protein levels of cell cycle regulators is found in different tumours - abnormal mitosis
- Contact inhibition of growth
- Attacking the machinery that regulates chromosome segregation is one of the most successful anti-cancer strategies in clinical use

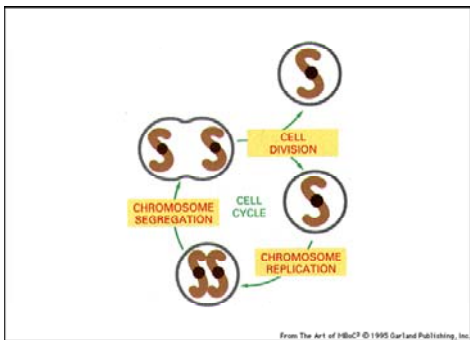
What is the cell cycle?

Orderly sequence of events in which a cell duplicates its contents and divides in two.

Duplication

Division

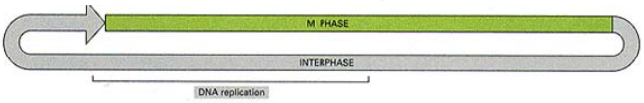
Co-ordination



Regulated progression through the cell cycle

M-phase: Mitosis (Division)

- Nuclear division
- Cell division (cytokinesis)



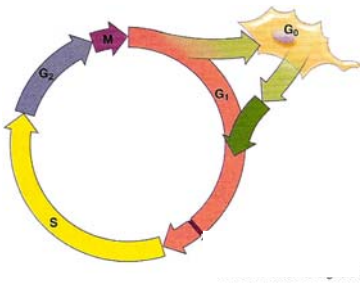
Interphase (Duplication)

- DNA
- organelles
- protein synthesis

Mitosis - most vulnerable period of cell cycle:

- Cells are more easily killed (irradiation, heat shock, chemicals)
- DNA damage can not be repaired
- Gene transcription silenced
- Metabolism?

The eukaryotic cell cycle



M phase - Mitosis

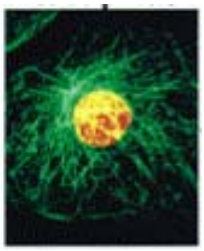
Interphase:

G_0 - cell cycle machinery dismantled

G_1 phase (Gap) - Decision point

S phase - Synthesis of DNA/protein

G_2 phase (Gap) - Decision point

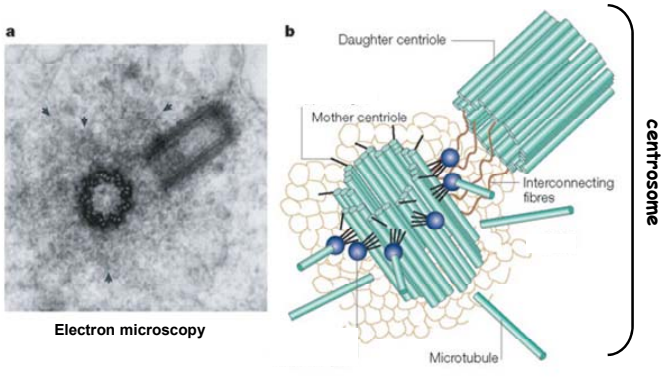


S phase – replication for division

- DNA replication
- Protein synthesis: initiation of translation and elongation increased; capacity is also increased
- Replication of organelles (centrosomes, mitochondria, Golgi, etc) in case of mitochondria, needs to coordinate with replication of mitochondrial DNA

The centrosome

- **Consists of two centrioles** (barrels of nine triplet microtubules)
- **Functions: microtubule organizing center** (MTOC) **and mitotic spindle**



Electron microscopy

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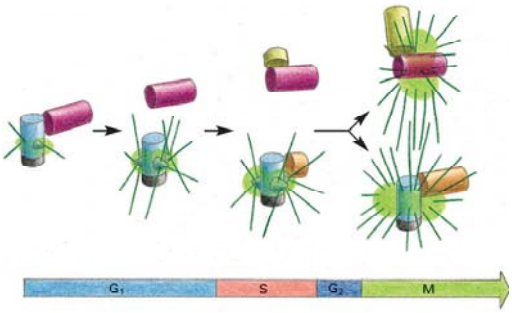
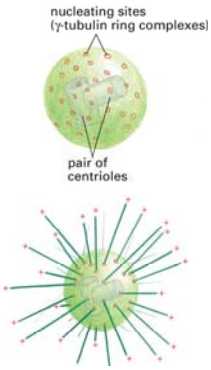
Sluder, 2005

Centrosomes – Duplication and Life cycle

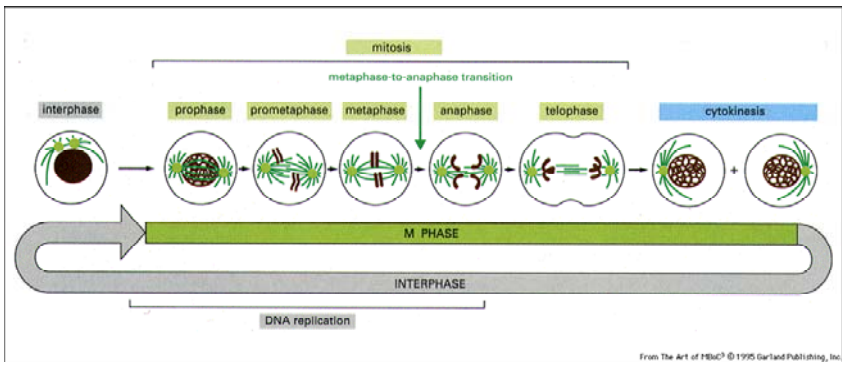
Life cycle of centrosomes during mitosis



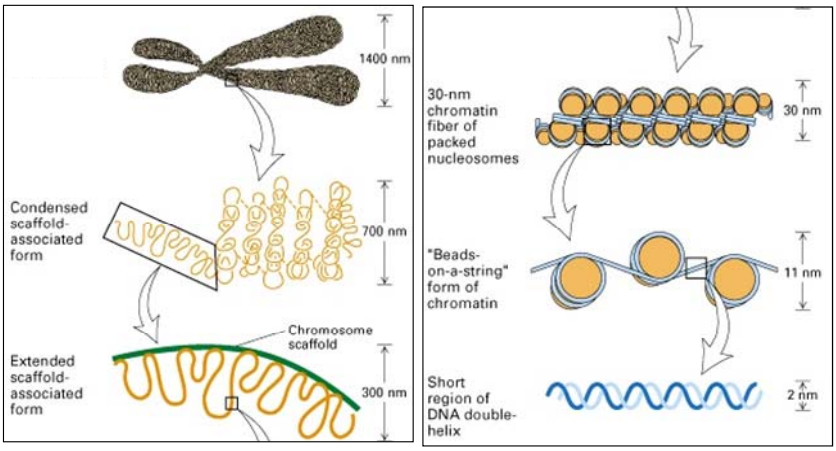
Microtubules growing from centrosomes



Mitosis: six different phases

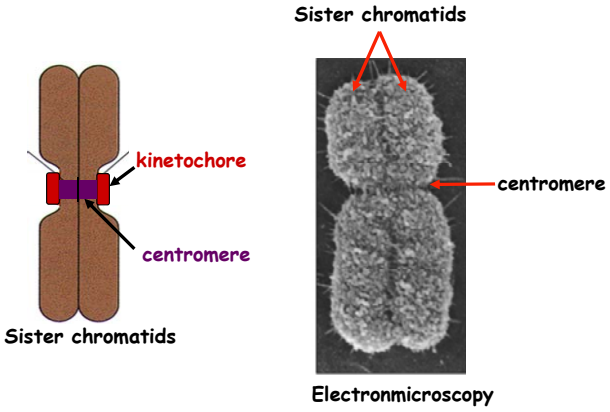


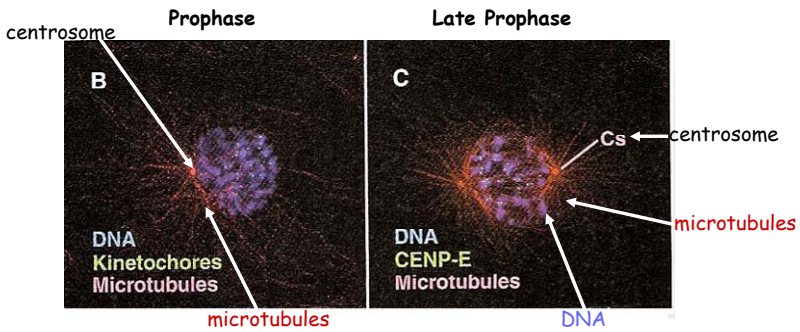
Prophase – condensation of chromatin



Prophase

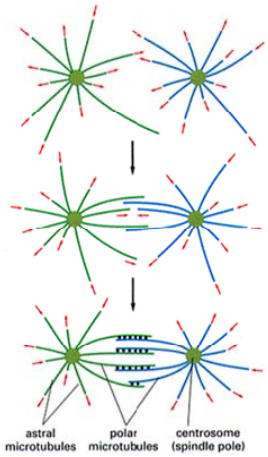
- Condensed chromosomes - each consists of 2 sister chromatids, each with a kinetochore





- Replicated chromosomes condense
- Duplicated centrosomes migrate to opposite sides of the nucleus and organize the assembly of spindle microtubules
- Mitotic spindle forms outside nucleus between the 2 centrosomes

Spindle formation



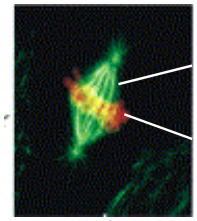
- **Radial microtubule arrays** (ASTERS) **form around each centrosome** (microtubule organizing centers - MTOC)
- **Radial arrays meet**
- **Polar microtubules form**

N.B. Microtubules are in a DYNAMIC state

Metaphase

Chromosomes aligned at equator of the spindle.

Prometaphase: **early prometaphase**
late prometaphase

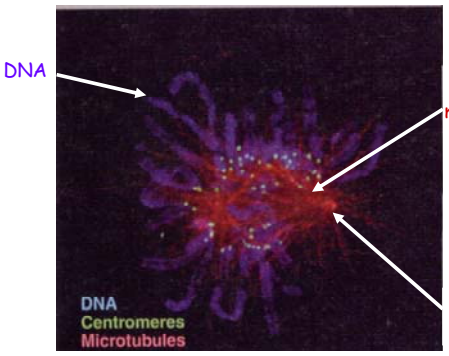


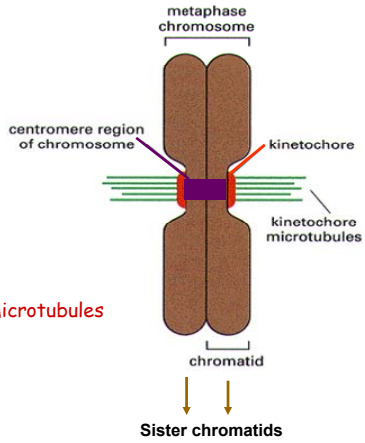
Metaphase

Prometaphase (1)

Early prometaphase

- **Breakdown of nuclear membrane**
- **Spindle formation largely complete**
- **Attachment of chromosomes to spindle via kinetochores (centromere region of chromosome)**

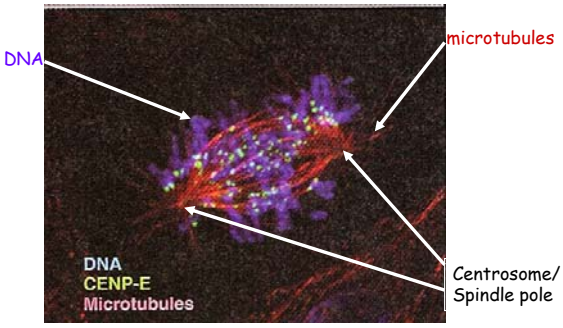




Prometaphase (2)

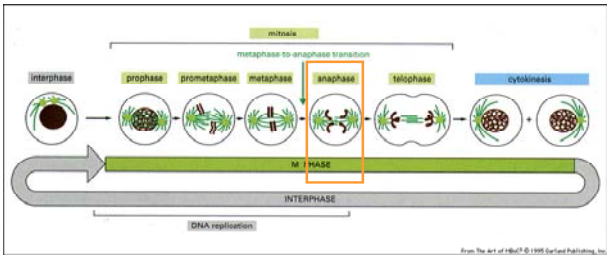
Late prometaphase:

- Microtubule from opposite pole is captured by sister kinetochore
- Chromosomes attached to each pole congress to the middle
- Chromosome slides rapidly towards center along microtubules



CENP-E = centromere protein E (kinetochore tension sensing)

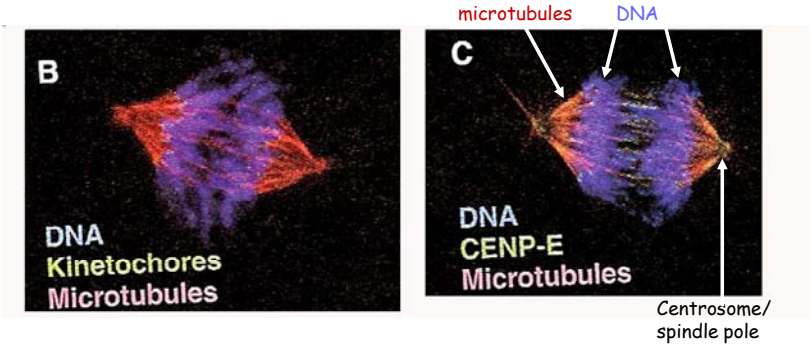
Anaphase



- Paired chromatids separate to form two daughter chromosomes
- Cohesin holds sister chromatids together
- Anaphase A and B

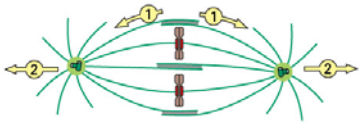
Anaphase A:

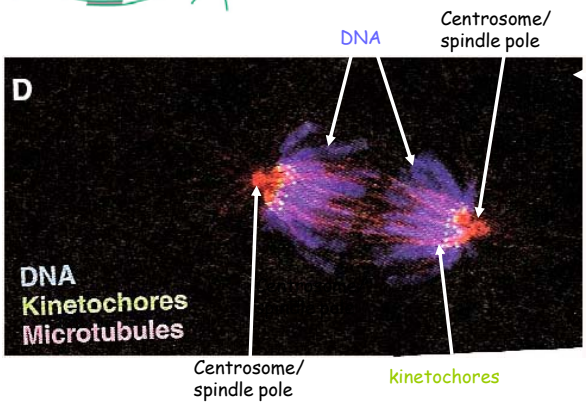
- Breakdown cohesin
- Microtubules get shorter
- Daughter chromosomes pulled toward opposite spindle poles



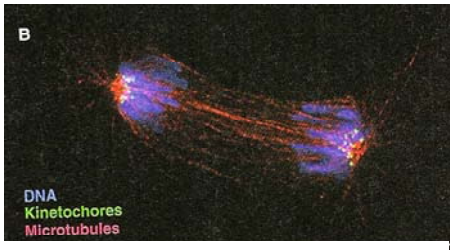
Anaphase B

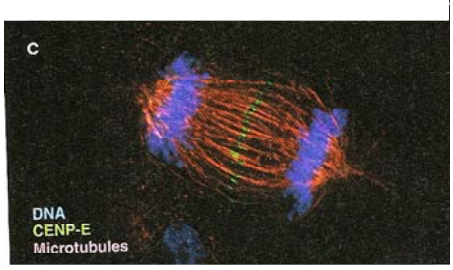
- 1-Daughter chromosomes migrate towards poles
- 2-Spindle poles (centrosomes) migrate apart



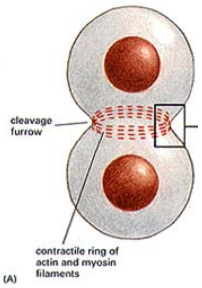


Telophase

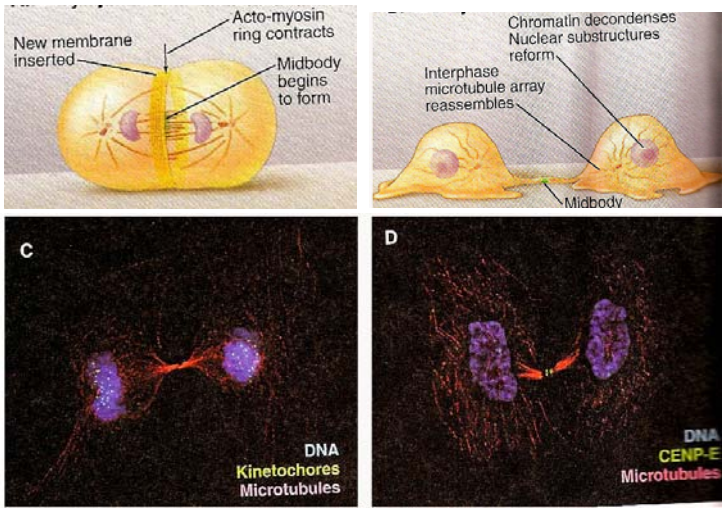




- Daughter chromosomes arrive at spindle
- Nuclear envelope reassembles at each pole
- Assembly of contractile ring

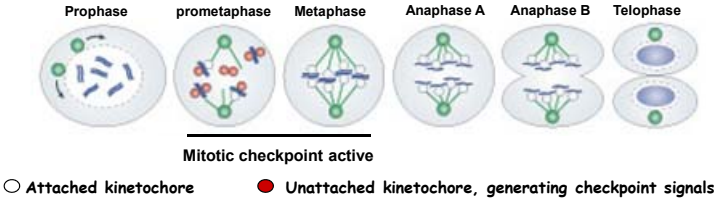


Cytokinesis



Transition out of metaphase: Spindle Assembly checkpoint

Senses completion of chromosome alignment and spindle assembly
(monitors kinetochore activity)



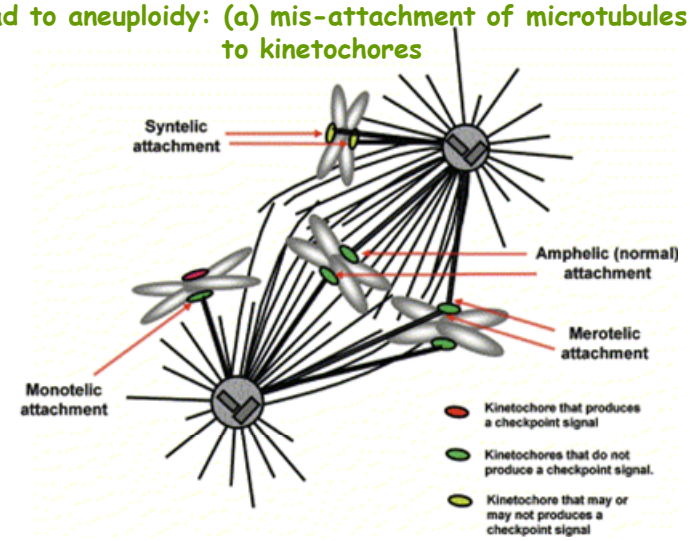
Requires:

CENP-E
BUB protein kinases

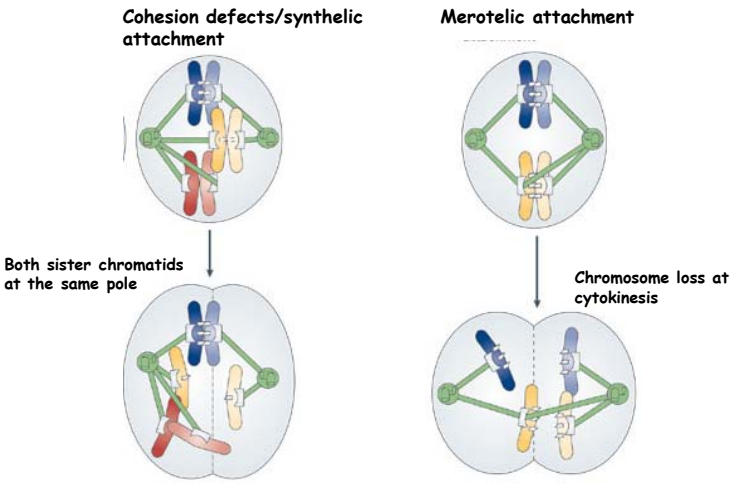
BUBs dissociate from kinetochore when chromosomes are properly attached to the spindle

When all dissociated, anaphase proceeds.

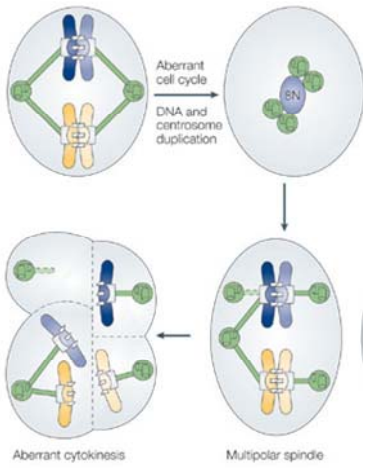
Road to aneuploidy: (a) mis-attachment of microtubules to kinetochores



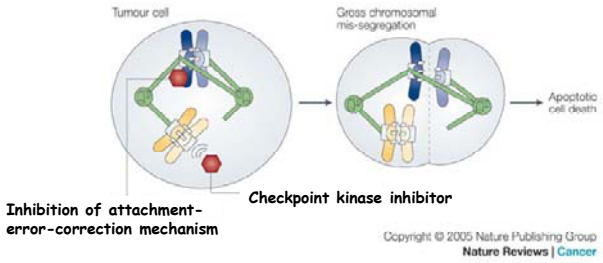
Road to aneuploidy: (a) mis-attachment of microtubules to kinetochores



Road to aneuploidy: (b) aberrant mitosis



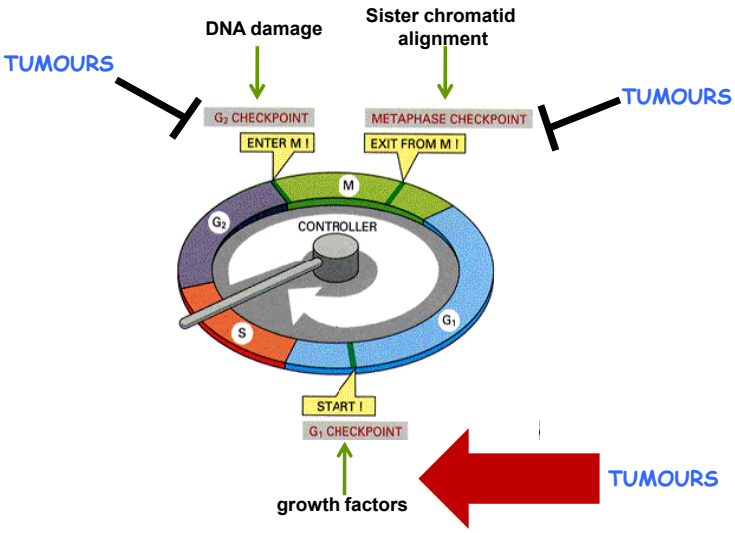
Anti-cancer therapy by inducing gross chromosome mis-segregations



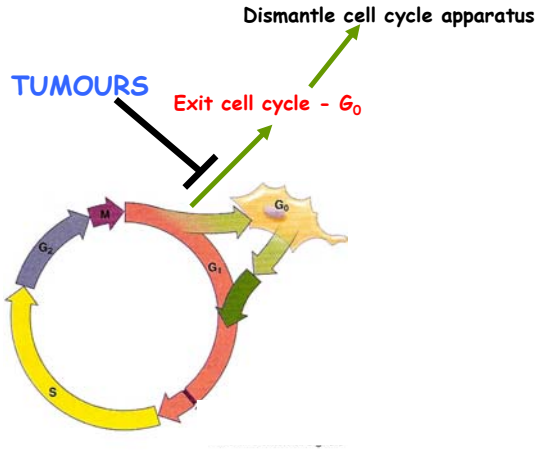
Taxanes and vinca alkaloids (breast and ovarian cancers)

- **Alters microtubule dynamics**
- **Produces unattached kinetochores**
- **Causes long-term mitotic arrest.**

Summary cell cycle checkpoints

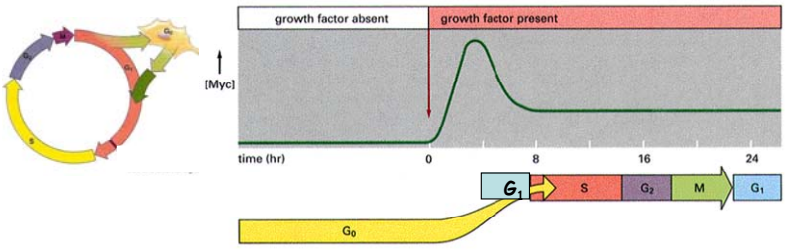


De-regulation of cell cycle during tumorigenesis



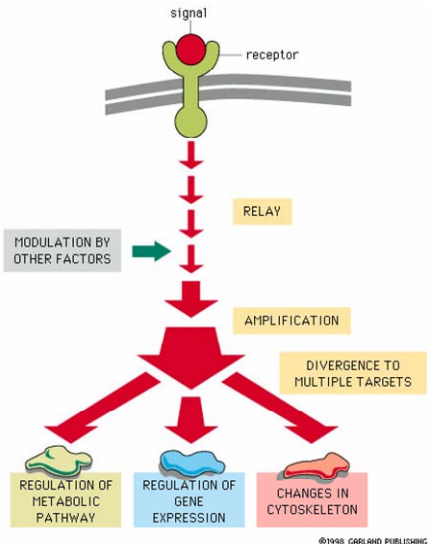
What triggers a cell to enter the cell cycle and divide?

- In the absence of stimulus, cells go into Go (quiescent phase)
- Most cells in the body which are differentiated to perform specific functions
- Cells are not dormant



- Exit from G_0 highly regulated - requires growth factors and intracellular signalling cascades

Signalling through the cell

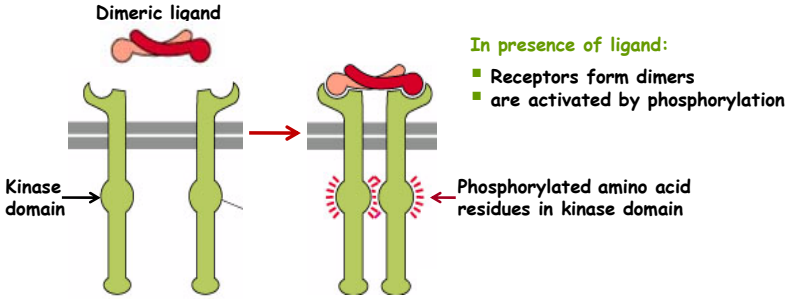


Signalling cascades:

- Response to extracellular factors
- Signal amplification
- Signal integration
- Modulation by other pathways
- Regulation of divergent responses

Signalling by peptide growth factors: Ligand binds and activates the receptor

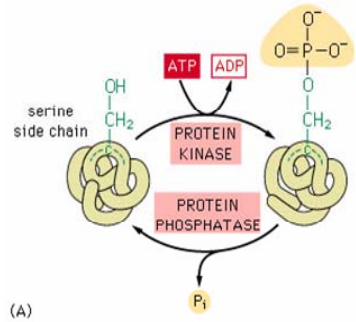
- Epidermal growth factor (EGF); Platelet-derived growth factor (PDGF)
- Respective receptors found as monomeric, inactive state
- Receptor Protein Tyrosine Kinase (RPTK)



In presence of ligand:

- Receptors form dimers
- are activated by phosphorylation

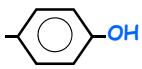
Protein phosphorylation: transfer of phosphate from ATP to a hydroxyl groups



Serine (Ser) $-CH_2OH$

Threonine (Thr) $-CH(OH)-CH_3$

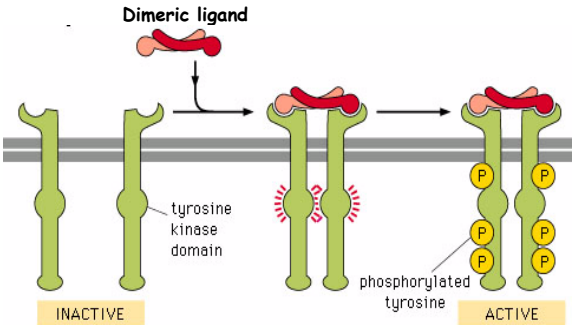
Tyrosine (Tyr)



The added phosphate group (negatively charged) can alter protein function by:

- causing a change in shape (conformation) leading to change in activity (+ve or –ve)
- creating a docking site for another protein

Signalling by peptide growth factors: receptor activation triggers different processes



In presence of ligand:

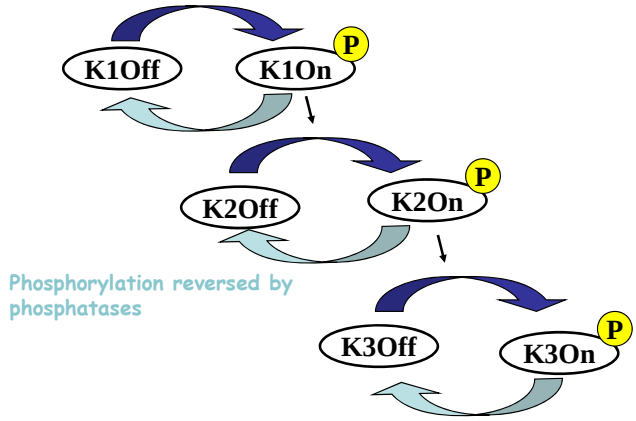
- Receptors form dimers
- are activated by phosphorylation

Receptor activation triggers:

- kinase cascades
- binding of adapter proteins

1. Protein kinase cascades

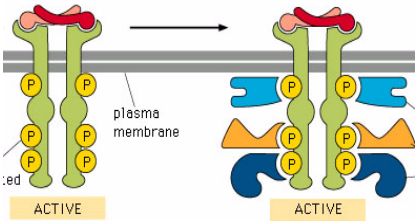
Frequently the protein regulated by a kinase is another kinase, and so on…



Leads to signal amplification, diversification and opportunity for regulation

2. Adapter proteins

- Tyrosine phosphorylation provides docking sites for adapter proteins
- Protein-protein interactions: protein binding – bringing proteins together



- Proteins are modular and contain domains, i.e. functional and structural units that are copied in many proteins
- Some domains are important in molecular recognition

Grb2: SH3 | SH2 | SH3 — Src Homology regions

Proline-rich regions (constitutive)

Phosphorylated tyrosines (inducible, specific sequence context)

What if something goes wrong during the cell cycle?

e.g. Cell is not big enough or DNA damage

1. Cell cycle arrest
   - at check points (G1 and spindle check point)
   - can be temporary (i.e. following DNA repair)

2. Programmed cell death (apoptosis)
   - DNA damage too great and cannot be repaired
   - Chromosomal abnormalities
   - Toxic agents

Cell cycle progression aborted and cell destroyed

CANCER 6

SIGNALING MECHANISMS IN GROWTH AND DIVISION

Professor Tony Magee

Intracellular signaling involves transmitting signals through the cell to control global cellular responses such as survival, division, differentiation and death. This frequently involves reception of an extracellular ligand by a cell surface receptor followed by production of a second messenger and signal transmission via:

- Regulation of protein function by reversible phosphorylation
- Signaling cascades
- G proteins (GTPases)
- Adapter proteins

This results in signal integration and amplification and regulation of divergent responses, and is modulated by other pathways.

Protein kinase cascades are characterised by the sequential phosphorylation of a series of kinases (at serine, threonine or tyrosine residues) ultimately resulting in the phosphorylation and activation or deactivation of target proteins. These cascades are regulated by the opposing action of specific protein phosphatases.

GTP-binding (G) proteins can be activated by certain cell surface receptors and exchange bound GDP for GTP, thus changing conformation and allowing them to interact with downstream effector molecules. This is reversed by intrinsic or stimulated GTPase activity.

Nucleotide exchange is frequently stimulated by exchange factors that are brought to activated receptors by so-called adaptor proteins (e.g. Grb2) that have no catalytic function but serve to couple receptor to downstream pathways, and also provide an additional point of regulation.

Examples of peptides involved in cell growth regulation are EGF and PDGF. These bind as dimers to their transmembrane tyrosine kinase receptors causing receptor dimerisation and transphosphorylation on tyrosine. The resulting phosphorylated tyrosines are docking sites for adaptors and downstream signaling molecules such as the Grb2/Sos exchange factor for the small G protein Ras, a key player in the regulation of normal cell growth and differentiation that is often mutationally activated in cancer cells.

Ras then brings the kinase Raf to the membrane, Raf is activated and triggers a kinase (MAPK or ERK) cascade involving several downstream kinases ultimately leading to phosphorylation of targets involved in regulation of gene transcription, protein synthesis and the cell cycle.

Cell cycle control is based on cyclically activated protein kinases, the cyclin-dependent kinases (Cdks), which are present in proliferating cells throughout the cell cycle and are regulated by interaction with cyclins and by complex phosphorylation. Cyclins are proteins whose expression is regulated transiently at specific points in the cell cycle by cyclical synthesis and degradation.

Different cyclin-Cdk complexes trigger different events in the cell cycle such as S phase (Cdk2/Cyclin A), mitosis (Cdc2/Cyclin B) and G1 progression (Cdk2/Cyclin E). Genes activated by the Ras-ERK pathway include the “immediate early” genes c-*jun*, c-*fos*,

and c*myc* encoding transcription factors which in turn activate other genes (e.g. cyclin D). Cyclin D then activates Cdk4 and Cdk6 to stimulate synthesis of cyclin E.

Cdks phosphorylate proteins (on serine or threonine) to effect cell cycle progression, including nuclear lamins (causes breakdown of nuclear envelope) and Retinoblastoma protein (Rb; activates gene transcription required for cell growth and division).

Thus, the cell cycle is controlled by a complex network of interacting proteins providing multiple checks and balances.

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CANCER 7

DNA DAMAGE AND REPAIR

Professor Nigel Gooderham

Learning objectives

- Describe how DNA can be damaged by radiation or chemicals (carcinogens) and the role metabolism can play in these reactions.
- Outline in general terms the role of p53 in the detection of, and response to, DNA damage.
- Summarise the natural repair mechanisms for damaged DNA.
- Explain how unrepaired or misrepaired DNA damage can become “fixed” as a mutation.
- Summarise how the potential of a chemical/agent to damage DNA can be assessed.

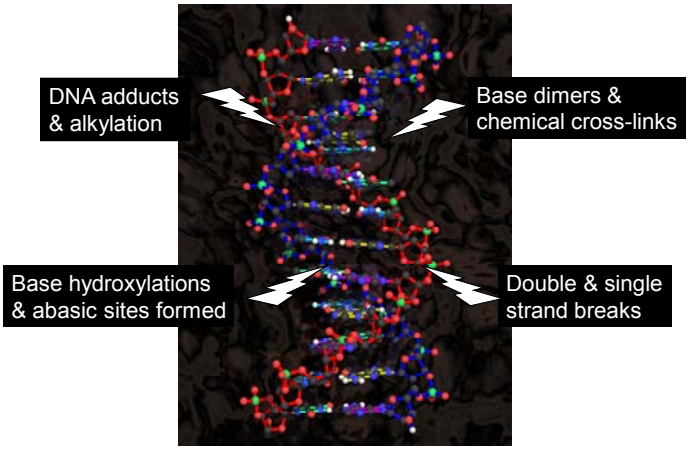
What can damage DNA?

- Chemicals (carcinogens)
  - dietary
  - lifestyle
  - environmental
  - occupational
  - medical
  - endogenous
- Radiation
  - ionizing
  - solar
  - cosmic

Why do we care?

- DNA damage can lead to mutation
- Mutation may lead to cancer

DNA damage by carcinogens

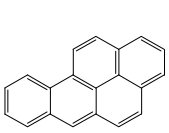


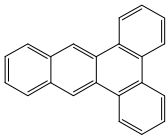
Mammalian metabolism

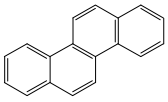
- Phase I
  - addition of functional groups
    - e.g. oxidations, reductions, hydrolysis
  - mainly cytochrome p450-mediated
- Phase II
  - conjugation of Phase I functional groups
    - e.g. sulphation, glucuronidation, acetylation, methylation, amino acid and glutathione conjugation
  - Generates polar (water soluble) metabolites.

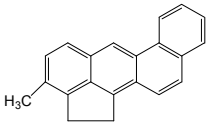
Polycyclic aromatic hydrocarbons

- Common environmental pollutants
- Formed from combustion of fossil fuels
- Formed from combustion of tobacco

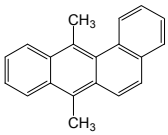

Benzo[a]pyrene


Dibenz[a,c]anthracene

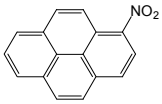

Chrysene



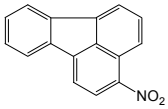
3-Methylcholanthrene



7,12-Dimethylbenz[a]anthracene

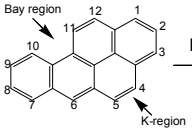


1-Nitropyrene



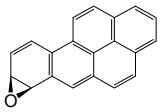
3-Nitrofluoranthene

Two step epoxidation of B[a]P



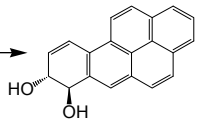
Benzo[a]pyrene

P450



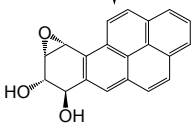
Benzo[a]pyrene-7,8-oxide

EH

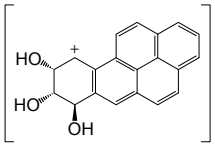


Benzo[a]pyrene-7,8-dihydrodiol

P450



Benzo[a]pyrene-7,8dihydrodiol-9,10-oxide

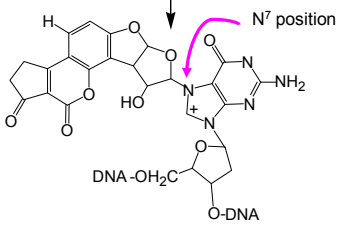


DNA adducts

Epoxidation of aflatoxin B_1

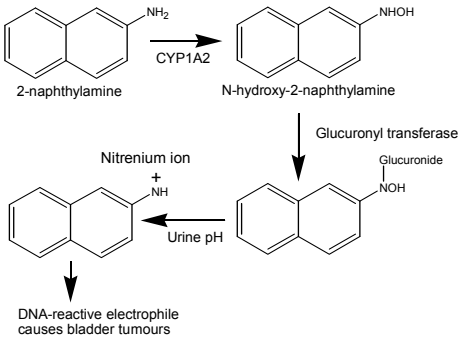
Aflatoxin B_1 — Aflatoxin B_1, 2,3-epoxide

- Formed by *Aspergillus flavus* mould
- Common on poorly stored grains and peanuts
- Aflatoxin B_1 is a potent human liver carcinogen, especially in Africa and Far-East



Metabolism of 2-naphthylamine

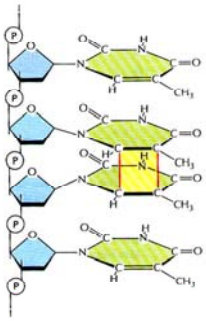
- Past components of dye-stuffs
- Include 2-naphthylamine and benzidine
- Potent human bladder carcinogens
- German dye industry epidemiology (1895 Rehn)



Other carcinogens

Solar (UV) radiation

- Pyrimidine (thymine) dimers
- Skin cancer



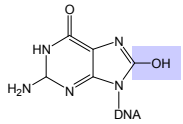
Other carcinogens

Ionising radiation

- Generates free radicals in cells
- Includes oxygen free radicals
  - super oxide radical: $O_2^{\bullet}$
  - hydroxyl radical: $HO^{\bullet}$
- Possess unpaired electrons
  - electrophilic and therefore seek out electron-rich DNA

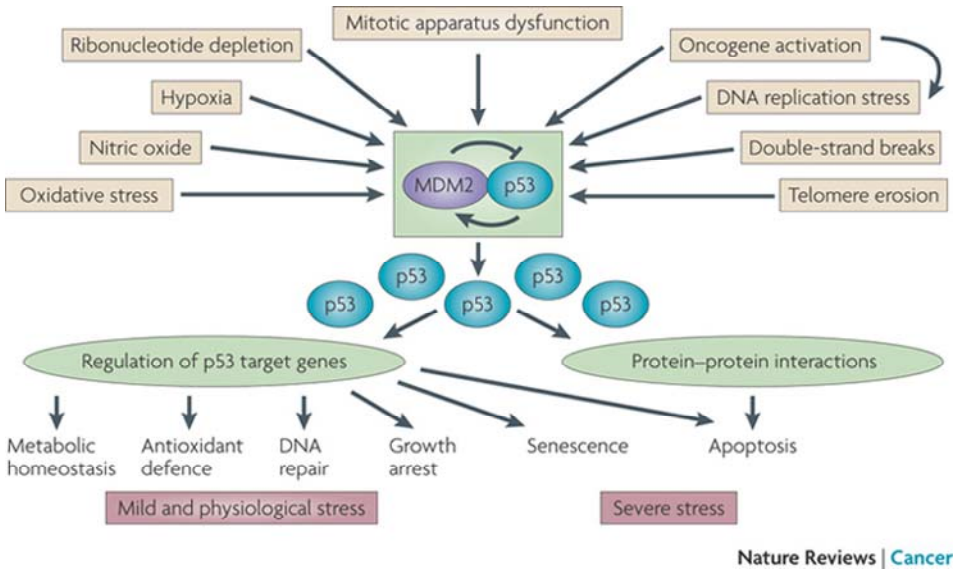
Oxygen free radical attack on DNA

- Double and single strand breaks
- Apurinic & apyrimidinic sites
- Base modifications
  - ring-opened guanine & adenine
  - thymine & cytosine glycols
  - 8-hydroxyadenine & 8-hydroxyguanine (mutagenic)



8-hydroxy guanine

Role of p53 in dealing with cellular stress

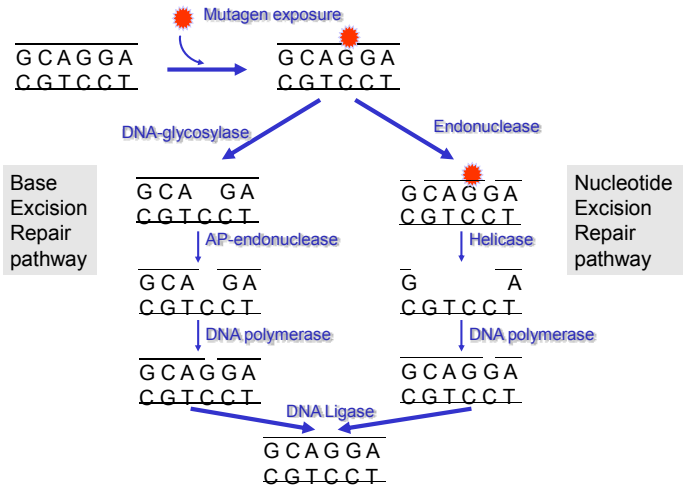


Repair is a key event

Types of DNA repair

- **Direct reversal of DNA damage**
  - photolyase splits cyclobutane pyrimidine-dimers
  - methyltransferases & alkyltransferases remove alkyl groups from bases
- **Base excision repair** (mainly for apurinic/apyridinic damage)
  - DNA glycosylases & apurinic/apyrimidinic endonucleases + other enzyme partners
  - A repair polymerase (e.g. Polβ) fills the gap and DNA ligase completes the repair.
- **Nucleotide excision repair** (mainly for bulky DNA adducts)
  - Xeroderma pigmentosum proteins (XP proteins) assemble at the damage. A stretch of nucleotides either side of the damage are excised.
  - Repair polymerases (e.g. Polδ/β) fill the gap and DNA ligase completes the repair.
- **During- or post-replication repair**
  - mismatch repair
  - recombinational repair

Excision repair of DNA damage

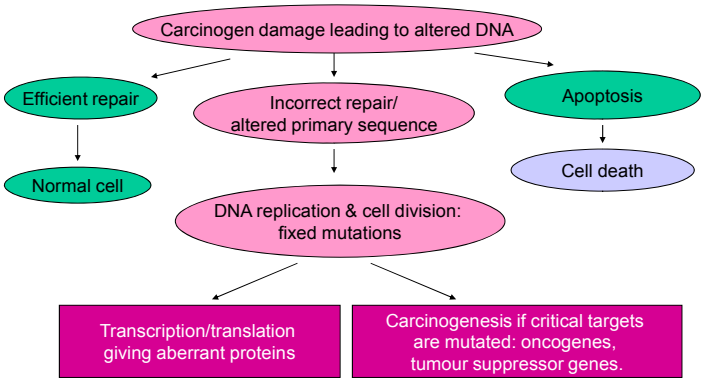


Estimated rates of endogenous damage and repair

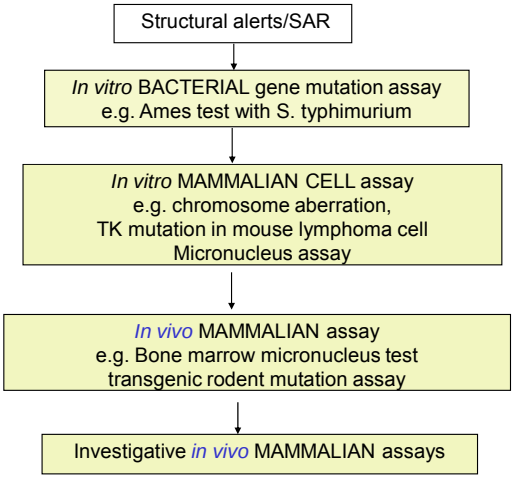
Type of damage	Damage per hour per cell	Max repair rate: BP/hour per cell
Depurination	1000	10,000
Depyrimidination	55	10,000
Single-strand breaks	5000	200,000
Alkylation (O^6-methylguanine)	130	10,000
Free radical base oxidations	120	100,000

The greater the persistence of damage then the greater the chance of a mutagenic event

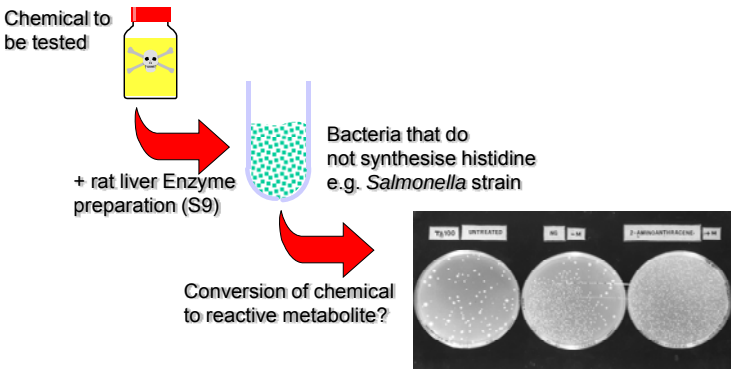
Fate of carcinogen-DNA damage



Testing for DNA damage



Bacterial (Ames) test for mutagenicity of chemicals



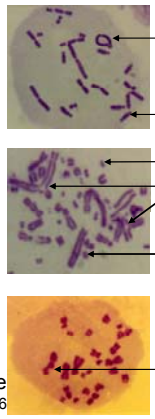
On histidine-free media: if mutations occur in bacterial genome then bacteria acquire ability to synthesise histidine = colonies

Detecting DNA damage in mammalian cells Chromosomal abberrations

Treat mammalian cells with chemical in presence of liver S9. Look for chromosomal damage

CHO Cell
Normal karyotype, 2n = 22

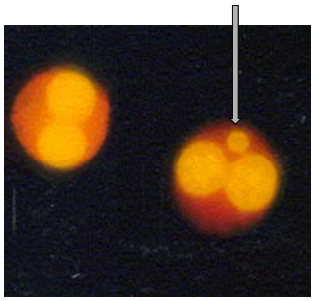
Human Lymphocyte
Normal karyotype 2n = 46



In vitro micronucleus assay

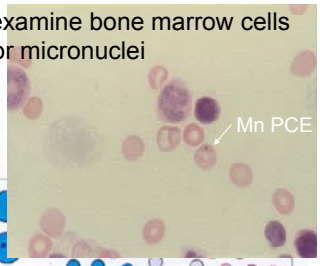
- Cells treated with chemical and allowed to divide
- Cytokinesis blocked using cytochalasin-B
- Binucleate cells assessed for presence of micronuclei
- Can stain the kinetochore proteins to determine if chemical treatment caused clastgenicity (chromosomal breakage) or aneuploidy (chromosomal loss)

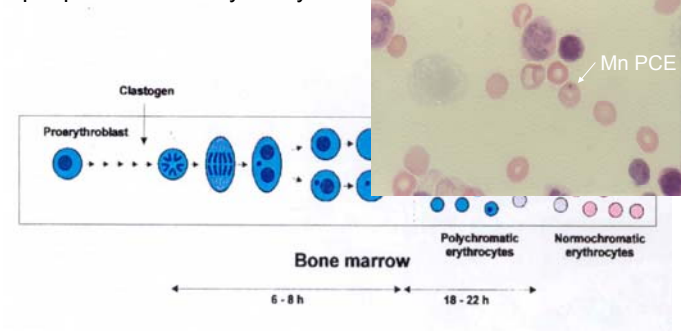
micronucleus



Bone marrow micronucleus assay in mice or rats

Treat animals with chemical and examine bone marrow cells or peripheral blood erythrocytes for micronuclei





Summary

- Chemicals and radiation can damage DNA.
- Chemicals often require metabolic activation (e.g. by cytochrome P450) before they are able to damage DNA.
- Radiation induces pyrimidine dimers, strand breaks, abasic sites and modified bases in DNA.
- Damaged DNA can be repaired by direct reversal or excision of damaged bases or nucleotides.
- Incorrect repair can lead to mutation and possibly neoplasia.
- Detecting the ability of agents (chemicals and radiation) to damage DNA is essential.

Reading material

- The Cancer handbook 2nd Ed (*MR Alison, 2007, Wiley pub*).
- Cells *(B. Lewin et al. 2007, Pub Jones and Bartlett)*
- Review articles in journals *Cell* and *Cancer Research.*

CANCER 8

EXTERNAL FACTORS CONTROLLING CELL DIVISION

Dr Peter Clark

This lecture aims to outline the diverse factors in the extracellular environment that affect cell growth and proliferation.

What external influences are detected by cells?

- **Chemical**:- hormones, growth factors, ion concs, ECM, molecules on other cells, nutrient and dissolved gas (O_2/CO_2) concs.
- **Physical**:- mechanical stresses, the topography or "layout" of the ECM and other cells

Although all external factors may potentially influence cell proliferation, the best understood, and the ones to be considered here are: Growth factors, cell-cell adhesion and cell-ECM adhesion.

Anchorage dependence

- in suspension, cells do not significantly synthesise protein or DNA
- cells require to be attached to ECM (and a degree of spreading is required) to begin protein synthesis and proliferation (DNA synthesis)
- attachment to ECM may be required for survival (e.g. epithelia, endothelia)

Anchorage dependence

Cell-ECM adhesion molecules

- Cells have receptors on their cell surface which bind specifically to ECM molecules
- these molecules are often linked, at their cytoplasmic domains, to the cytoskeleton
- this arrangement means that there is mechanical continuity between ECM and the cell interior

Integrin structure

Integrins

- are the most important ECM receptors
- αβ heterodimers (both sub-units span the plasma membrane?are β known? α and 8?once), ~10
- α5β1?recognise short, specific peptide sequences (e.g. fibronectin receptor binds asp-gly-arg (RGD)
- known? α/β ?more than 20 combinations of
- each combination specifically binds a particular peptide sequence
- such peptide sequences found in more than one ECM molecule, e.g. RGD found in fibronectin, vitronectin, fibrinogen plus others
- linked, via actin-binding proteins, to the actin cytoskeleton (most integrins)
- α6β4 intergin complex found in epithelial hemidesmosomes,?the linked to the cytokeratin (intermediate filament) network
- integrin complexes cluster to form focal adhesions (most) or)?hemidesmosomes (α6β4
- these clusters are involved in signal transduction
- integrins also bind to specific adhesion molecules on some cells αvβ3 binds to PECAM-1(CD31) on endothelial cells in inflammation)?(e.g.

Signalling to and from ECM receptors

- ECM receptors (e.g. integrins) can act to transduce signals
- e.g. ECM binding to an integrin complex can stimulate the complex to produce a signal inside the cell, i.e. “outside-in” signalling

Signalling to and from ECM receptors (I)

- a signal generated inside the cell (e.g. as the result of hormone binding to receptor) can act on an integrin complex to alter the affinity of an integrin (i.e. alter its affinity for its ECM binding)

this is “inside-out” signalling (e.g. in inflammation or blood-clotting, switching on adhesion of circulating leukocytes)

“Outside-in” signalling

- a cell can receive information about its surroundings from its adhesion to ECM
- e.g. the composition of the ECM will determine which integrin complexes bind and which signals it receives
- this can alter the phenotype of the cell

Control of proliferation

When cells in culture form a confluent monolayer, they cease proliferating and slow down many other metabolic activities. This used to be known as contact inhibition of cell division. Another set of experiments suggest that it is competition for external growth factors and not cell-cell contact responsible:- density dependence of cell division.

Mechanism of anchorage dependence?

- growth factor receptors and integrin signalling complexes can activate identical signalling pathways (e.g. MAPK)
- individually, this activation is weak and/or transient
- together, activation is strong and sustained
- the separate signalling pathways act synergistically

Interactions between cells

- long term, stable interactions resulting in formation of cell-cell junctions
- short-term transient interactions between cells which do not form cell-cell junctions

Cell-cell contact between non-epithelial cells

When non-epithelial cells “collide”, they do not form stable cell-cell contacts. They actually “repel” one another by paralysing motility at the contact site, promoting the formation of a motile front at another site on the cell, and moving off in the opposite direction.

This is contact inhibition of locomotion and is partly responsible for preventing the multilayering of cells in culture and in vivo.

Long-term cell-cell contacts

Upon contact, some cell types strongly adhere and form specific cell-cell junctions (adherens junctions, desmosomes, tight junctions, gap junctions) (see 1st year MCD lecture).

This is true of epithelial cells and endothelial cells, which form layers, and neurones forming synapses.

β-catenin dynamics in cells: a mechanism for contact inhibition of proliferation?

- ?when bound to cadherin at the membrane, **β-**catenin not available for LEF-1 binding and nuclear effects
- β?normally, cytoplasmic-catenin rapidly degraded
- β-catenin cytoplasmic levels rise as a result of?if ?inhibition of degradation or loss of cadherin-mediated adhesion, β-catenin/LEF-1 complex enters nucleus and influences gene expression, leading to proliferation.

Cells can lose their social skills

Under certain conditions, cells lose their behavioural restraints. As a result, they will

- proliferate uncontrollably (lose density dependence of proliferation)
- are less adherant and will multilayer (lose contact inhibition of locomotion and anchorage dependence)
- epithelia breakdown cell-cell contacts
- not Hayflick-limited, express telomerase i.e. cancer

Many components of signal transduction pathways are proto-oncogenes

- if the gene coding for a component of a signalling pathway is mutated so that the protein is constitutively active, that pathway will be permanently 'on'
- receptors, signalling intermediates and signalling targets (e.g. transcription factors) are proto-oncogenes
- this is the mechanism of short-circuiting leading to uncontrolled proliferation as a result of loss of growth factor dependence etc.

Metastasis

- in addition to deregulated proliferation, a major feature of cancerous tumours is their ability to spread
- most human cancers are carcinomas (i.e. of epithelial origin)
- in order to spread to other sites (metastasis), cells must break away from the primary tumour, travel to a blood or lymph vessel, enter the vessel, lodge at a distant site, leave the vessel, and ultimately establish a secondary tumour

How does a primary carcinoma cell metastasise?

- cell-cell adhesion must be down-regulated (e.g. cadherin levels reduced)
- the cells must be motile
- degradation of ECM must take place; (matrix metalloproteinase (MMP) levels increased in order to migrate through basal lamina and interstitial ECM
- <u>the degree of carcinoma cell-cell adhesion is an indicator of how differentiated the primary tumour is, and indicates its invasiveness and the prognosis for the patient</u>.

CANCER 9

THE CYTOSKELETON
Professor Mike Ferenczi

Principal components of the cytoskeleton

Principal components of the cytoskeleton

Review previous outline knowledge of the cytoskeleton and the three filament systems which make it up.
Describe the assembly and organisation of subunits in intermediate filaments.
Define the role of intermediate filaments, their size and localisation.
Describe how the structure of microtubules results from polymerisation of subunits consisting of α,β-tubulin dimers.
Describe the polymerisation dynamics of microtubules in terms of preferred (+) and non-preferred (-) ends and GTP hydrolysis.
Summarise the functions of motor proteins associated with the microtubule cytoskeleton.
Describe the structure of monomeric actin.
Summarise the structure of F-actin filaments and describe their polymerisation from monomeric (G-) actin.
Explain treadmilling in the context of filament polymerisation
Describe what is meant by molecular motors
Describe the role of molecular motors

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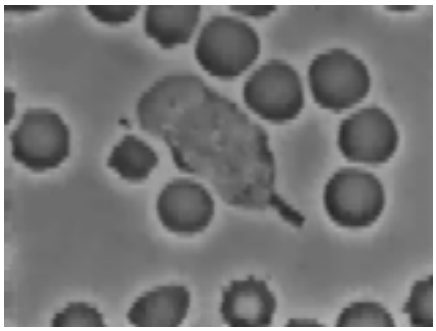
Chemotaxis: Crawling Neutrophil Chasing a Bacterium



Human polymorphonuclear leukocyte (neutrophil) on a blood film "chasing" *Staphylococcus aureus* microorganisms, added to the film.

16mm movie made in the 1950s by David Rogers at Vanderbilt University. Written by Tom Stossel, June 22, 1999. Cell Biology & Cytoskeleton Group Division of Hematology, Brigham & Women's Hospital, Harvard Medical School

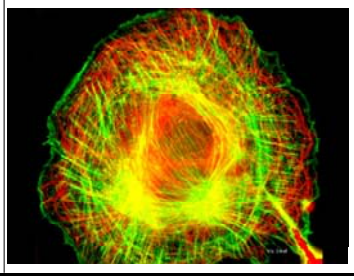
Imperial College London

The cytoskeleton consists of three types of filaments

Three types of filaments form the cytoskeletal system (skeleton of the cell):

1. Intermediate filaments. ∅: 8-12 nm
2. Microtubules (MT): ∅: 25 nm
3. Actin filaments – microfilaments and stress fibres: ∅: 7 nm

Each filament type consists of a predominant protein that polymerises to form the filament.



Each filament may also contain additional proteins that give the filaments particular properties: binding specificity, regulation, localisation

LO: Review previous outline knowledge of the cytoskeleton and the three filament systems which make it up.

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Filament systems

Microfilaments. Thin filaments in muscle, made up of *actin, troponin* and *tropomyosin*,

Microtubules (MTs), made up of *tubulin*, and

Intermediate filaments, made up of a number of proteins depending on the cell type.

Each is formed by polymerisation of subunits
There is a wide variety of filament-associated proteins:

- Control polymerisation/depolymerisation
- Link filaments to membranes, organelles, extracellular material via other proteins
- Move organelles, or the cell itself : motor proteins
- Control the movement of motor proteins

Highly dynamic.
Controls cell shape, rigidity and motility.
Cross-linking turns cytoplasm from liquid to gel

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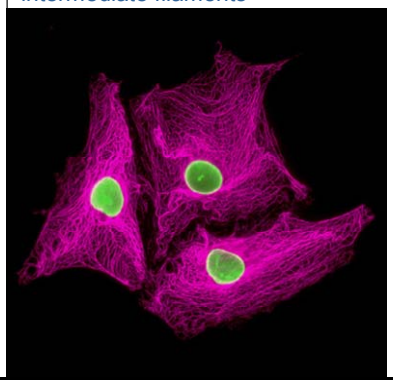
Cytoskeletal filament dynamics

All 3 filament types are made by polymerisation of monomer

Free monomer must be in equilibrium with polymer - but the equilibrium can be altered by other proteins that bind to either free monomers or to filaments near the site of monomer addition

The equilibrium dynamics are similar in principle for actin filaments and microtubules (they differ in detail though), but rather different for intermediate filaments



The intermediate filament cytoskeleton in epithelial cells: keratins (purple) give the cell resilience by making cytoplasmic networks and lamins (green) protect the DNA by reinforcing the nuclear envelope.
Professor Birgit Lane FRSE, University of Dundee

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Intermediate filaments

www.colorado.edu/MCDB/MCDB1150/ohd/overhead.html

Intermediate filaments are stable, durable. Diameter from 8-12 nm (intermediate in size compared with thin filaments and microtubules).
Prominent in cells that withstand mechanical stress
The most insoluble part of the cell. Can be dissociated by urea.
Highly regulated – polymerisation - depolymerisation
Distribution is cell-type specific .
Can be phosphorylated.
Mutations lead to degenerative diseases.

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Intermediate Filaments types

Types I and II: *Acidic Keratin* and *Basic Keratin*, respectively: in epithelial cells (bladder, skin, etc).

Type III. Number of cell types, including:
vimentin in fibroblasts, endothelial cells and leukocytes;
desmins found in desmosomes which link cells together (e.g. cardiac muscle, skeletal muscle);
glial fibrillary acidic protein (GFAP) in astrocytes and other types of glia (binds prion protein), and
peripherin in peripheral nerve fibers.

Type IV: *Neurofilament H* (heavy), *M* (medium) and *L* (low). Modifiers refer to the molecular weight of the NF proteins;
mainly in axonal cells.
internexin and some nonstandard IV's are found in lens fibres of the eye (*filensin* and *phakinin*).

Type V: *lamins* which have a nuclear signal sequence so they can form a filamentous support inside the inner nuclear membrane. *Lamins* are vital to the re-formation of the nuclear envelope after cell division.

Some unclassified protein filaments

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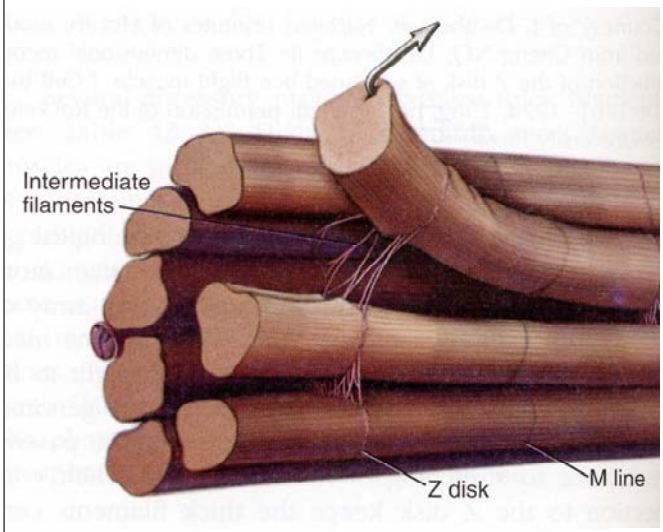
A function of desmin in a muscle cell



In a skeletal muscle cell, desmin filaments join myofibrils, to help maintain sarcomere alignment

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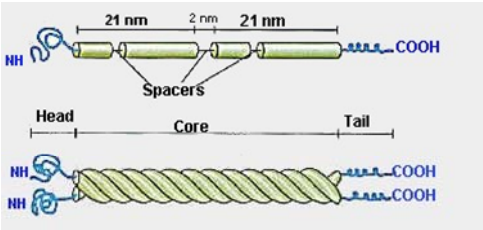
Intermediate filament assembly

The polymerising subunits are tissue specific (though they all have stretches of homologous structure), e.g. cytokeratins in epithelial cells, vimentin in connective tissue cells, neurofilament proteins in neurons, etc.

Usually found as a cytoplasmic network in the central region of the cell Provide mechanical strength to cells. In epithelial layers links at desmosomes connect intermediate filaments in adjoining cells to create strength of cell sheet



Intermediate filament subunit

Filament start forming by **parallel** assembly of monomers

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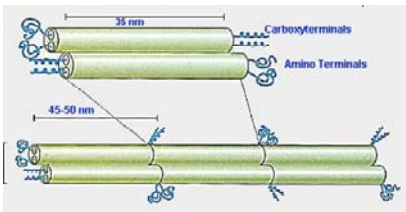
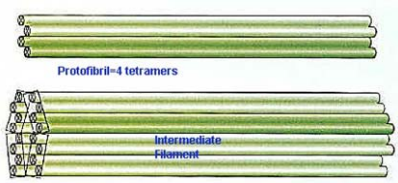
Intermediate filament assembly



Alberts et al, Molecular Biology of the Cell, Garland Publishing, NY, 1996)

Dimers form staggered **anti-parallel** tetramers

Four tetramers (16 filaments) form **protofilaments**
Which then assemble into **intermediate filaments**



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Microtubules (MTs)

•Microtubules are polymers of tubulin.

Microtubules are hollow cylinders. Their circumference is usually made up of 13 tubulin monomers

•The microtubules have polarity (the two ends are not identical)

•One end is called the 'plus' end, the other is the 'minus' end

•Elongation occurs preferentially at the 'plus' end.

•Tubulin dimers bind to MTs. The dimers are made of one molecule of α-tubulin and one of β-tubulin.

•The β-tubulin is at the 'plus end'.

•They are found 'singly' or in more complex structures: cilia, flagella or centrioles.

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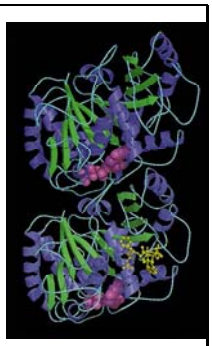
Tubulin

- Tubulin is a highly conserved protein with subdomains which are required for subunit interactions during microtubule assembly
- Tubulin has sites for:
  - GTP binding
  - interaction with MAPs
  - drug binding sites

α-tubulin with GTP

β-tubulin with GDP and taxol



Nogales et al. 1998. Nature 391:199-203

Two type of tubulins:
α-tubulin has a bound GTP that does not hydrolyse.
β-tubulin has a bound GTP or GDP. The bound GTP can be hydrolysed, releasing P_i. GDP can be released and exchanged for GTP
γ-tubulin is found in centrosomes and act as nucleating sites for microtubule assembly

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Microtubule (MT) Assembly

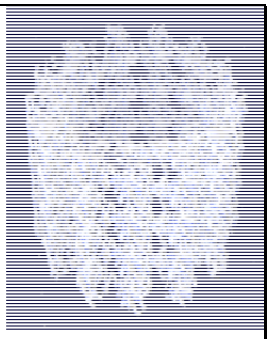
Polymerisation occurs in 2 phases:

1. Nucleation: The process requires tubulin, Mg^{++} and GTP and also proceeds at 37ºC. This stage is relatively slow until the microtubule is initially formed. An α and a β tubulin molecule join to form a heterodimer.

2. Elongation: Made by polymerisation of tubulin (αβ dimer, 2 × 55kDa proteins) to form a hollow filament, 25 nm diameter. Around the periphery are 13 tubulin dimers, forming longitudinal, staggered 13 protofilaments.

GTP bound to β-tubulin is hydrolysed to produce GDP and P_i. GTP bound to α-tubulin is not. Hydrolysis stops at 4 ºC.

MTs have an inherent polarity, determined by polarity of tubulin assembly.



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Microtubule (MT) Assembly

During microtubule assembly GTP binds to tubulin β-subunits and is subsequently hydrolysed to GDP. Tubulin subunits are added to GTP-capped microtubules much more efficiently than to GDP-microtubules

So, if GTP-containing subunits are present at the 'plus' end, then the filament is relatively stable and further polymerisation will occur.

If polymerisation is slow so that hydrolysis occurs faster than new subunit addition, GDP-containing subunits may be present at the end in which case depolymerisation is favoured.

Replacing GTP with a non-hydrolysable analogue of GTP does not prevent polymerisation, but does prevent depolymerisation.

In a healthy cell, the presence of GTP maintains microtubules.

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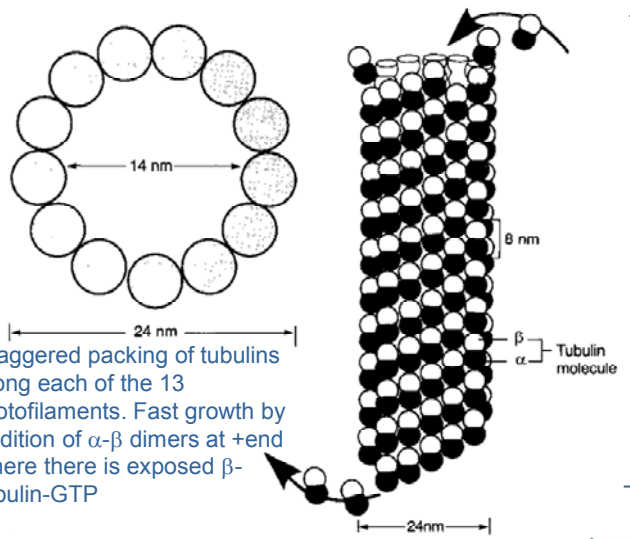
Microtubule structure



Staggered packing of tubulins along each of the 13 protofilaments. Fast growth by addition of α-β dimers at +end where there is exposed β-tubulin-GTP

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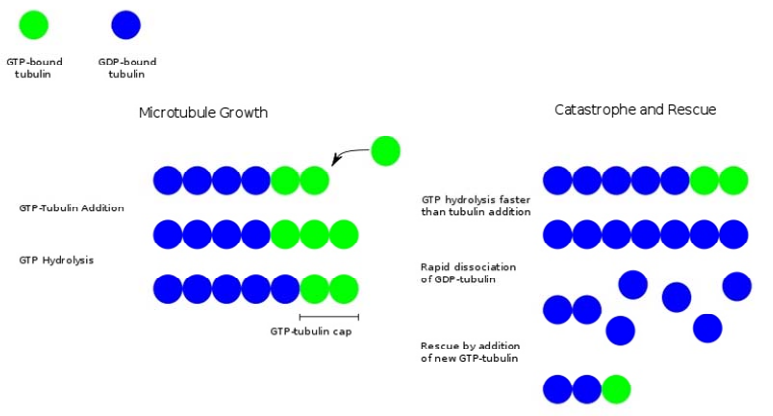
Filament nucleotides



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Filament nucleotides

Microtubule (and actin filament) subunits contain a nucleotide

For tubulin this is GTP

After polymerisation this hydrolyses slowly to GDP

[In the case of actin filaments the nucleotide is ATP/ADP]

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Dynamic instability

The switch between filament growth and depolymerisation is known as dynamic instability.

Polymerisation/depolymerisation of MTs depend on cellular concentrations of MTs, GTP, GDP, tubulin and **microtubule associated proteins** (MAPs) which affect the stability of the plus and minus-ends of MTs.

Free GDP-subunits resulting from depolymerisation are converted to GTP-subunits by nucleotide exchange

Since GTP hydrolysis is energetically favoured, microtubule polymerisation can do work in the cell

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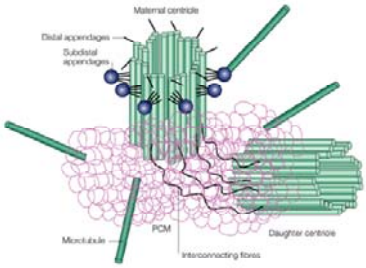
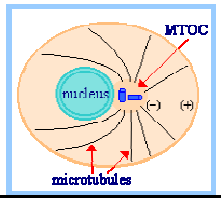
Cellular organisation of microtubules

In interphase cells, MTs extend radially from the microtubule organising centre (MTOC) associated with the centrosome. The minus ends are capped by the **centrosome** (2 centrioles arranged at right angles to each other from which 9 triplet MTs radiate). The plus-end of MTs is at the periphery. Capping of MTs by pericentriolar material (PCM) gives stability to MTs even at low tubulin concentration.

Ability of spindle microtubules to be broken down by cell during mitosis is essential for chromosomal separation





Doxsey (2001) Re-evaluating centrosome function Nature Reviews Molecular Cell Biology 2(9), 688-698.

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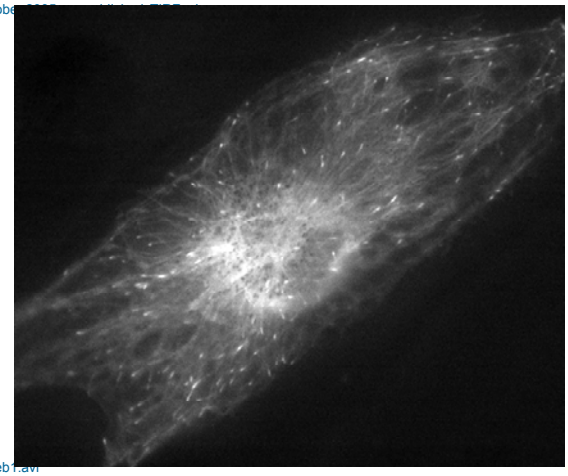
Microtubule elongation

Melanocytes labelled with GFP-EB1 protein. EB1 binds to the tips of microtubules. Alistair Hume, Dmitryi Ushakov, Miguel Seabra, Michael Ferenczi, Imperial College – Octobe



eb1.avi

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Microtubules during mitosis

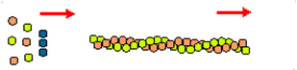
During mitotic spindle assembly, some microtubules are stabilized by the proteins of the kinetochore

During metaphase, subunits are added to the plus end of a microtubule at the kinetochore and are removed from the minus end at the spindle pole (microtubules maintain constant length)

At anaphase the chromatid is released from attachment to its sister at the metaphase plate and the kinetochore moves up the microtubule, removing subunits from its plus end as it goes (chromatid carried to spindle pole).

Part of chromatid movement is due to the simultaneous loss of tubulin subunits from the minus end of the microtubules at the pole.

Treadmilling: growth at one end, and depolymerisation at the other end. Proteins attached to MTs eventually end up at the minus end of the MTs.



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Microtubule associated proteins (MAPs)

Microtubules are essential for many critical cellular processes including cell division. Their polymerisation/depolymerisation is controlled by a number of regulatory systems and accessory proteins.

The microtubule system is a target for anti-cancer drugs: colchicine, vinblastine, nocodazole, taxol and vincristine, and a target for herbicides and pesticides

MAPs bind to MTs to
•Stabilize them
•Cross-link them
•Attach them to other cellular components: membranes, intermediate filaments, other microtubules

•Microtubule-dependent motor proteins (ATPases): kinesins (anterograde – towards periphery) and dyneins or ncd (retrograde – towards centre)
Tau protein (plaques in Alzheimer)

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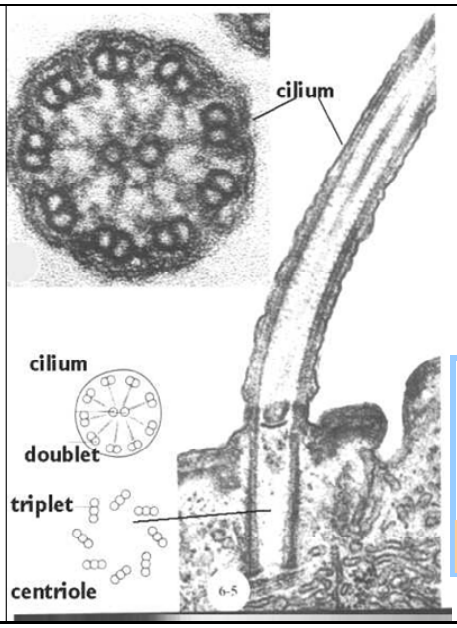
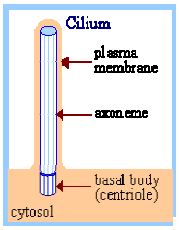
Cilia and flagella



Composed of microtubules

flagella (longer than cilia) - beating provides the force that allows sperm to swim

cilia – hair-like appendages found in respiratory tract (function to clear mucus) and epithelia of oviduct (function to transport ova toward the uterus)



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Microtubule diseases

Inhibition of the axonal transport in the brain accelerates neurodegenerative diseases, such as Alzheimer's disease (AD). Loss of cellular function and cell death might result from an accumulation of microtubule associated proteins (MAP) like tau in brain tissues.

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Actin filament system

EM-view of negatively stained F-actin filaments (Julie Hodgkinson, IC)

Filaments made by polymerisation of G-actin (43 kDa globular protein)

In most cells found at the periphery, underlying the cell surface

Examples include thin filaments of muscle; core of each microvillus in a brush border; involved in cell surface shape changes including those underlying cell migration

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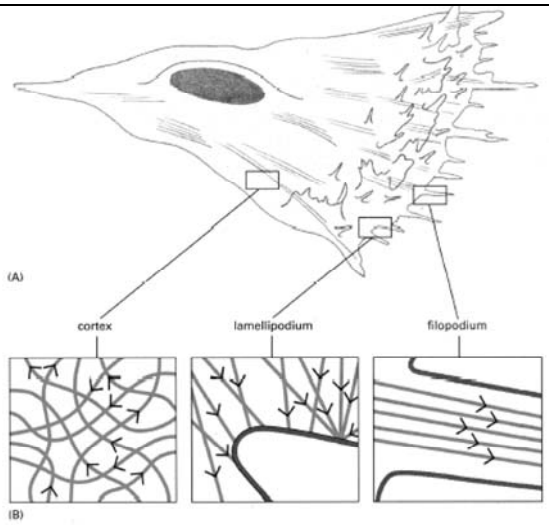
Stress fibres



Stress fibres are cables of actin
Stress fibres are found in fibroblasts and other cells where cell adhesion is important.

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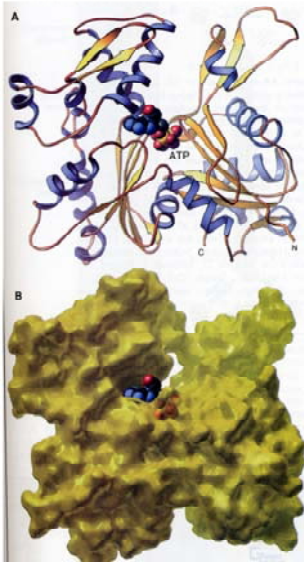
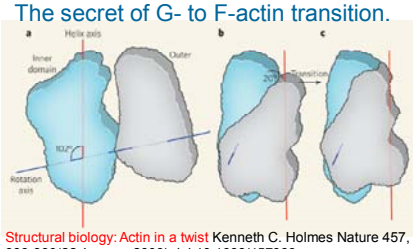
Actin structure



Actin monomers (G-actin for Globular actin) contain ATP or ADP. ATP is slowly hydrolysed upon polymerisation.

The secret of G- to F-actin transition.



Structural biology: Actin in a twist Kenneth C. Holmes Nature 457, 389-390(22 January 2009) doi:10.1038/457389a

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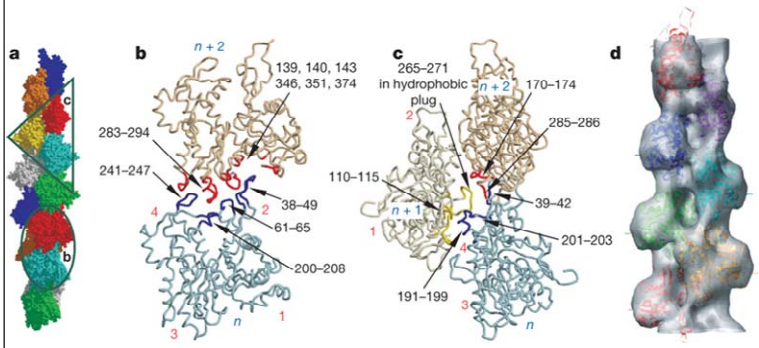
Actin structure



The nature of the globular- to fibrous-actin transition Toshiro Oda, Mitsusada Iwasa, Tomoki Aihara, Yuichiro Maéda & Akihiro Narita Nature 457, 441-445(22 January 2008) doi:10.1038/nature07685

Each repeat contains 13 molecules (35.7 nm).
Rotation/molecule = 167°. Looks like two long-pitch helices slowly winding around each other.

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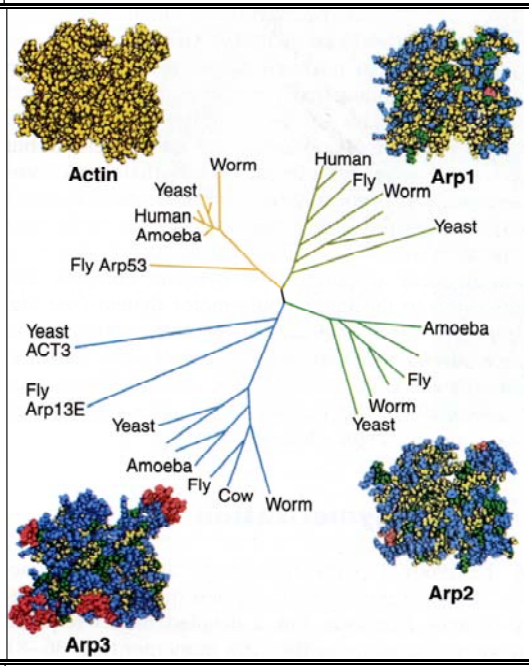
Actin phylogenetic tree



Actin (the amino acid sequence of actin) is highly conserved.

Several actin-like proteins are found. These usually bind actin and co-polymerise with it.

The flat F-actin structure is very much like the bacterial analogue of actin MreB. Actin is a bridge between eukaryotes and prokaryotes.

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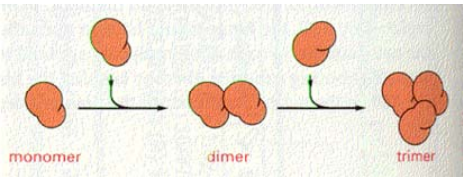
Actin polymerisation

Actin exists in two forms:
G-actin (globular actin) are actin monomers
F-actin (filamentous actin) are actin filaments
Actin monomers bind ATP. The ATP can be hydrolysed to form ADP-actin and P_i.
Two step polymerisation:

1. **Nucleation:** requires ATP-actin monomers at a high concentration to form trimers of ATP-actin. Under some conditions, actin dimers are formed. This allows the formation of branching actin structures. Nucleation rate is proportional to $[actin]^3$.

In motile cells, the leading edge nucleates actin polymerisation by the Arp2/3 complex and WASP family proteins



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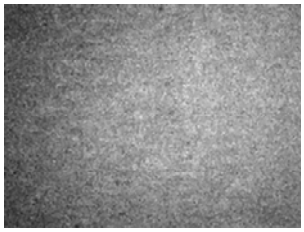
Actin elongation

2. **Elongation:** this is fast in the presence of ATP-actin. ATP-actin monomers are added to actin trimers to form new filaments and at the 'barbed'-end of actin filaments to elongate existing actin filaments. All subunits have identical polarity. Filament is helical. Polymerisation at 'barbed' end is 10x faster than at 'pointed'-end.

Elongation at the leading edge may push the membrane forward



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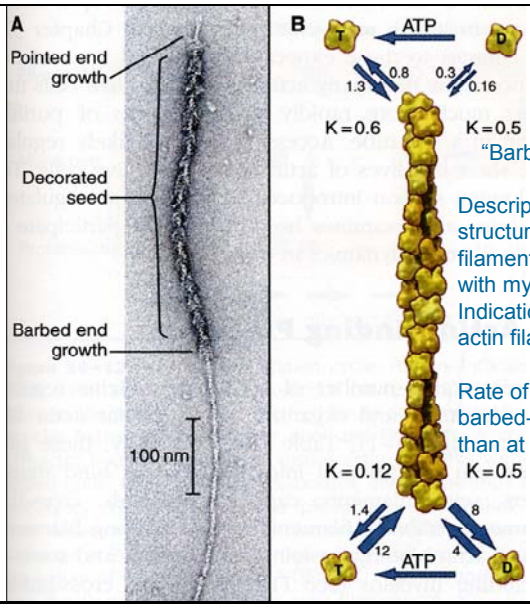
Actin elongation



"Barbed" and "pointed" ends:
Description of herring-bone structure seen when actin filaments are 'decorated' with myosin molecules. Indication of polarity of the actin filaments.

Rate of growth at the barbed-end is ~10x faster than at the pointed end.

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Actin depolymerisation - shortening of filaments

The ATP in F-actin hydrolyses so that the bulk of actin filaments contains ADP-actins.

At low concentrations of ATP-actin monomers, Actin monomers at the 'barbed'-ends of F-actin will end up with ADP-actins. These will dissociate from F-actin, causing shortening, unless stabilised by ligands.

ADP in ADP-actin monomers can be exchanged for ATP.

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Actin-binding proteins (ABPs) in muscle

Examples:
Myosin motors: many classes

Tropomyosin: part of thin filaments in smooth and striated muscle. Involved in regulation

Troponin: confers ca-regulation of contraction to thin filaments in skeletal muscle.

Caldesmon: smooth muscle regulation of contraction

Calponin: smooth muscle regulation

α-actinin: cross-linking protein

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Examples of actin binding proteins in the cytoplasm

α-actinin: cross-linking protein

Gelsolin: capping, nucleating and severing activity. Calcium activated capping and severing. Also bundles actin filaments

Villin: nucleates, severs and caps actin filament. Similar to Profilin: binds actin monomers (ATP-actin) and provides pool for actin elongation at barbed end

Cofilin: regulated by phosphorylation. Binds to G- and F-actin. Increases filament turnover 20-30x

Fimbrin: α-actinin-like domains. Can bundle actin filaments.

Vimentin: found in intermediate filaments. Maintains myofibril alignment in striated muscle

Vinculin: actin cross-linking and bundling

Ezrin, Radixin, Moesin (ERM): regulation by phosphoinositide lipids (membrane components). Active in unfolded tail conformation.

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Actin binding molecules

Cellular poisons active at low concentrations: used as research tools

Cytochalasin: from aspergillus clavatus fungus, inhibit polymerisation/depolymerisation

Latrunculin: from sea sponge: inhibits polymerisation

Phalloidin: toxin from amanita fungus. Binds to and stabilises F-actin. Fluorescent derivative (eg rhodamine-phalloidin) used to stain F-actin in vitro.

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Cell motility examples

Migration of phagocytic cells towards site of infection

•Migration of cancerous cells away from site of primary tumour - invasion

•Migration of cells during embryological development

•Cytoplasmic streaming

•Muscle contraction

•Swimming: waving of cilia/flagellae; movement of liquids

•Transport of organelles, Movement of vesicles

•Phagocytosis

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Cellular motors

Microtubule-dependent motors:
kinesins
dyneins
ncd (nonclaret disjunctional – name of a drosophila gene)

Actin-dependent motors:
myosins

Polymerisation engines: microtubules, actin (eg listeria)

Treadmilling: when filaments shorten at one end and grow at the other.

Rotary motors:
flagellar engine
F1 ATPase

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How do cells move?

•Involves coordinated shape changes due to cytoskeleton (actin mainly)

•Needs appropriate signalling to coordinate parts of cell and control direction

•May depend on extracellular signals and receptor pathways

•Cells become polarised - line up with a thin actin-containing extension (lamellipodium) forming the leading edge

•Microtubule system also is aligned - MTOC (microtubule organising centre) is forward of the nucleus, as is the Golgi

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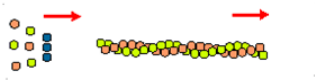
Treadmilling:

Movement of polymers brought about by polymerisation at one end, and depolymerisation at the other.

Treadmilling is observed in the laboratory for microtubules and actin filaments.



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Molecular motors

Molecular Motors:

These are proteins which have evolved to move cells or parts of cells.
The movement requires energy, so molecular motors are usually ATPases (hydrolyse MgATP to MgADP and inorganic phosphate).
Sometimes they harness ion gradients (bacterial flagellae).

ATP hydrolysis results in ADP, P^i, work and heat

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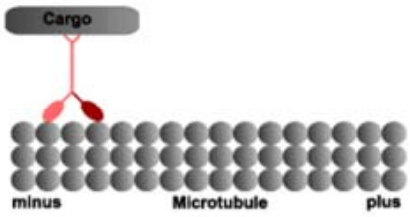
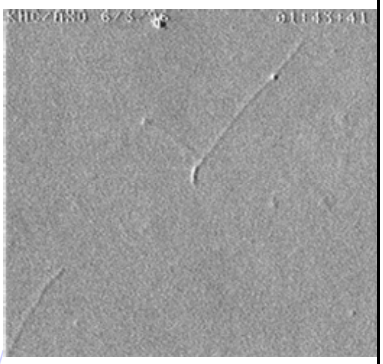
Microtubule based motors

Kinesin moves vesicles along microtubules



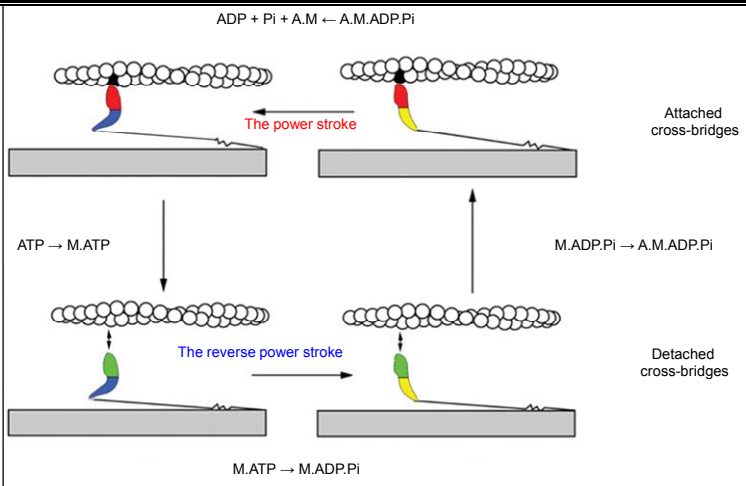
Konrad J . Böhm www.imb-jena.de/~kboehm/

Kinesin plated on a slide move microtubules



Imperial College London

Lymn-Taylor scheme of cross-bridge cycling



What happens in the absence of ATP?

Rigor mortis

Imperial College London

CANCER 10

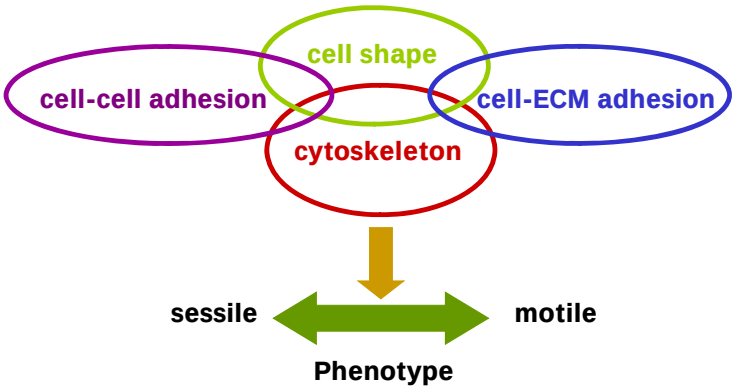
INVASION: REGULATION OF CELL MIGRATION

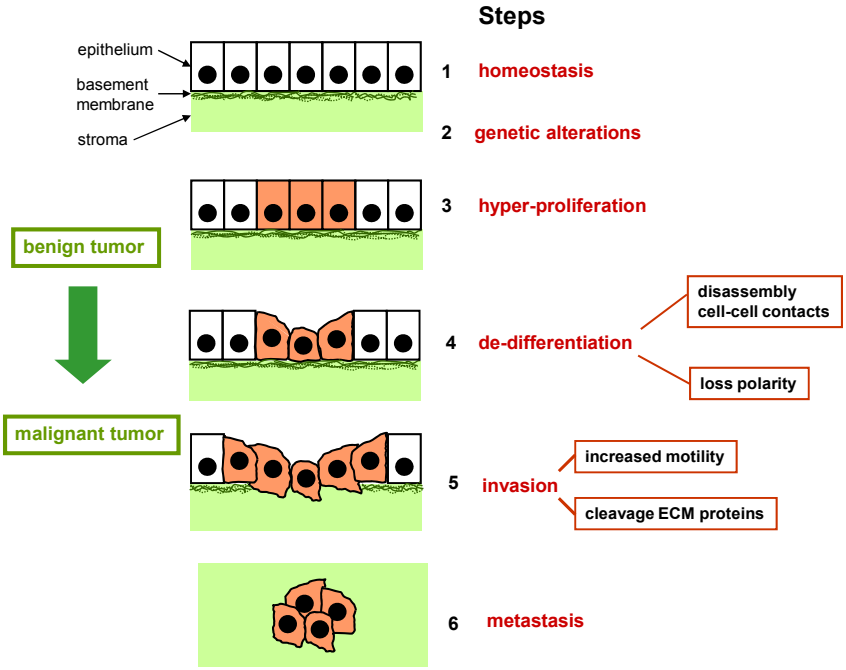
Dr Vania Braga

1) How does detachment from primary tumour and migration occur?

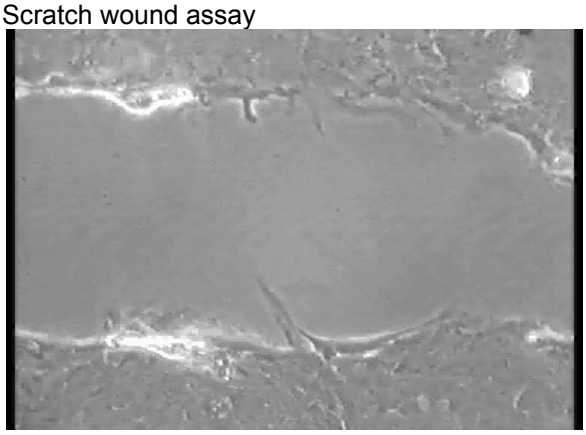
2) What are the molecular mechanisms that regulate motility?

- microfilaments
- regulation of actin dynamics
- cytoskeletal proteins
- signalling proteins

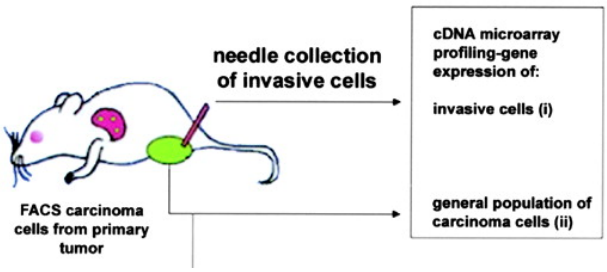




Migration of primary glial cells
Scratch wound assay



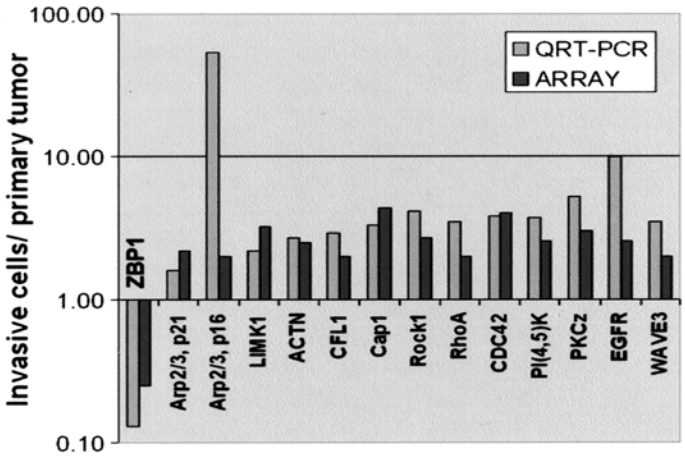
Migration of a glial tumour cell line



Comparison of expression profile of invasive cells Vs primary tumours

Upregulation of genes involved in:

- **Cytoskeleton regulation**
- **Motility machinery**



mRNA levels

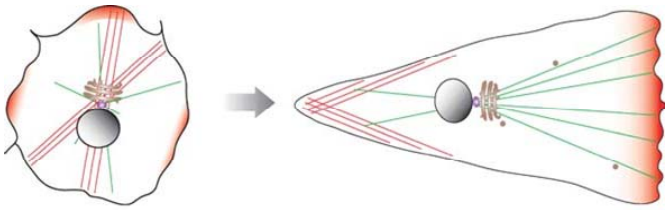
Wang et al., 04

Cell movement:

Stimuli to move

organogenesis and morphogenesis
wounding
growth factors/chemoattractants
dedifferentiation (tumours)

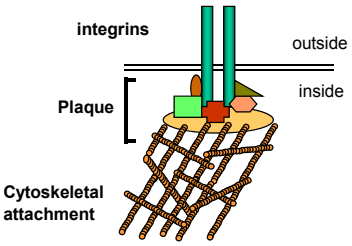
Where to go? **directionality** (polarity) **When to stop?** **contact-inhibition motility**



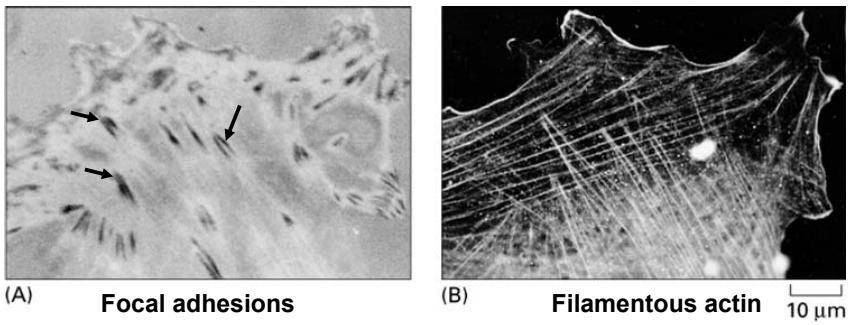
How to move? **specialized structures** (focal adhesion, lamellae, filopodium)

REGULATION?

Jaffe and Hall, 2005



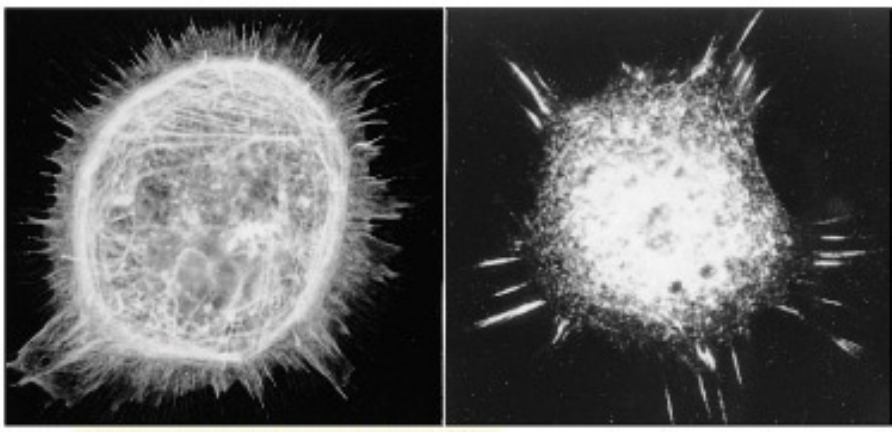
Attachment to substratum (ECM proteins)



Molecular Biology of the Cell, 4th Edition.

Filopodia
Finger-like protusions rich in actin filaments

actin **vinculin**



Molecular Biology of the Cell

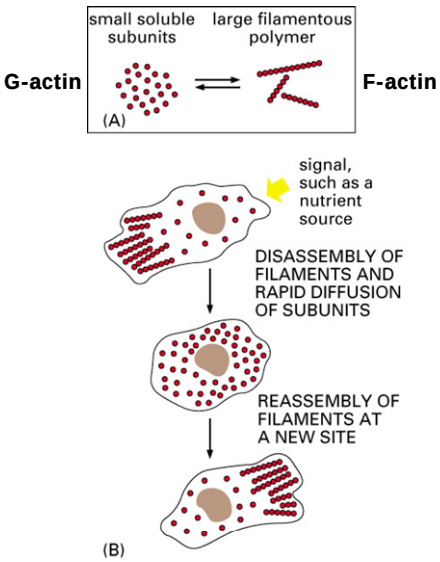
Lamellipodia
Sheet-like protusions rich is actin filaments

Cell Movement
Control is needed:

- within a cell to coordinate what is happening in different parts
- regulate adhesion/release of cell-extracellular matrix receptors
- from outside to respond to external influences –
  sensors
  directionality

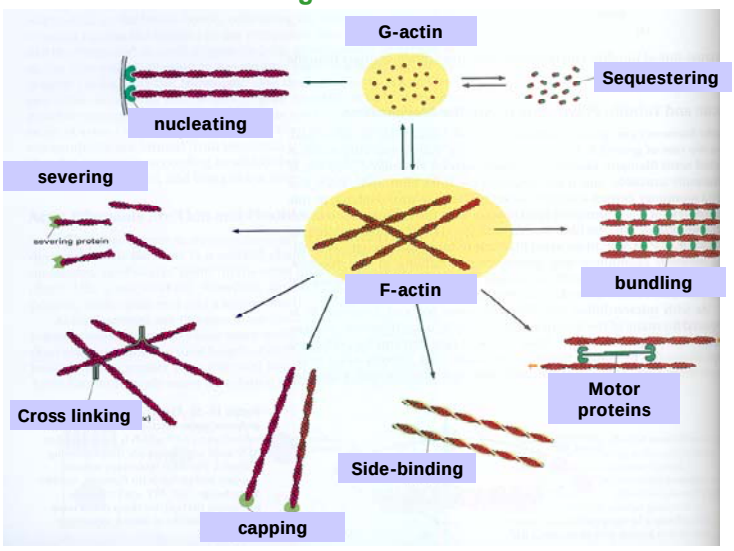
  Motility: hapoptatic *versus* chemotatic

  Cell movement = changing cell shape

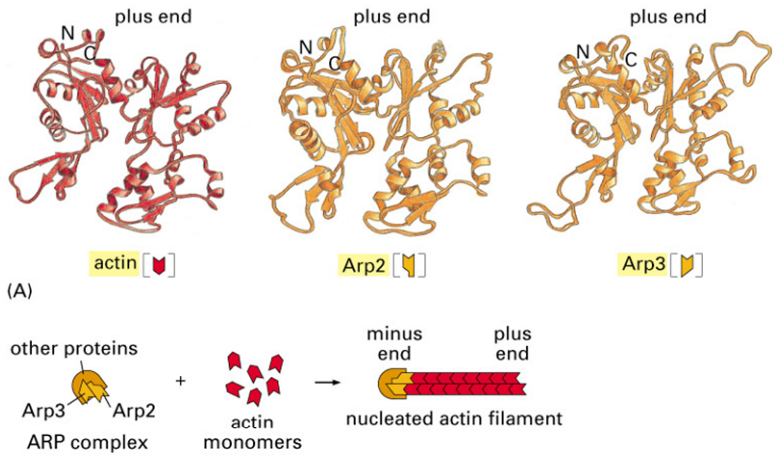


Molecular Biology of the Cell, 4th Edition.

Remodelling of actin filaments:

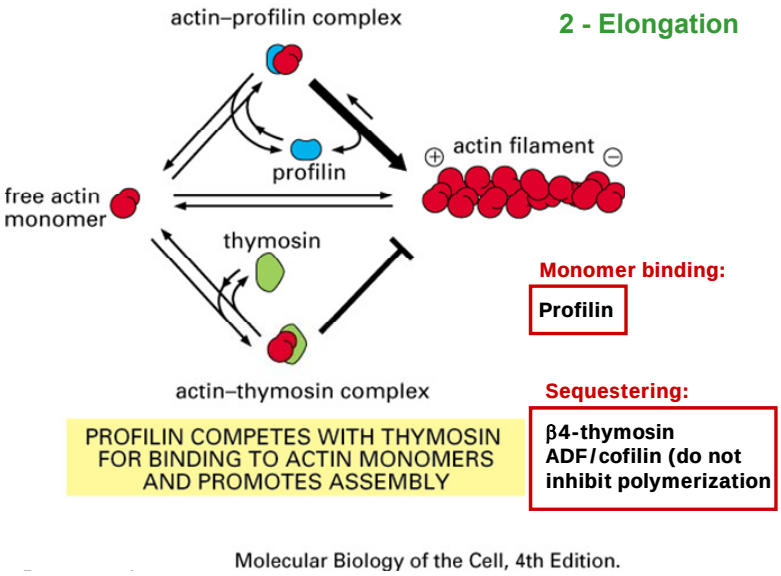


1 - Nucleation



Limiting step in actin dynamics – formation of trimers to initiate polymerization

2 - Elongation



Molecular Biology of the Cell, 4th Edition.

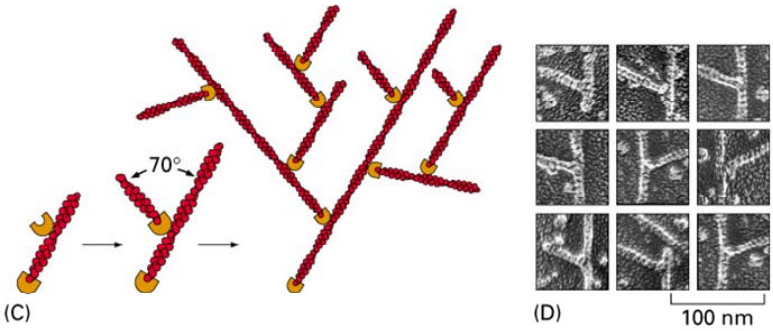
Monomer binding:

Profilin

Sequestering:

β4-thymosin
ADF/cofilin (do not inhibit polymerization

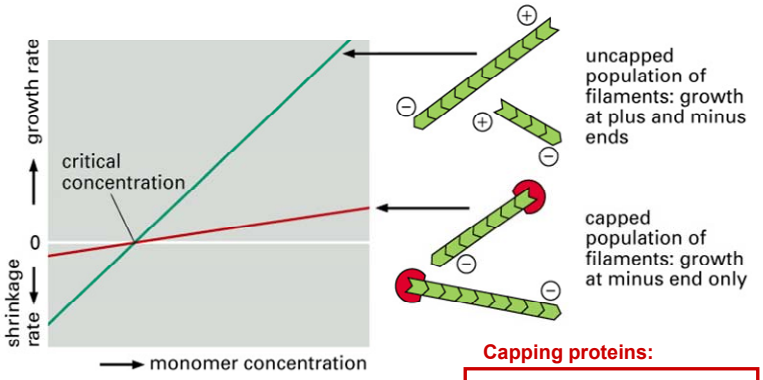
3 - Branching



Molecular Biology of the Cell, 4th Edition.

Branching protein: **Arp complex**

4 - Capping

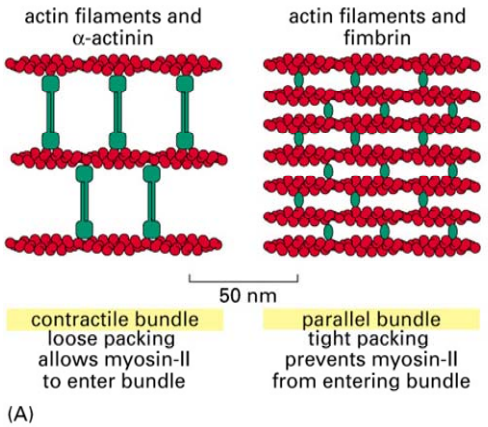


Molecular Biology of the Cell, 4th Edition.

Capping proteins:

+ end: Cap Z
Gelsolin
Fragmin/severin
- end: Tropomodulin
Arp complex

5 - Cross-linking and bundling

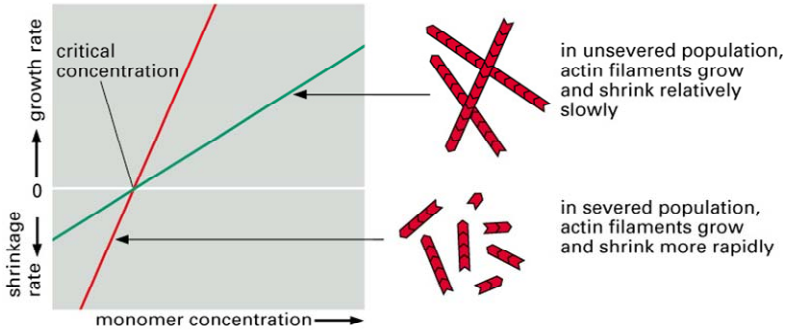


Molecular Biology of the Cell, 4th Edition.

Proteins involved:

α-actinin
fimbrin
filamin
spectrin
villin
vinculin

6 - Severing

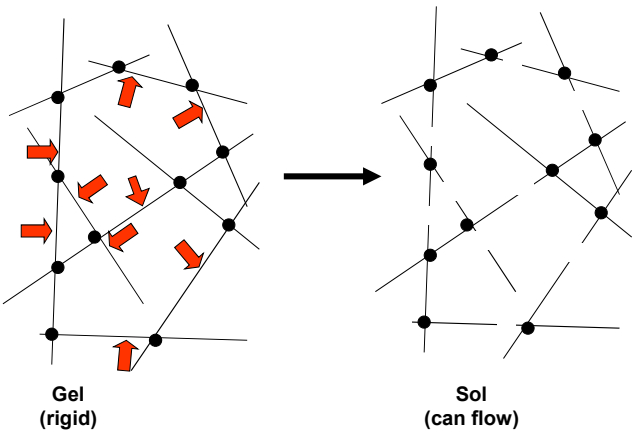


Molecular Biology of the Cell, 4th Edition.

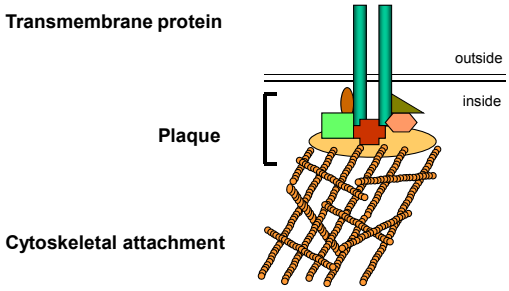
Severing proteins

gelsolin
ADF/cofilin
fragmin/severin

Gel-sol transition by actin filament severing



7 - Membrane connections



Connection to transmembrane proteins - **cell-cell and cell-ECM receptors**
nucleation, elongantion, cross-linking, bundling
recruitment of signalling complexes
points at membrane to generate tension

Proteins involved: **Talin, α-catenin, spectrin, Ezrin/radixin/moesin**

Why is it important to learn about cytoskeletal proteins?
Which one of these diseases is not caused by deregulation of actin cytoskeleton?

(a) High blood pressure

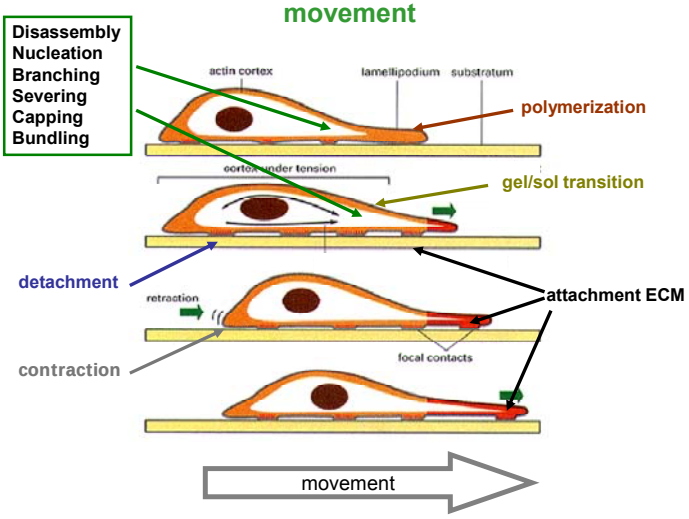
(b) Wiskott-Aldrich Syndrome – WAS (immunodeficiency, eczma, autoimmunity)

(c) Epidermolysis Bullosa (hereditary blistering diseases)

(d) Bullous Pemphigoid (autoimmune disease)

(e) Alzheimer (neurodegenerative)

Participation of different actin activities during cell movement



Lamellae protrusion

Polymerization, disassembly, branching, capping

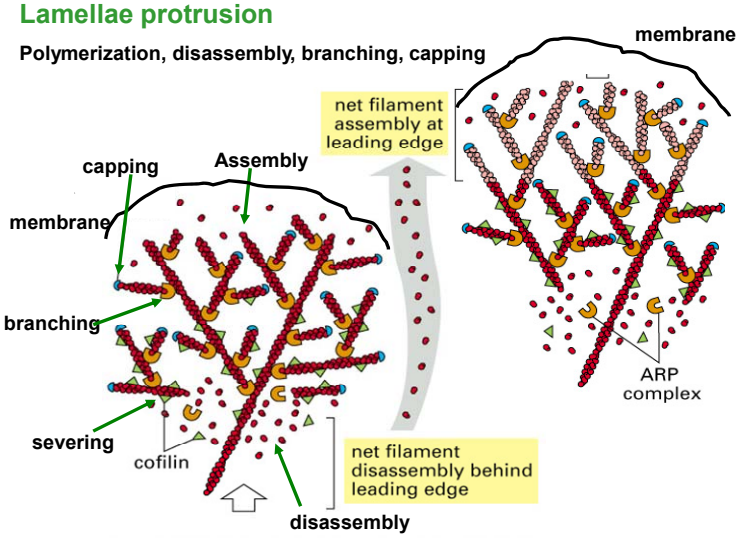
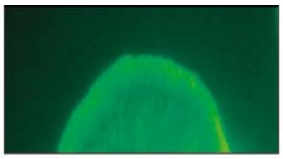


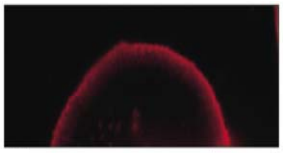
Figure 16–90. Molecular Biology of the Cell, 4th Editior

Lamellae protrusion

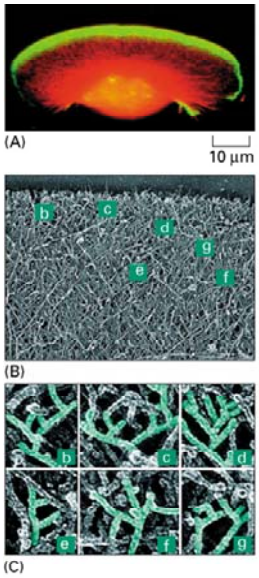
Phalloidin – F-actin



Newly incorporated actin



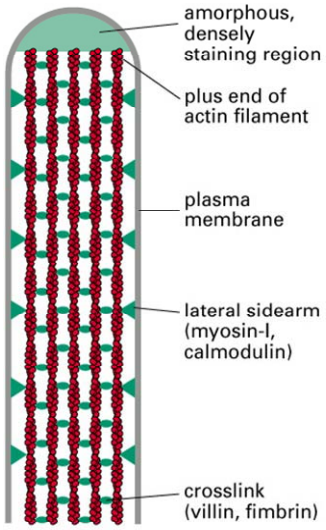
5 µm



actin

Arp complex

Molecular Biology of the Cell, 4th Edition.Figure 16–88. Molecular Biology of the Cell, 4th Edition.



(A)

Molecular Biology of the Cell, 4th Edition.

Filopodia

Actin polymerization

Bundling

Crosslinking

Signalling mechanisms that regulate the actin cytoskeleton:

1 - ion flux changes (i.e. intracellular calcium)
2 – control by phosphoinositide signalling
3 - signalling cascades via small GTPases

Control of actin filament networks by Ca^{2+}

Gelsolin

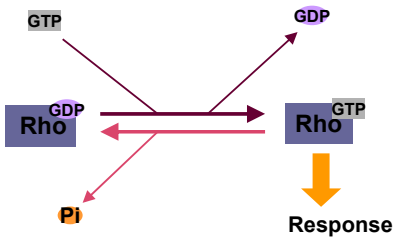
- Ca^{2+} dependent severing
- exposes - (fast depolymerising) end

α-actinin

- Crosslinks actin filaments
- binding decreased at high Ca^{2+}

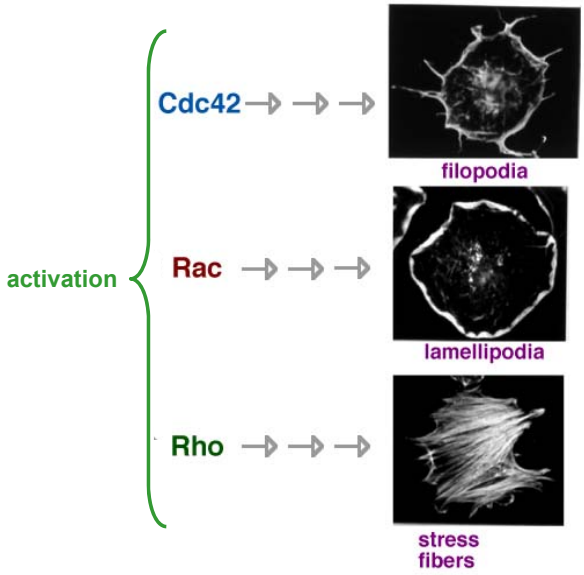
Control of actin cytoskeleton by small G proteins

- Rho subfamily of small GTPases belongs to the Ras super-family
  Family members: Rac, Rho, Cdc42 best known

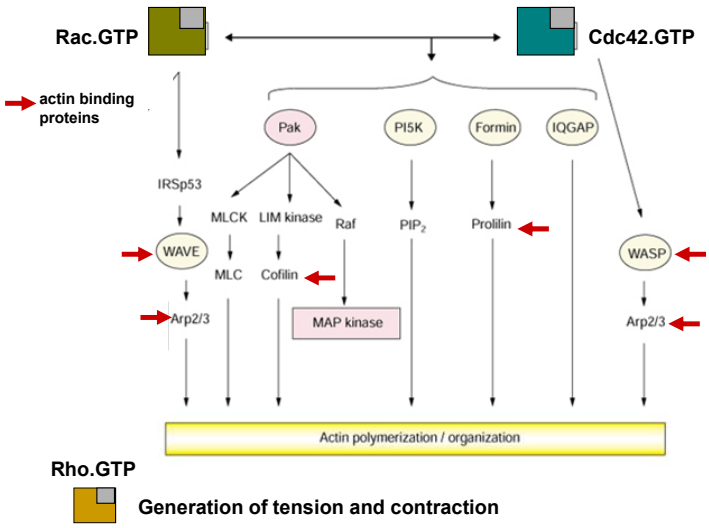


- Participate in a variety of cytoskeletal processes.
- These proteins are activated by receptor tyrosine kinase, adhesion receptors and signal transduction pathways.
- Expression levels up-regulated in different human tumours.

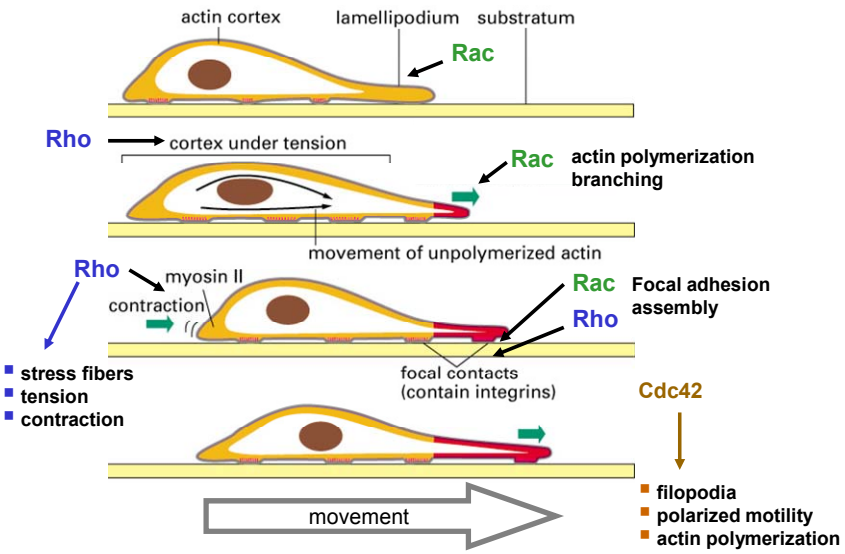
Rho family of small GTPases



How does signalling from Rho small GTPases regulate actin cytoskeleton and motility?



Participation of small GTPases on cell migration



Further reading:

1) Molecular Biology of the Cell

2) Cell Biology. 2002. T.Pollard & W.Earnshaw. pg 551-670, Elsevier Science

3) Condeelis J, Singer RH, Segall JE. THE GREAT ESCAPE: When Cancer Cells Hijack the Genes for Chemotaxis and Motility. Ann Rev Cell Dev Biol. 2005;21:695-718.

4) Jaffe AB, Hall A. RHO GTPASES: Biochemistry and Biology.
Ann Rev Cell Dev Biol. 2005, 21:247-269.

5) Sahai E. Mechanisms of cancer cell invasion. Curr Opin Genet Dev. 2005, 15(1):87-96.

CANCER 11

CANCER AS A DISEASE -COLORECTAL CANCER

Dr Michael Osborn
St Mary's campus
(handout prepared by Professor Gordon Stamp**)**

LEARNING OBJECTIVES:

- Describe the different modes of clinical presentation of colorectal carcinoma
- List the principles of the adenoma-carcinoma sequence
- Define the Duke's and TNM staging systems
- Develop an understanding of molecular pathogenesis of colorectal carcinoma
- Describe the major pathological features which are associated with aggressive malignant behaviour of colorectal carcinoma

Colonic Function (what is it there for?)
The colon has evolved to serve not only as a reservoir but to compact faecal residues, reducing frequency of defaecation which would have obvious evolutionary advantages. The large intestine is also very efficient at water and electrolyte resorption.

Colonic Anatomy
The colon is divided into:

- the caecum which includes the ileocaecal valve and appendix
- ascending ("right") colon
- the transverse colon
- descending (left) colon
- sigmoid colon and
- rectum

The hepatic flexure is at the junction of the ascending and transverse colon while the splenic flexure is at the junction of transverse and descending colon. The rectum is below the level of the peritoneum surface ranging between 8-15 cm. in length.

The anatomy is important as the blood supply to these areas differs and also that colon cancers present in different ways when they arise in different parts of the colon. There is also evidence that there are some differences in molecular genetic abnormalities and behaviours in tumours arising in right side versus left side of the colon.

The colonic mucosal epithelium, from which carcinomas arise, consists of tubular crypts (of Lieberkuhn) lined by predominantly mucin secreting cells with intervening surface cells which are mainly absorptive cells. The absorptive cells have basally located nuclei and do not secret mucin, whereas in the crypts goblet cells synthesise and secrete mucin. Each crypt has 5-10 enteroendocrine cells (neuroendocrine cells) and a few stem cells. Occasional Paneth cells are seen in the base of the crypts of the caecum and ascending colon.

Adenomas
Colonic adenomas are very common lesions and increase with age. Most adenomas present in the 30-60 year age group and may be incidental findings at colonoscopy or cause symptoms by bleeding. They range in size from very tiny to large masses which can obstruct the colon.

Sometimes gastroenterologists see colonic lesions and describe them as polyps, but the term polyp is just applied to a mass lesion in the bowel which may or may not have a stalk. There are many different types of polyp, most of which are benign, and most are not adenomas. The majority of small colonic polyps are benign so called hyperplastic polyps. A minority are adenomas which are true neoplasms with genetic abnormalities.

Up to the age of 60 almost half of the population may be harbouring a small adenomatous polyp. There is a greater risk of developing polyps if you have first-degree relatives with colorectal carcinoma or adenomas, particularly if they are multiple.

There are 4 main morphological patterns of adenomas:

- tubular
- villous
- tubulovillous (a mixture of the two)
- serrated (a more recently recognised category)

The important features are that tubular adenomas are often well defined and pedundulated and whereas villous adenomas are often larger, more diffuse ('carpet papillomas') and difficult to sample in the centre where carcinomas develop.
Some very large villous adenomas more than 5cms in diameter have a high risk of harbouring a cancer (more likely than not).

Dysplasia in adenomas
Dysplasia is a term often used by pathologists and literally means "bad moulding".
In the context of neoplasia, it indicates cells show features associated with precancerous change, such as enlarged nuclei with more heavily staining chromatin (hyper chromasia) and a coarser chromatin pattern, often accompanied by large nucleoli.

In the tubular adenomas nuclei are often enlarged and elongated (cigar shaped).
In higher grades of dysplasia the nuclei enlarge to an irregular ovoid pattern with thick, irregular nuclear membranes and increased numbers of mitotic figures.
Along with this the glandular structure tends to become more complicated with buds and branches and a greater degree of irregularity in the architecture. The changes are accompanied by an increasing number of genetic abnormalities. It is the excess and irregularly distributed chromosomes and DNA that gives the cells their features.

Pathologists often divide adenomas into low-grade and high-grade dysplasia and there is a much greater risk of invasive cancer developing with the high-grade dysplasia.

The essential difference between an adenoma and a carcinoma is an absence or presence of invasion, which in most circumstances implies that the malignant epithelial cells have acquired 3 abilities

- Extracellular (stromal) matrix degradation (especially basement membranes)
- Adhesion to degraded or new extracellular matrix (ECM)
- Ability to move into the newly degraded ECM

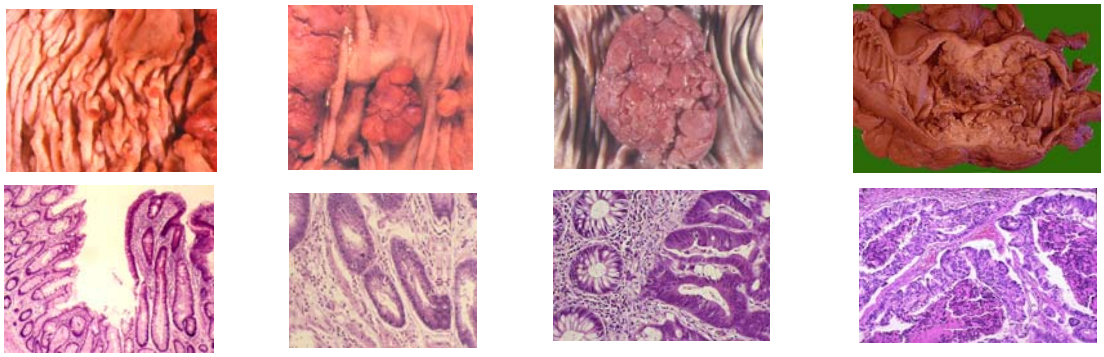
The hallmark of invasion in adenomas is penetration of the thin layer of muscle (the muscularis mucosae) that separates the epithelial containing compartment (lamina propria) from the underlying submucosa which lies above the thicker muscle layer, the muscularis propria.

Once the tumour cells have got beyond the muscularis mucosae they then have access to the vascular system (lymphatic and capillaries) and thus potentially can spread further.

The distinction between and adenoma and carcinoma is very difficult using radiology and endoscopy, but generally speaking the larger the lesion the higher the risk that malignancy has developed.

On rare occasions there may be small early carcinomas encountered without any obvious adenoma. Generally speaking most cancers will develop in a setting of an adenoma.

Progression of colonic cancer - Paradigm for molecular cancer genetics



APC	Hypomethylation	ras mutation 18q tsg	p53 mutation	others

Normal colon crypt cell	Hyperplasia	Early Adenoma	Late Ademoma	Carcinoma	Metastasis

A **'Vogelgram'**
Each stage accumulates a new set of molecular genetic abnormalities.

The adenocarcinoma sequence
The progression of adenomas to carcinomas is accompanied by an increasing degree of genetic abnormalities. Clues to the progression of colorectal carcinoma came from careful study of patients with hereditary colorectal cancers and comparing them to the patients with sporadic colorectal carcinomas. It is also known that there are some early and some later events. In general the accumulation of mutations is more important than any specific order in which order they occur. The main genes affected are listed in table 3 but include APC, mismatch repair genes, P53, K-RAS, DCC (deleted in colorectal cancer), SMAD (loss) and E cadherin mutation.

Molecular pathogenesis of colorectal carcinoma
There are relatively rare families that have a greatly increased risk of developing colonic adenocarcinoma. The best known is familial adenomatous polyposis FAP) syndrome, due to a mutation of the APC gene on chromosome 5q21. Depending where the mutation is in the gene, you can see different variants of this condition including Gardner and Turcot syndromes.

In the classical FAP syndrome, patients may have up to 2500 adenomas over the whole colon although predominantly in the left colon. If left unmanaged, almost all patients will develop cancer by the age of 30 years. There are some mutation variants where the risk is less and patients have relatively few polyps. Generally speaking if a patient has more than adenomatous polyps in their colon they are almost certain to have an hereditary syndrome. Always think of possible hereditable tendencies when people<30 develop cancers that usually affect older generations.

Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Syndrome is a different autosomal dominant familial syndrome where there are fewer numbers of adenomas, usually on the right side of the colon. Here the mutation lies in DNA repair genes leading to micro satellite instability.

There are a few other familial syndromes which may predispose to colorectal cancer but these are far less common (Multiple Juvenile Polyposis, Peutz-Jeghers syndrome and Cowden's syndrome)

Colorectal Cancer
Most colorectal cancers occur sporadically and the risk increases with age.
The patients usually present with:

- altered bowel habit (constipation or diarrhoea or a mixture of the two)
- rectal bleeding (fresh blood or altered blood)
- discharge of mucus
- intermittent (colicky) abdominal pain
- intermittent obstruction leading to swelling of the abdomen
- tiredness and malaise due to and unexplained iron deficiency anaemia

There is often a considerable delay in the diagnosis of colorectal cancer and General Practitioners have to be alert to the possibility of colorectal cancer in any middle aged to elderly patient who presents with vague abdominal symptoms and especially rectal bleeding. Occasionally patients will present with acute intestinal obstruction or with due to the blockage of the lumen of the colon by an advanced cancer, or with peritonitis due to perforation of the centre of an ulcerated carcinoma and rarely with bowel fistula's due to the carcinoma invading other organs such as bladder or small bowel.

Half of all cancers will occur in the rectosigmoid area, the remainder are distributed throughout the colon. Minority of patients (5%) will have more than one carcinoma developing. It should also be remembered that patients who have been treated for colonic carcinoma are at risk of developing others.

Tumours occurring in the caecum and right colon often present later and with vaguer symptoms, partly due to the capacity of the caecum to expand before getting blocked, and also they more often mucinous and soft in nature so obstruction occurs later.

Diagnosis is usually by a combination of radiology (plain abdominal x-ray, barium enema and CT scan, but particularly endoscopy using a sigmotoscope or colonoscope.) As yet, there are no reliable markers of colorectal cancer than can be measured in the blood although carcinoembryonic antigen has been used as a response marker in advanced colorectal carcinoma. However it is elevated in many other conditions.

Types of Colorectal Cancer
Pathologists usually assess colorectal carcinomas by their resemblance to the normal cells in the crypts and how abnormal the glands and cells are arranged, and describe this as **differentiation**.

Usually carcinomas are assessed as well, moderately or poorly differentiated but this is a relatively subjective assessment. In addition, some cancers may secrete a large amount of mucin (so called mucoid/colloid carcinomas). Others may rarely have a high content of endocrine cells and there are very rare pure endocrine carcinomas (carcinoids and high grade neuroendocrine cancers).

Staging
Aside from assessing differentiation, the most important clinical indicator is the extent to which the tumour has progressed.

There are two main systems that pathologists use to describe this progression.
The classical Dukes staging was described by Cuthbert Dukes from St Marks Hospital in the 1937 and is elegantly simple, describing whether the tumour is

- Dukes' A - confined within the bowel wall (including the muscle)
- Dukes' B - extended beyond the muscle into fat or serosa (peritoneal surface)
- Dukes' C - metastasis in lymph nodes

If the tumour is still confined within the wall and involves lymph nodes it is nevertheless classified as Dukes' C.

At a later stage some authorities have recommended a Dukes' D classification for liver metastasis.

More recently the TNM classification has been used (see Table 1).

Table 1 - TNM Classification of Carcinoma of the Colon and Rectum

Tumour Stage	Histologic Features of the Neoplasm
Tis	Carcinoma in situ (high grade dysplasia) or intramucosal carcinoma (lamina propria invasion)
T1	Tumour invades submucosa
T2	Extending into the muscularis propria but nor penetrating through it
T3	Penetrating through the muscularis propria into subserosa
T4	Tumour directly invades other organs or structures
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1 to 3 lymph nodes
N2	Metastasis in 4 or more lymph nodes
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant Metastasis

Table 2 -

Dukes' Stage	TNM Stage	Extent of Invasion	5-yr Survival
A	T1N0M0 or T2N0M0	Mucosa	100%
B1	T3N0M0	Muscularis propia	65%
B2	T4N0M0	Serosa	50%
C1	Any TN1M0	Muscularis propia + lymph nodes	40%
C2	Any TN2, N3M0	Serosa + lymph nodes	25%
D	Any T, NM1	Distant metastases	5%

Spread and metastasis
Colorectal carcinomas go to the regional lymph nodes and liver in the first instance and subsequently may spread to the lungs and elsewhere. Most surgeons aim to clear the local lymph nodes in order to stage the tumours. The Dukes' system was also adapted to try and asses whether lymph nodes near the tumour were involved (C1) or more distant nodes at the point of the mesenteric blood vessel ligature which represented more distant spread (C2). These seem to have some prognostic value.

In the past, involvement of the liver was very difficult to treat, but with modern surgical techniques including so called bloodless surgery with radio frequency ablation or laser techniques, it is possible to resect colorectal cancer metastases and allow the liver to regenerate hopefully to affect a cure or allow a greater window for chemotherapeutic effect.

On occasion colorectal cancers will spread across the abdominal cavity to the small bowel or the ovaries/uterus, particularly if they are of the mucinous type.

Prognostic features
After curative resection for colorectal carcinoma about half of the patients will survive 5 years. Survival is increasing with advances in surgery and chemotherapy and more recently there have been promising developments in new drugs which target rogue factors best on colorectal cancer cells. It is a little early to see what impact these will have but early results are promising.

Apart from stage and type, the factors which affect how a colorectal carcinoma will develop and behave include:

- **Age extremes** – some very young patients (20-30 years old) present with colorectal carcinoma although it is extremely rare. In a few of these this is in a background of a pre-malignant condition such as ulcerative colitis or Crohn's disease and many of these tumours are aggressive mucinous types. Added to this is the difficulty in making the diagnosis in such a young patient with what is effectively a rare tumour in that age group. Similarly in the very elderly patient, the vagueness of the presenting symptoms means the tumours often present at an extremely advanced or untreatable stage.
- **Sex** – the prognosis is better for females than males. There is a slight female preponderance of the less common right-sided colorectal carcinomas which paradoxically have a worse prognosis. There is a good correlation between tumour size and extent which is reflected in the staging and outcome
- **Obstruction and perforation** – These complications are often accompanied by advanced tumour and access to the vascular system and peritoneum and therefore not surprisingly associated with worse prognosis.
- **Inflammatory reaction** – A host immune response consisting of lymphocytes and plasma cells is associated with a better prognosis.
- **Tumour type** – Mucinous and very undifferentiated carcinomas have a worst outlook.
- **Angioinvasion** – penetration of muscular walled blood vessels carries a worse prognosis

Screening for colorectal cancer
The government has recently proposed a new screening programme using the Fecal Occult Blood Test. The disadvantage is that this is relatively insensitive and produces some false positives but in pilot studies it has been shown to produce some benefit in picking up asymptomatic cancers. Many other molecular genetic tests have been proposed including screening for mutations by PCR-based techniques on fecal samples but many of these mutations are encountered in other carcinomas and may occasionally occur in the absence of a cancer. So, a robust technique has not yet been developed. Screening by sigmoidoscopy or colonoscopy is usually reserved for those with a significant family history (usually in a first degree relative) and obviously for those with known hereditary bowel cancer in the family.

Table 3 **Major molecular genetic abnormalities in colorectal cancer**

1 APC gene (5q)

2 DNA hypomethylation

3 K-RAS mutation (12p)

4 Deleted in DCC (18q loss)

5 P53 (17p)

6 Mismatch repair (HMSH2 {2p}HMLH {3p})

7 TGF beta (receptor 2 mutation) (3p)

8 Beta catenin mutation (3p)

IMMUNOLOGY 1

TUMOUR IMMUNOLOGY

Professor Henning Walczak
Department of Medicine, Hammersmith Campus

CANCER 12

BIOLOGICAL BASIS OF CANCER THERAPY

Professor Justin Stebbing

Objectives

- Describe the main chemotherapeutic and radiotherapeutic approaches to treating cancer
- Explain why many cancer treatments cause side effects such as nausea, hair loss, anaemia and immunosuppression, and indicate the approaches which have been tried to minimise these
- Explain the rationale for the newer drugs in cancer therapy
- Discuss the prospects for new therapies based on the biology of cancer development

Hallmarks of a cancer cells
Keep on growing
Keep on dividing
(Invade & Spread)

Targeting cancer cells
Keep on growing - target DNA synthesis
Keep on dividing - target mitotic spindle

The discovery of chemotherapy
By mistake
By luck
By trial and error
Cytotoxic discovery by mistake

Mustard Gas
Bis (2-chloroethyl) sulphide ($C_4H_8Cl_2S$)
Mustard gas in warfare
1925 Geneva protocol signed
1925 Spain against Morocco
1930 Soviet Union against China
1935 Italy against Ethiopia
1937 Japan against China
1965 Egypt against North Yemen
1985 Iraq against Iran
1988 Iraq against Kurds

US Chemical Warfare Service
Established 1940
'Chemical warfare antidotes'

Director Cornelius Rhoads
(later founded Memorial Sloane Kettering Hospital)
1943 Bari Harbour

German raid on US fleet in Bari harbour sunk USS John Harvey
USS John Harvey was carrying 2000 M47A1 bomb containing a total of 100 tonnes of mustard gas
Bari Harbour victims
Survivors developed conjunctivitis & skin blistering
4 died the next day, 9 the following day…..etc
83 of 617 survivors died within a month

Colonel Stewart Alexander noted steep fall in white cell count on day 3-4 after exposure…mustard gas was stopping bone marrow cells dividing to make white cells

Sulphur mustard (Mustard gas)	**Nitrogen mustard (mechlorethamine)**
CH_2-CH_2-Cl	CH_2-CH_2-Cl
S	N
CH_2-CH_2-Cl	CH_2-CH_2-Cl

US Military research programme
1942 Alfred Gilman & Louis Goodman (Yale)
Nitrogen mustard in mouse with lymphoma led to tumour regression
The first chemo patient
1944 Mr JD (48 year old silversmith) with non-Hodgkin's lymphoma
Treated with nitrogen mustard
Tumours regressed and he lived 2 months before dying of marrow failure

Drug discovery by luck
Cisplatin
1965 Barnett Rosenberg (physicist at Michigan State university)
Studied effects of electric currents on E.coli using platinum electrodes in a water bath

Cisplatin
E coli stopped dividing but not growing leading to long bacteria up to 300 times longer than normal
Cisplatin a product from the platinum electrodes is responsible

Drug discovery by trial and error

Nixon declares "War on Cancer"

Drug testing program at NIH

Classes of chemotherapy from nature
Vinca alkaloids
Taxanes
Camptothecans
Anthracyclines
Bleomycin
Epipodophyllotoxins
Actinomycin

How does chemotherapy work?
Cancer cells divide too fast:
1. Target formation of DNA bases
2. Target DNA to inhibit synthesis
3. Target mitotic spindle

Antimetabolites – inhibit base synthesis
<u>False bases</u>
Purine analogues
Pyrimidine analogues
<u>Inhibit enzymes that synthesise DNA bases</u>
Dihydrofolate reductase inhibitors
Thymidylate synthetase inhibitors

Purine analogues

Pyrimidine analogues

Antimetabolites
1933 Dr Lucy Wills reversed anaemia in Bombay textile workers by giving Marmite
The anaemia was due to folic acid deficiency which was described in 1943

Anti-folates
1945 Sidney Farber (Harvard) gave folic acid to patients with acute leukaemia where it accelerated the leukaemia
Dr Subba Row of Lederle commissioned to make folic acid antagonists

Aminopterin
First anti-folate synthesised
1947 Faber treated 16 children with acute leukaemia with aminopterin and 10 achieved temporary remission

Methotrexate
Synthesised in 1949 and superseded aminopterin
First ever cures (durable remissions) with chemotherapy alone reported with methotrexate for choriocarcinoma in 1963 by Roy Hertz & Min Chiu Li

Disrupt DNA double helix & interfere with DNA synthesis

Alkylating agents	-bind to DNA bases
Intercalating agents	-disrupt double helix
Topoisomerase inhibitors	-disrupt DNA supercoiling

Alkylating agents

Consequences of DNA alkylation

Actions of topoisomerases
E*topo*side – a topoisomerase inhibitor
Cancer- too many cells dividing
Mitosis

Microtubule growth
Taxanes
Paclitaxel -Pacific Yew bark
Docetaxel -European Yew needles
Taxanes bind β-tubulin

Why the drugs don't work
Multi-drug resistance
- MDR-1 Pglycoprotein efflux pump overexpression
- Pumps out toxins from cells including most cytotoxics

Cytotoxic drug specific resistance
Methotrexate
- Actions of methotrexate
- Antifolate in pyrimidine (A,T) synthesis
- Inhibits DHFR

DHFR gene amplification
Methotrexate resistance due to amplification of DHFR gene as double minutes (d min*) and homogenously staining regions (HSR)*

The good, the bad and the ugly
Predictable side effects

Fast growing normal cells
1. Inhibit cell division
2. Cell cycle specific drugs
    Bone marrow, GI tract epithelium, hair & nails,
    Spermatogonia

Slow growing normal cells
1. Introduce DNA mutations
2. Cell cycle independent (alkylating agents)
    Secondary tumours

The commonest side effect ?
Side effects ranked by severity by patients

- Nausea
- Tiredness
- Hair loss
- Concern about effect on friends & family
- Vomiting

Classification of toxicities

Immediate:	Nausea & Vomiting
(Hours after)	Anaphylaxis (allergy)
	Extravasation (tissue damage)
Delayed:	Myelosuppression (low blood cells)
(Days-weeks)	Stomatitis (sore mouth)
	Alopecia (hair loss)
	Neuropathy (nerve damage)
Late:	Infertility
(Years)	2nd tumours

How to be struck off and do time….
Wayne Jowett of Nottingham

Manslaughter
Dr Feda Mulhem was only 3 days into his first post as SpR in haematology was supervising Dr David Morton (SHO), who had been at the hospital for 4 months and had never given the treatment before.
Dr Feda Mulhem was given 8 months in jail for manslaughter.

How to be struck off and do time….
Since 1985 at least 13 patients in the UK have died
Radiotherapy
Topical radium
Linear accelerator in 1940s
How radiotherapy kills
Radiation damage to DNA
Radiation dose & effect
1 Gray is the dose absorbed when 1 joule is deposited in 1kg of tissue

Each Gray per cell causes:

10,000 damaged DNA bases
1,000 damaged deoxyribose sugars
1,000 single strand breaks
40 double strand breaks
150 DNA-protein cross links
30 DNA-DNA cross links

8:15 am August 6, 1945

Hiroshima

Kengo Futagawa was crossing the Kannon Bridge (1,600 meters from the hypocenter). He jumped into the river, terribly burned. This is his watch – stopped at 8.15am when the bomb landed.
Effects of nuclear bomb irraditation
New targets, so new drugs…
(There's more to cancer than DNA replication and mitosis)

Six Steps to Cancer

1. Self sufficiency in growth stimuli
2. Insensitivity to inhibitory stimuli
3. Evasion of apoptosis
4. Immortalisation
5. Neoangiogenesis
6. Invasion and metastasis

1. Autonomous growth signals

Targeting EGFR
Antibodies = -mab
"Humanisation" = -zumab
cerbB2/Her2 in breast cancer
Anti-cErbB2 (Her 2) staining

Herceptin (Trastuzumab)

Cost: 1 year adjuvant trastuzumab £21,000

Benefit:

HERA trial Increase DFS 8.4%.
No difference in overall survival.
US studies Increase DFS 12%
Increase in overall survival at three years is 2.5%

Risks:

HERA trial Cardiac events 0.5-2.2%. Treatment withdrawals due to cardiac events in 5.5%
Combined US studies Heart failure/death from cardiac causes was 0.8% in control group and 4.1% in Herceptin group.

2. Ignore cell cycle arrest points

Cell cycle

3. Evade apoptosis

Bcl2 prevents apoptosis and is over produced by cancer cells
Turning off bcl2 production should lead to programmed cell death
Antisense oligonucleotide to bcl-2

4. Immortalisation
Cancer cells (and normal germinal cells) avoid senescence by producing telomerase (hTERT) that restores the telomere ends.

5. Neo-angiogenesis
Angiogenesis inhibitors

6. Invasion & metastasis
26 human matrix metalloproteinases

How can you tell if it works?

Phase I trials
Aim: Determine toxicity & dose scheduling
Who: Few patients for whom no known alternative therapy is available
Endpoints: Activity in humans
Maximum tolerated dose
Pharmakokinetics
Side effects related to different doses
Phase II trials
Aim: Identify promising tumour types
Who: More patients than phase I trials treated at the dose and schedules determined in phase I
Endpoints: Activity in human tumour types
Side effects

RECIST criteria for response
Complete response: disappearance of all known disease
Partial response: >50% reduction in measurable lesions and no new ones
Stable disease: lesions unchanged (<50% smaller or <25% larger)
Progressive disease: new lesions or measurable lesions >25% larger

A sustained complete response

A partial response

What are the consequences for a cancer survivor?
Cancer prevalence in UK
2% of the population of the UK (1.2 million people) are alive having received a diagnosis of cancer
Mainly breast cancer (180,000)

Childhood cancer survivors
1 in 600 children (often inherited risk)
7 in 10 are cured (3 in 10 in 1960s)
In UK 55,000 young adults (16-40y) who are cancer survivors

Late side effects
- Late effects of surgery
- Late effects of radiotherapy
- Late effects of chemotherapy
- Psychological consequences

Common psychological problems
Lazarus syndrome
(difficulty with returning to normal life)
Damocles syndrome
(fear of recurrence and terror of minor symptoms)
Survivor syndrome
(guilt about surviving where others have died)

Conclusions 1- Biological basis of chemotherapy
Molecular basis of chemotherapy – DNA and mitotic spindle
5 Classes of cytotoxics -
- Antimetabolites
- Alkylating agents
- Intercalating agents
- Topoisomerase inhibitors
- Spindle poisons

Conclusions 2- Biological basis of radiotherapy
High energy Ionising Radiation damages DNA
Ionisation
Delivery of radiotherapy
- External beam
- Brachytherapy
- Radio-isotopes

Conclusions 3- Biological basis of new treatments
Target 6 molecular mechanisms
-Nibs & -Mabs

Further reading
Bower, M. & Waxman, J. Oncology, Blackwell Publishing

IMMUNOLOGY 2

TOLERANCE AND AUTOIMMUNITY

Dr Keith Gould

Learning Objectives:

- To understand the concept of immunological tolerance
- To understand the mechanisms underlying immunological tolerance
- To understand how defects in tolerance lead to autoimmune diseases and know examples of these

Tolerance

The immune system mounts specific responses to proteins from microorganisms and has the capacity to retain a memory of those proteins.

How does the immune system avoid recognition of proteins from the individual itself? This is critical since such recognition could potentially lead to the destructive immune responses against one's own tissues.

Tolerance is defined as the *acquired* **inability** to respond with an immune reaction to an antigen to which the organism normally responds.

There are two major mechanisms which achieve tolerance to self proteins.
1. Central tolerance
2. Peripheral tolerance

Tolerance is required for both T-cells (which help the responses of other immune cells) and B-cells (which produce specific antibodies).

Central tolerance

- Central tolerance for **T**-cells
- occurs in the **T**hymus, where cells excluded or retained according to the affinity of their receptors for peptide antigen and the abundance of antigen.
- dependent on MHC: peptide: T-cell receptor interaction
- Most cells die by neglect: no or very weak recognition
- **Negative selection** occurs in ca. 5% of cells: high affinity, high abundance
- Cells die by apoptosis (possibly other mechanisms also)
- Surviving cells undergo **positive selection** on low affinity, low abundance self peptides

- Central tolerance for **B**-cells
- occurs in **B**one marrow
- crosslinking of surface Immunoglobulin by polyvalent antigens expressed on bone marrow stromal cells facilitates **deletion.**

Peripheral Tolerance

- Many antigens may not be expressed in the thymus or in serum and are sometimes expressed only after the immune system has matured.
- Mechanisms are required to prevent selected T-cells, which may have low Affinity for self peptide-MHC complexes becoming auto-reactive.

Tolerance in the periphery is controlled by a number of different mechanisms:

- Anergy: a refractory state resulting from antigenic stimulation under unusual conditions.
- Ignorance: expression of self antigen at immunologically privileged sites
- Suppression: negative regulation of potentially autoreactive cells by specialised factors or cells.

Autoimmunity

- Results from failures in central or peripheral tolerance

Failure in central tolerance can occur:

- When a particular self protein is absent or mutated. Subsequent exposure to the native form of this protein will promote an auto-immune response.
- When the mechanisms involved in the removal of autoreactive cells during T- or B-cell selection are faulty.

Failure in peripheral tolerance can occur

- When mechanisms of peripheral tolerance are faulty.
- As a result of infection.

Clinical examples will be given.

CANCER 13

CANCER AS A DISEASE - SKIN CANCER
Dr Tony Chu

Epidermis provides our first line of defence against harmful solar UV radiation. It is exposed to much higher levels of solar radiation than any other organ. This exposure increases the risk to skin cells of damage by UV and to DNA of UV-mediated mutagenesis and consequently to **UV-induced cancer**.

Many & varied types
Epidermis keratinocytes –basal cell carcinomas, squamous cell carcinomas
Melanocytes – malignant melanoma
Blood vessels haemangiomas, Karposi's sarcoma. Also tumours of adnexae, etc.

Main Causes
Genetic (rare) – familial melanoma, Gorlin's syndrome (BCC's), xeroderma pigmentosa, dysplastic naevus syndrome, epidermodysplasia verruciformis
Viral – Karposi' sarcoma – human herpes virus 8, Human papilloma virus in squamous cell carcinoma
Ultraviolet Light BCC, SCC, MM,
Cases of all types of UV-induced skin cancer are increasing in the UK.

This lecture: **keratinocytes & melanocytes** – "non-melanoma skin cancer" (SCC's, BCC's) & malignant melanoma

Ultraviolet Light as a Mutagen
Solar UV primarily, but also UV used in phototherapy for some inflammatory dermatoses

Wavelengths
<280nm UVC – mostly filtered by atmosphere, little reaches earth's surface
280-320nm UVB
320-400nm UVA
400nm+ visible spectrum
Photon wavelengths 250 to 300nm are absorbed by ring structures and linear repeats found in DNA and other complex macromolecules such as proteins. Energy absorbed by bonds in these structures makes them highly reactive and easily modified or broken.

As UVC mostly filtered out by the atmosphere, primarily UVB is responsible for this kind of DNA damage.

Changes in DNA molecules caused by UVB
These affect pyrimidines Cytosine (C) & Thymine (T) bases
Most commonly 6-4 pyrimidones from pyrimidines, also formation of cyclobutane pyrimidine dimers from two adjacent pyrimidines *e.g.* -T-T-, -C-T-

Other structural effects of UV (A and or B) on cells
Photon energy can cause many molecules in the cell to become reactive. Releasing, for example, highly reactive free radicals that cause oxidative damage to DNA, proteins, etc. (contribute to photoageing of skin).

Consequences of these chemical changes - Damaged DNA!
Where one of the two strands of is damaged DNA can be removed and replaced by the cell's normal nucleotide excision repair system. In Xeroderma pigmentosum this pathway is inactive because of mutations in genes encoding repair enzymes – DNA damage is unrepaired → skin cancer can result if mutated cells persist.

"UV signature mutations" Structurally-altered C and T bases are "mis-read" by DNA repair and replication enzymes and replaced with different bases. e.g. damaged CC in DNA replaced by TT, or damaged CT replaced by TT. Thus, the coding sequence of a gene is altered, the mRNA is changed and this is translated into a protein with an altered function or which does not function at all or the mRNA may not even be transcribed fully so no protein is made.

What kinds of mutations can start a cell down the route to becoming a cancer?

1. Mutations that stimulate uncontrolled cell proliferation e.g. by abolishing control of the normal "cell cycle" – p53
2. Mutations which alter responses to growth stimulating/repressing factors by altering structures of signalling pathway proteins e.g. pathways permanently signalling "proliferate" or rendering cells "blind" to inhibitory signals.
3. Mutations that inhibit programmed cell death (apoptosis)

Mutations in genes involved in these types of pathways may initiate or continue the pathway towards a keratinocytes or melanocytes becoming malignantly transformed.

Melanin & Protection from UV

Melanocytes – neural crest origin, resident in basal layer of the epidermis
Eumelanin - (the dark brown/black pigment) granules shield the nuclei of keratinocytes in the basal and germinative layers from UV. Eumelanin absorbs UV.

Tanning Mechanism**: Solar UV stimulates melanin production by melanocytes.**
Usually UV stimulates keratinocytes to release a number of growth factors & hormones such as αMSH which stimulate melanocytes to make more melanin.

Also, Damaged DNA/RNA can stimulate melanogenesis. Evidence from *in vitro* experiments indicates that fragments removed by DNA during excision repair, etc. in melanocytes (or released from UV damaged keratinocytes) also stimulate melanogenesis.

Variation in Rates, Amounts and Types of Melanin Produced in response to UV
Epidemiology: fairer skin types more prone to UV induced skin cancers.

Fairer skin type skin contains less eumelanin - needed for photoprotection. Less is produced in response to UV.

One reason is genetic variation in the receptor for MSH – the melanocortin 1 receptor (MC1R). There are more than 20 known MC1R? gene polymorphisms. These can affect: Efficiency of response to αMSH (melanosome activity). Type of melanin produced in response to UV. Result is variation photoprotection.

Efficient Immunosurveillance is Essential for Defence Against Skin Cancer
An efficient immune system is important for defeating skin and other tumours early in their development. In individuals who are immunocompetent many very early cancers are probably resolved (unknown to and undetected by us) very early by our own immune systems.

In patients who are immunosuppressed - either through long term immunosuppressive therapy (e.g. transplant patients or patients with severe psoriasis) or through disease (e.g. HIV infection); there is an increased occurrence of keratinocyte-derived skin tumours on sun-exposed sites.

Immunomodulatory Effects of UV Light
UVA and UVB light can affect the expression of genes that are involved in various aspects of normal skin immunity – resulting in reduced skin immunocompetence.
This is the basis of some forms of UV phototherapy for treatment of some inflammatory skin diseases (e.g. psoriasis).

UV exposure: Increases expression of some down-regulatory cytokines such as interleukin 1Ra - suppresses Langerhans cell activity. Inhibits expression of adhesion molecules such as ICAM-1 (inflammatory cell adhesion molecule 1) decrease the migration of T cells, etc. into the skin. Depletes the number of Langerhans cells in the epidermis, decreasing epidermal immunosurveillance.

UV-induced immunosuppression combined with the ability of UVB to cause cancer-generating mutations further increases the cancer-causing potential of sun exposure.

Programmed Cell Death – APOPTOSIS
Mutations leading to failure of normal PCD are generally considered to be one of the most important events in triggering transformation of a healthy cell into a cancer cell. Cells that are damaged beyond repair by normal mechanisms (e.g. by UVB) usually undergo PCD. It is an important pathway by which the immune system kills “unusual” (e.g. cancer) cells.

PCD is a normal part of our biology. It is a process by which cells are renewed or replaced and by which organs are shaped during development.

In the normal epidermis, the end stage of keratinocyte differentiation is programmed cell death.

What is Programmed Cell Death?
This is not the same as necrosis! The apoptotic cell undergoes a series of defined steps – a specific biochemical programme is activated.

Trigger Types
1. Severe DNA or protein damage
2. Withdrawal of survival/factors
3. Binding of specific ligands to specific cell surface receptors (e.g. Fas/Fas ligand) also known as CD95/CD95 ligand)

These events can all trigger a cascade of intracellular signals that initiate the programme. Mutations which affect genes involved in or in responding to any of these trigger types can result in failure of a cell to follow the apoptosis programme.

The Programme
Apoptosis is characterised by cytoskeletal disruption, cell shrinkage & formation of apoptotic cell envelope, cell membrane blebbing, nuclear fragmentation – DNA broken down into oligonucleosome fragments. Apoptosed cell phagocytosed by surrounding cells or breaks up into apoptotic cell bodies. There is no inflammatory response.

Necrosis is characterised by changes that include membrane damage, cell swelling, release of cell contents; all of which are combined with an inflammatory response.

SUNBURN – UV leads to keratinocyte cell apoptosis "Sunburn Cells" are apoptotic cells in UV overexposed skin. Apoptosis removes UV damaged cells in the skin which otherwise might become cancer cells. How?

> **UVA** causes formation of free radicals: oxygen or superoxide anions these can damage mitochondrial membranes. This damage triggers PCD.
>
> **UVB** induced DNA damage stimulates apoptosis: e.g. DNA damage activates transcription factor p53 and AP1. p53 stimulates expression of the Bax gene which initiates the PCD pathway. AP1 stimulates expression of Fas ligand. FasL binds to Fas which initiates PCD.

Mutations in p53 have been associated with keratinocyte cancers – failure of p53 expression results in loss of one DNA damage-activated pathway, damaged/mutated & potentially cancerous cells may survive!

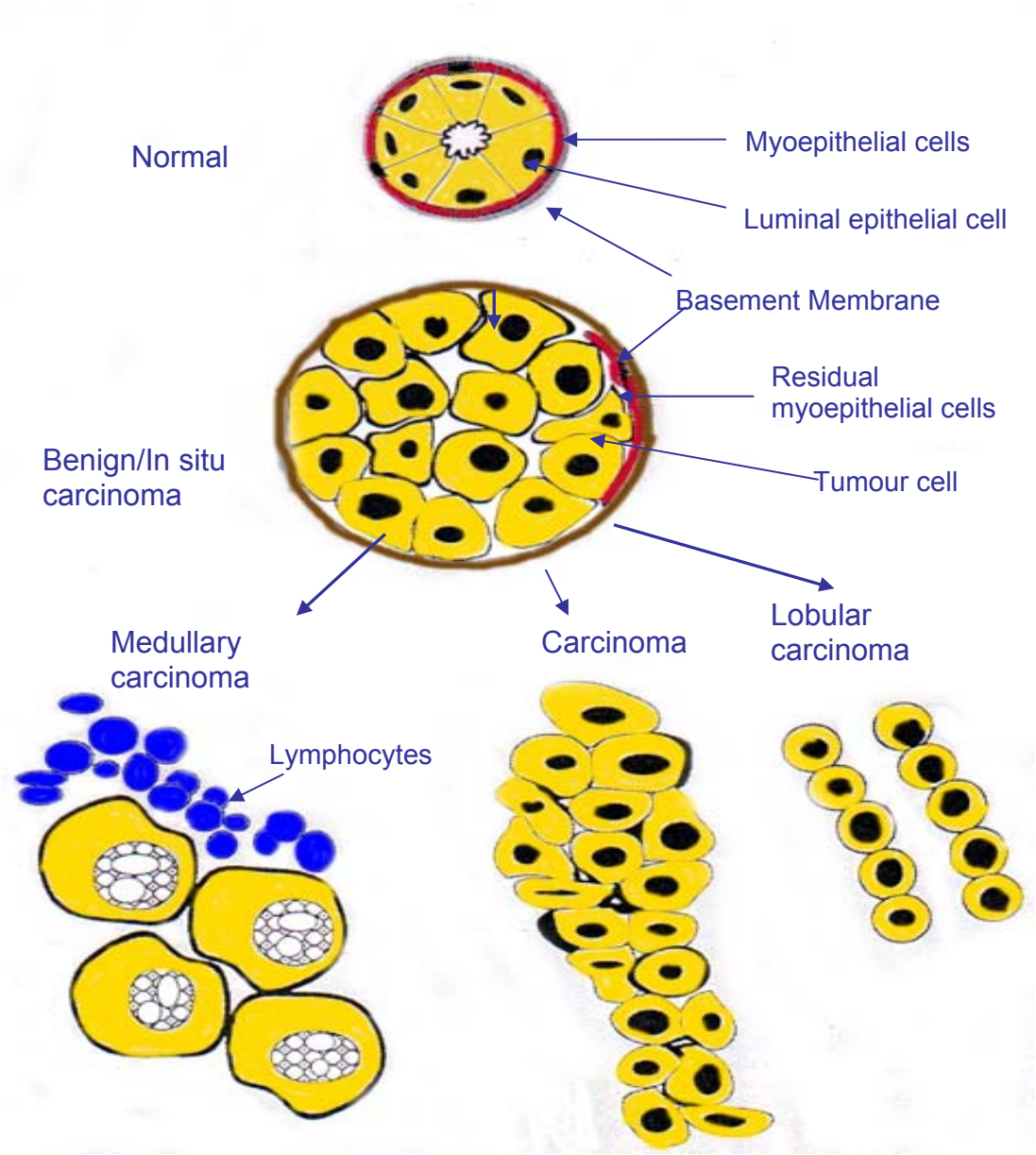
Mutations which lead to Fas L overexpression has been found in malignant melanoma and other cancers. Expression of FasL enables the malignant cells to avoid immune attack: The FasL binds to Fas-bearing lymphocytes triggering lymphocyte PCD.

CANCER 14

CANCER AS A DISEASE – BREAST CANCER

Dr Laki Buluwela

Schematic diagram of the progression of normal to malignant breast



Breast Cancer Growth is Estrogen-Regulated
1870s: A. Schinzinger noted that atrophy of the breast follows cessation of ovarian function and proposed ovariectomy as a treatment for breast cancer

1886: George Beatson demonstrated that ovariectomy in pre-menopausal women resulted in disease regression and improved prognosis.

Subsequent studies confirmed that ovarian hormones stimulate breast cancer development and identified estrogen as the hormone responsible.

Important risk factors include

- lifetime of exposure to estrogens:
- age of onset of menarche,
- age to first full-term pregnancy,
- some contraceptive pills,
- some hormone-replacement therapies

Further studies have elucidated the mechanisms by which estrogen synthesis is regulated.

Oestrogen and Receptor (ER) in Breast Cancer

Some breast cancers like normal breast, are sensitive to the effects of oestrogen.

Approximately one-third of premenopausal women with advanced breast cancer will respond to oophorectomy. Paradoxically, breast cancer in postmenopausal women responds to high-dose therapy with synthetic estrogens *ie* causes breast tumour regression,

ER is over expressed in around 50% of breast cancers. Presence is indicative of a better prognosis.

In ER-positive case,oestrogen regulates the expression of genes involved in cellular proliferation leading to breast cancer.

Oestrogen withdrawal or competition for binding to the ER using anti-oestrogens results in a response in about 70% of ER-positive cancers, 5-10% of ER-negative cancers also respond.

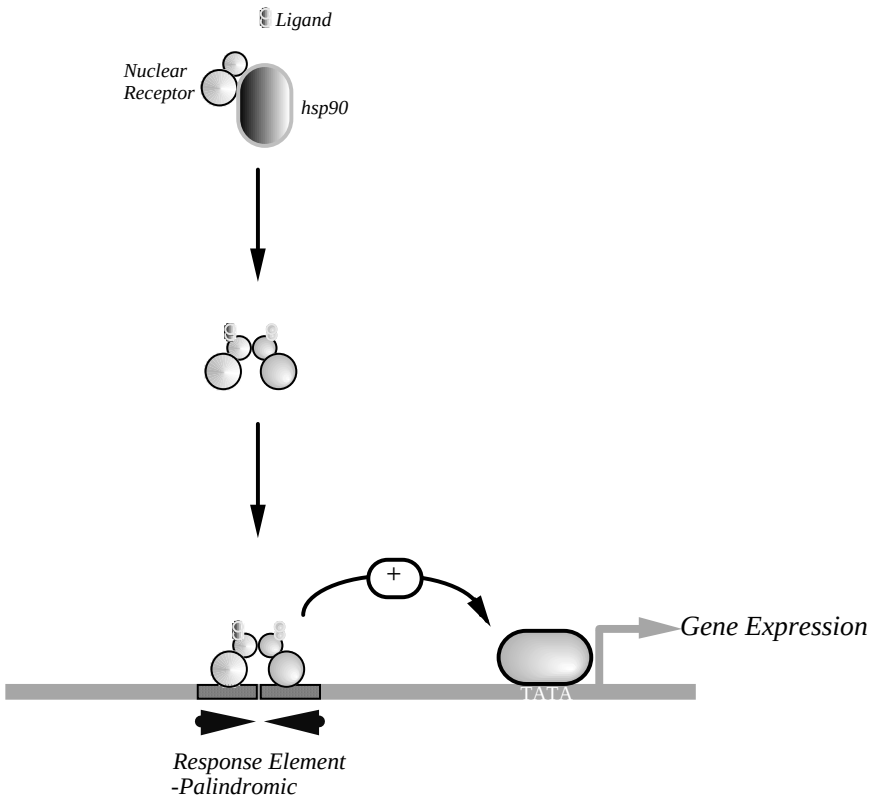
An increased level of expression of ER indicates a good prognosis in female breast cancer but a worse prognosis in male breast cancer

Oestrogen receptor

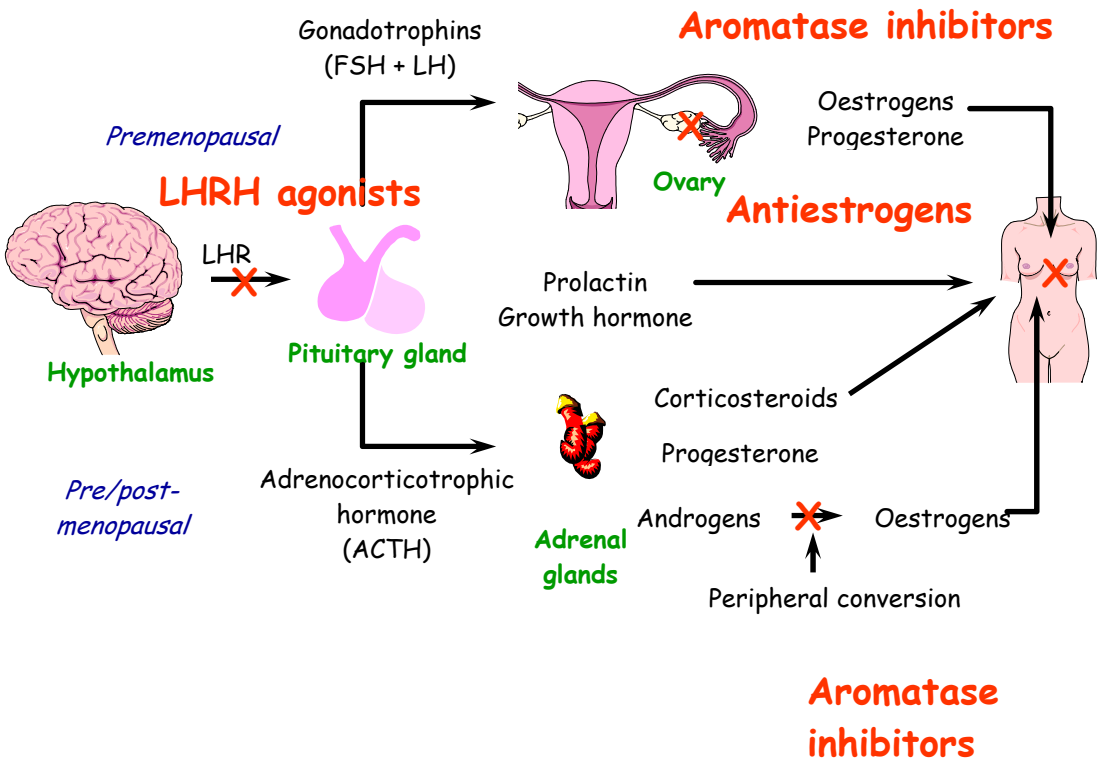
The Oestrogen Receptor is Activated upon binding Oestrogen

Gene Expression is Induced by Binding to Specific DNA Sequences called Oestrogen Response Elements

The Oestrogen-Induced Gene Products Increase Cell Proliferation, Resulting in Breast Cancer



Targets for Breast Cancer Treatment

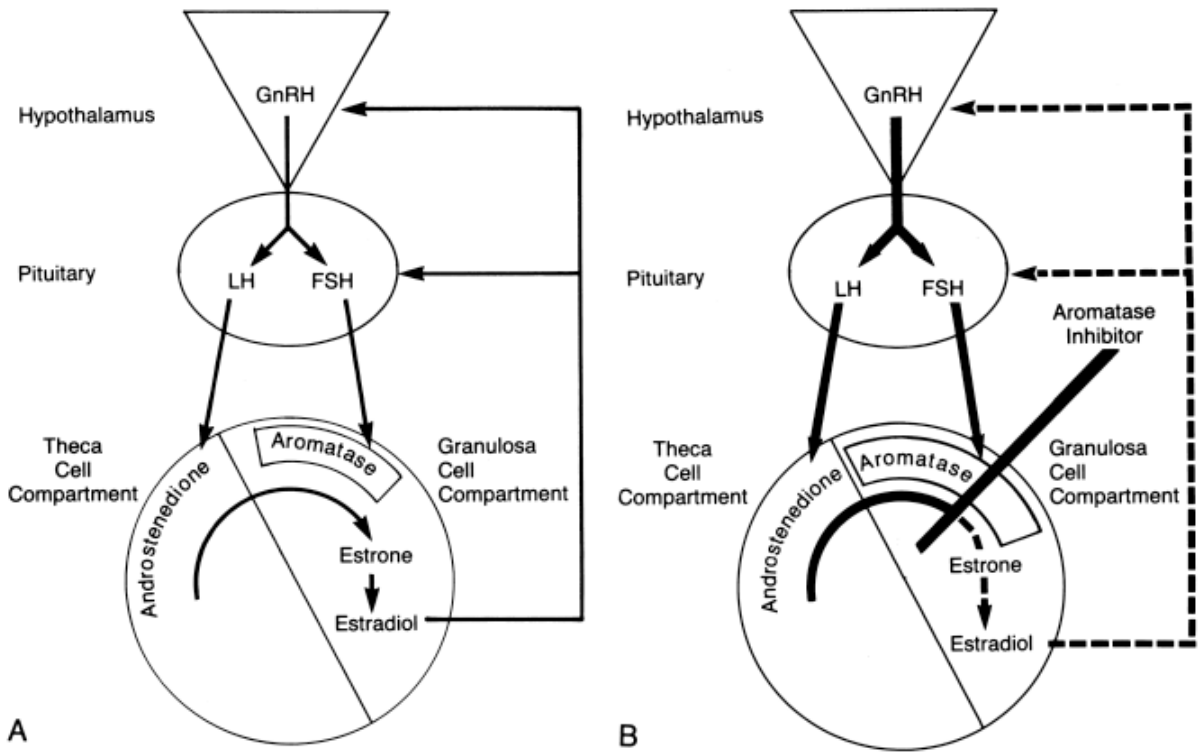


Estrogen Target Tissues & Tamoxifen

TAMOXIFEN	ESTROGEN'S DESIRABLE EFFECTS	ESTROGEN'S NEGATIVE EFFECTS	TAMOXIFEN
BREAST Reduces breast cancer **LIVER & HEART** Lowers cholesterol, reduces atherosclerosis and heart attacks **BONE** Maintains density to help prevent bone loss	**BRAIN** Improves cognitive function **BREAST** Programs glands to produce milk **LIVER & HEART** Lowers cholesterol, reduces atherosclerosis and heart attacks **UTERUS** Programs uterus to nourish a foetus **BONE** Maintains density to help prevent bone loss	**BREAST** Promotes breast cancer **LIVER** Increases thromboembolism **UTERUS** Promotes endometrial cancer	**HYPOTHALAMUS** Increases vasomotor symptoms **EYE** Increases cataracts **LIVER** Increases thromboembolism **UTERUS** Promotes endometrial cancer, fibroids, polyps & vaginal discharge

Aromatase Inhibitors in Breast Carcinoma

- In postmenopausal women, the major source of estrogen derives not from the ovaries but from the conversion of the adrenal hormones androstenedione and, to a lesser extent, testosterone to estrone.
- This enzymatic conversion occurs at extra-adrenal or peripheral sites such as fat, liver, and muscle.
- This conversion is catalyzed by the aromatase enzyme complex.



Progestins in Breast Cancer

- Progesterone is the dominant naturally occurring progestin
- Progestin response in the human breast is complex and influences both proliferation and differentiated function.
- Progestins are used in the endocrine treatment of uterine and breast cancer with clinically proven antineoplastic properties.
- The poor absorption of progesterone has been overcome with some of the synthetic derivative progestins.
- Progestin therapy for metastatic breast cancer has been used principally as a second- or third-line therapy following selective estrogen.
- The principal progestin used for metastatic breast cancer has been megestrol acetate

Breast Cancer – Risks and Causes

Established risks include:

- Age
- Family History
- Early age of menarche
- Late Menopause
- Having no children, or children late in life
- A history of benign disease
- Lobular Carcinoma in-situ

Possible risks include:

- Contraceptive pill
- HRT
- Diet
- Weight
- Alcohol
- Being tall

Patient History of Breast Cancer

- "Lump" detected by Self Examination or GP
- Referred to Hospital
- Examined by surgical team (mammogram, FNA)
- Surgery performed (lumpectomy/mastectomy)
- Tumour examined pathologically (ER/PR)
- ER^+ (90%) or ER^-
- See Physician for first time
- ER^+ Tamoxifen (5 years) or ER^- Chemotherapy (6 months)
- Disease-free period
- Patient returns with secondary tumour (no cure)

References

http://www.cancerresearchuk.org/
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An introduction to the Cellular and Molecular Basis of Cancer - 3rd Edition.
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IMMUNOLOGY 3

HYPERSENSITIVITY & ALLERGY

Professor Sebastian L Johnston
Department of Respiratory Medicine, National Heart and Lung Institute

Learning objectives

By the end of this lecture you should be able to:

- Outline the mechanisms by which IgE, antibodies, immune complexes and T cells can cause tissue damage and inflammation (the four types of hypersensitivity), giving examples of the clinical syndromes associated with each
- Outline the factors underlying the development of atopic/allergic diseases
- Describe the important clinical features of asthma, hay fever, allergic eczema and anaphylaxis
- Briefly describe the approach to investigation and management of patients with these disorders

This lecture is intended to give you an overview of the immunopathology of hypersensitivity. The slides will be posted on the intranet.

By the end of the lecture you should understand the mechanisms leading to and the clinical manifestations of hypersensitivity reactions (4 types). These are tissue damaging immune responses to auto or alloantigens:-

- Type 1: Immediate Hypersensitivity
- Type 2: Antibody-dependent Cytotoxic Hypersensitivity
- Type 3: Immune Complex Mediated Hypersensitivity
- Type 4: Delayed Cell Mediated Hypersensitivity

Gell & Coombs

Allergy

Allergy occurs when a damaging immune response develops to an otherwise innocuous foreign substance, the antigen involved is referred to as the allergen.

Most allergic disease is produced by mixed type I and type IV hypersensitivity reactions
Type I is immediate hypersensitivity/IgE- mediated

Type IV is cell mediated chronic inflammation

Atopy is the tendency to produce abnormally high IgE responses to otherwise harmless foreign environmental substances.

ALLERGIC DISEASE IS THE EXPRESSION OF A DISEASE CAUSED BY ATOPY

GENERAL POINTS

- common - 50% of young adult population are atopic, prevalence has increased dramatically and may still be increasing
- severity varies e.g. mild occasional symptoms, severe chronic illness to fatal anaphylactic shock
- risk factors for allergy include genetic and environmental:

Genetic factors

~80% of atopics have family history of allergy (20% of general population)
Polygenic – many genes implicated:
Genes on chromosome 5 (IL-4 gene cluster) implicated in regulation of IgE
Genes on chromosome 11q (IgE receptor) linked to the atopic phenotype

Environmental Factors:	***Atopy***
age	increases in children, peaks in teens, then decreases
gender	commoner in boys in children, females in adults
family size	less in large families
dietary factors	anti-oxidants, fatty acids may protect
infections	early life infections protect
animals	early exposure protects

Expression of disease *requires*

- sensitisation (usually in childhood), then
- exposure to the allergen

Immunopathogenesis:

- Specific IgE produced on first exposure
- IgE binds to Fc receptors on mast cells
- Re-exposure to allergen cross-links IgE with mast cell activation (synthesis of prostaglandins/leukotrienes) and degranulation (histamine and other mediators) produce vasodilation, and permeability of blood vessels with fluid entering mucosal tissues with swelling (oedema), increased mucus secretion (rhinorrhoea, sputum production), neural stimulation (cough, sneezing, itch).
- Chronic inflammation with lymphocyte and eosinophil activation and infiltration

Clinical manifestation

relates to the organ exposed to and responding to the allergen:

Organ	**Disease**	**Route of exposure**
nose	allergic rhinitis	inhaled
bronchi	allergic asthma	inhaled
blood circulation	anaphylactic shock	oral/mucosal contact/inhaled
skin	allergic eczema	skin contact

ALLERGIC RHINITIS

- seasonal e.g. hayfever due to grass/tree pollen
- perennial e.g. house dust mite, animal dander
- often associated with: sinusitis, otitis media, allergic conjunctivitis, asthma

ALLERGIC ASTHMA

Asthma is characterised by: Reversible generalised airway obstruction
Bronchial hyper-responsiveness
Airway inflammation

Asthma is common (4-20% UK population)

Asthma may be: Perennial (eg house dust mite, cat/dog dander)
Seasonal (eg pollens)
Occupational

Onset often in childhood, usually associated with other atopic disorders and family history of allergy

Chronic, variable disease with acute exacerbations

Immunopathogenesis:

Combination of:
acute **mast cell** activation:

- mucosal oedema
- mucous secretion
- smooth muscle contraction

leading to acute, rapidly reversible narrowing of airways.

chronic **TH2 lymphocyte** and **eosinophil** activation:

- inflammatory cellular infiltration
- tissue damage
- epithelial cell shedding
- subepithelial fibrosis
- smooth muscle hypertrophy

- **Factors that may precipitate acute attack of asthma:** Viral infectionAllergen exposureCold air / Exercise
  Irritants
  Emotional stress

ANAPHYLAXIS

Uncommon but potentially fatal.
Most severe and dramatic form of allergy.
Generalised degranulation of IgE-sensitised mast cells and basophils.

Clinical features of anaphylaxis

Organ	**Feature**
Cardiovascular system	Cardiovascular collapse
Respiratory system	Bronchospasm, laryngeal oedema
Skin	Erythema, urticaria, angioedema
Gastrointestinal system	Vomiting, diarrhoea

Common causes of anaphylaxis

Bee and wasp stings ie venom allergy
Food e.g. peanut allergy, shellfish, fruits & veg
Drug allergy e.g. penicillin

Where allergen is introduced directly into the blood e.g. bee sting, the reaction can be almost instantaneous with cardiovascular collapse the predominant feature.

Where the allergen is absorbed through skin or mucosa e.g. peanut allergy, the reaction may develop more slowly, but is still very rapid.

Milder forms of urticaria/angioedema much more common that full anaphylaxis.

Anaphylactoid/anaphylaxis-like reactions are clinically identical to anaphylaxis but the mast cell activation is not due to IgE-mediated allergy. No prior exposure is required. A number of other mast cell triggers may operate instead e.g. complement activation by radiocontrast media, direct stimulation of mast cells by opiates, alcoholic drink constituents or food colours e.g. tartrazine.

The treatment of the acute episode is the same.

FOOD ALLERGY

Less common than is thought, but increasing in prevalence
Common clinical features of food allergy:

- Gut: nausea/vomiting/pain/diarrhoea
- Skin: urticaria (itchy ‘wheal and flare’ type rash)
- Angioedema (soft tissue swelling)

Common allergens implicated include peanuts. Cow’s milk, egg (in young children), nuts, fruit & veg, shellfish

ATOPIC/ALLERGIC ECZEMA

- Chronic itchy skin rash in atopic persons
- Common - in 10% under 2 years of age and 2% of adults
- In infants affects face first then flexures of arms and legs
- 50% clears by 7 years, 90% by late teens
- Complicated by bacterial infection, severe herpes simplex
- 90% have high serum IgE
- House dust mite allergy may be important, food allergy is controversial

INVESTIGATION AND DIAGNOSIS OF ALLERGY

- Careful history is essential
- Immediate hypersensitivity (type I) skin prick tests very useful to confirm presence of atopy and identify possible causative allergens
- Specific IgE measurement (RAST) only necessary if:
  - On antihistamines
  - Extensive skin disease
  - Presence of dermatographism
  - Very young baby
  - Previous anaphylaxis
  - Skin test solution not available
- Challenge with allergen e.g. for food allergy, occupational allergy
- Other tests e.g. total IgE, lung function tests for asthma

APPROACH TO MANAGEMENT OF ALLERGY

Allergen avoidance (difficult even with peanuts!)

Topical treatment:

- Nasal sprays for allergic rhinitis: corticosteroids, cromoglycate
- Inhalers for asthma: β_2-adrenoceptor agonists (reliever), corticosteroids (preventer)
- Corticosteroid cream/ointments for atopic eczema

Oral medication:

- Antihistamines e.g. for allergic rhinitis, urticaria
- Corticosteroids: only for severe disease, avoid long-term use

Medic alert bracelet
Allergen immunotherapy/desensitisation e.g. for bee or wasp allergy

Anaphylaxis:
Adrenaline (epinephrine) IMI 500mcg (0.5mL of 1:1000), repeated at 5 min intervals as necessary. IVI 500mcg (5mL of 1:10,000), by slow injection.
Antihistamines (chlorpheniramine 10-20mg IM or slow IV)
Corticosteroids (hydrocortisone 100-300mg IM/IV)

Anaphylaxis kit (Epipen, 300mcg prefilled adrenaline)

IMMUNOLOGY 4

TRANSPLANTATION

Dr Candice Roufosse Department of Histopathology (Renal Pathology)

OBJECTIVES

To understand which organs can be transplanted, why, and where the transplanted organs come from
To understand some of the ethical and structural/organisational issues surrounding organ transplantation
To understand the immunological issues in transplantation and their impact on organ allocation and rejection after transplantation (including the main types of transplant rejection)
To understand the risks and complications associated with transplantation and transplant immunosuppression

STRUCTURE OF THE LECTURE

- Which organs can we transplant?
- Why do we transplant them?
- Where do the organs come from?
- Clinical practice of transplantation
    - Transplantation programme
    - Transplant immunology and immunosuppression
    - Complications of transplantation

Which organs can we transplant?

- Autografts
    - within the same individual
- Isografts
    - between genetically identical individuals of the same species
- Allografts
    - between different individuals of the same species
- Xenografts
    - between individuals of different species

<u>Autografts</u>

- Hair
- Skin
- Teeth
- Arteries and veins
- Pericardium and fascia
- Bone and cartilage
- Tendons
- Nerves
- Bowel
- Parathyroid
- Bone marrow
- ?Stem cell derived organs

Xenografts
- Skin
- Heart valves
- Bone and cartilage

Allografts
Solid organ
  - kidney
  - liver
  - heart
  - lung
  - pancreas
  - small bowel
- Free cellular grafts
  - bone marrow stem cells
  - pancreatic islets
- Privileged sites
  - cornea
- Temporary
  - blood transfusion
  - skin
- Framework for new host tissue
  - bone and cartilage
  - tendon and fascia
  - nerve

Transplantation in the UK, 2008-09

	Deceased donor	Live donor
Kidney	1570	927
Liver	667	34
Heart	130	
Lung	143	
Heart and lung	3	
Pancreas	216	
TOTAL	2552	961

Why do we transplant them?
Organ failure

Organ vital for survival, no long-term replacement therapy: lung, heart, liver

Organ transplantation has benefits over other replacement therapy, including prolonged survival: kidney, bone marrow, pancreas

Organ not vital but improved quality of life: cornea, reconstructive surgery

For example: Kidney transplantation
- Quality of life on renal replacement therapy
  - "Time trade-off" method

0 = death; 1 = perfect health
- dialysis patients average score: 0.41
- transplant patient average score: 0.74
- 27% increase in employment rate (38% in males)

Where do the organs come from?

The deceased donor
Heart-beating (donor after brain death DBD) – brain dead (usually intracranial haemorrage or head injury), organs perfused until last minute by life support (ventilation)

- Stringent criteria
  - irremediable structural brain damage - KNOWN cause
  - apnoeic coma NOT due to
    - depressant drugs
    - metabolic or endocrine disturbance
    - hypothermia
    - neuromuscular blockers
  - demonstrate lack of brain stem function
    - pupils both fixed to light
    - corneal reflex absent
    - no eye movements with cold caloric test
    - no cranial nerve motor responses
    - no gag reflex
    - no respiratory movements on disconnection (with $PaCO_2$>50 mmHg)

Non-heart beating (donor after cardiac death DCD) – in addition to brain, heart has also stopped for variable length of time before organs are harvested (leads to delayed graft function after transplantation related to ischaemic acute tubular injury)

Removed organs rapidly cooled and perfused
- absolute maximum cold ischaemia time for kidney 60h (ideally <24h)
- much shorter for other organs
- except cornea 96hr (longer with cryopreservation)

Exclude:
- viral infection (HIV, HBV, HCV)
- malignancy
- drug abuse, overdose or poison
- disease of the transplanted organ

"Supply" of organs for transplantation
- The increasing shortage of organs relative to need: mainly a problem for kidney, lungs, and combined heart/lungs
- Cadaveric donor rate (pmp) differs by country

Strategies for reducing the shortfall
- Decrease the demand for organs (improve preventative and interventional therapy)
- Increase cadaveric donation – Organ Donation Tackforce 2008
  - Review of the role of the transplant co-ordinator
  - donor card campaigns
  - public information
  - "presumed consent"?

- Look for other sources of organs ("marginal donors" (>60, hypertension, smokers,…), high immunological risk transplantation, develop paired exchange programmes, xenotransplantation?stem cell biology?)
- Optimise use of currently available organs
  - improve the half-life of an allograft = decrease need for re-transplantation
    - improved surgical preparation/storage
    - improved organ sharing
    - improved immunological work-up
    - improved immunosuppression
      - recent burgeoning of number of drugs available

<u>Live organ donation (kidney and liver)</u>
- Live-related
- Live-unrelated/emotionally related
- Altruistic donation

Risks of Live Donation (figures for kidney donation)
- Risk of perioperative mortality - 0.03% - 0.06%
  - Comparable with the risk in USA of dying in a road traffic accident in one year (0.02%)
  - There have been at least 2 perioperative donor deaths in the UK
- Perioperative morbidity
  - major perioperative complication rate: c.2%

- Late mortality - unilateral nephrectomy in healthy individuals does not have an adverse affect (up to 45 years of follow-up)
- Associated with asymptomatic, non-progressive proteinuria in 25-35% cases
- No convincing evidence of increased risk of hypertension
- Long term renal function – c 75% of normal.

THE CLINICAL PRACTICE OF TRANSPLANTATION

Pre-transplantation management
Waiting list
Immunological investigations
Other investigations
Transplantation surgery
Post-transplantation management

<u>Immunosuppression</u>
- Induction agents
- Corticosteroids (current tendency for reduction of steroids)
  PLUS
- Other immunosuppressive drugs

<u>Complications of transplantation</u>
- Rejection
- Infection
  - early period
    - typical post-operative bacterial infection
    - oropharyngeal candidiasis
    - aspergillosis
  - medium term
    - CMV (after 1-2 months) - seronegative recipients should have prophylaxis

        - pneumocystis
        - tuberculosis
- Drug side effects
- Malignancy
    - UV-induced skin cancer
    - post-transplant lymphoproliferative disease (PTCLD; mostly B cell, EBV-driven)

<u>Why a graft fails</u>

- Surgical complications
- Bad quality organ
- Rejection
- Recurrence of original disease
- Others: infection, drug toxicity, bad vascular supply, ureteric obstruction etc.

<u>Rejection</u>

Recognition and destruction by recipient immune system (immune-mediated damage)

Acute cellular rejection (T-cell mediated)
Acute antibody-mediated rejection (antibody-mediated, B cells)

Gold standard for diagnosis of rejection is the biopsy of the transplanted organ (easy for kidneys, more problematic for other organs)

Hyperacute rejection

- Caused by pre-existing antibodies
- Historical sensitization: the patient has “seen” the antigen before
    - previous transplant
    - previous transfusion
    - pregnancy
- Bind to graft endothelium in minutes
- Destroys graft in hours
- Every patient screened by direct crossmatch

Acute rejection

T or B-cell mediated, or both

Chronic rejection

T or B-cell mediated, or both

RENAL TRANSPLANTATION

- Matching
    - ABO blood group matching
    - HLA matching
- 60-80% kidneys produce urine “on the table”
    - others have “delayed graft function”
- 10-25% suffer acute rejection
    - usually reversible
- Monitoring for good blood and urine flow and rejection
    - serum creatinine
    - DTPA perfusion scan/ultrasound
    - serial renal biopsy
- Outcome
    - 5-year graft survival rate currently around 89% (living) – 83% (deceased)
    - 10-year survival around 60-70%
    - current “life-expectancy” of first kidney: 11yr

    - in ideal conditions, could be up to 40 yr

CORNEA TRANSPLANTATION

- Supply and demand
    - most patients never get on waiting list, but corneas are “ordered”
- Cornea is “immunologically privileged site”
    - immunosuppression not required
    - rejection does occur
- Matching
    - historically not considered important
- Outcome
    - first graft: c75% 5 year survival

LIVER TRANSPLANTATION

- Supply and demand
    - relatively well matched

- Options for transplantation
    - Orthotopic transplantation - replacing the diseased liver
    - Heterotopic transplantation - placing a new liver in a different place (right subhepatic region)
    - Live lobe transplantation

- Organ-specific issues
    - surgical technique is very difficult and complications are common
    - thrombosis of hepatic artery or portal vein may occur
    - haemorrhage is very common
- Matching
    - ABO blood group
    - size
    - HLA matching controversial and not practical

- Rejection
    - liver is less aggressively rejected than many other organs
- Outcome

3 year survival:	cirrhosis	69%
	emergency	56%
	cancer	39%

HEART TRANSPLANTATION

- Supply and demand
    - Short-fall of supply
    - predicted demand: 20-60/million population
    - current supply: 6/million in UK
- Matching
    - appropriate blood group
    - size
    - ?HLA matching
- Rejection
    - only reliable way to detect is regular endomyocardial biopsy
- Outcome
    - 85-90% 1 year survival
    - 75% 5 year survival
    - 50% live 10-12 years

    - functional rehabilitation excellent

LUNG TRANSPLANTATION

- Supply and demand
    - approx 200 suitable donors per year in UK
    - 111 on heart/lung waiting list and 205 on lung waiting list at 31.12.2000
- Options for transplantation
    - heart-lung transplant
    - single lung transplant
    - sequential double lung transplant
- Matching
    - ABO match
    - size
- Outcome
    - for heart-lung and single lung transplant
        - 80% 1 year survival
        - 50% 5 year survival
    - less good for double lung transplant

CONCLUSIONS

Transplantation is:

- highly successful
- potentially life-saving
- cost-effective

The major problems are the lack of donor organs and the immunological rejection of transplanted organs.

CANCER 15

CANCER AS A DISEASE - LEUKAEMIA

Professor Barbara Bain

LEARNING OBJECTIVES

The student should be able to

- Explain what “leukaemia” is
- Explain the difference between lymphoid and myeloid leukaemias and between acute and chronic leukaemias
- Outline the clinical and haematological features and representative cytogenetic and molecular genetic abnormalities of acute lymphoblastic leukaemia

“Leukaemia” was first described, almost simultaneously, in Edinburgh and Berlin, in 1845. The striking abnormality that was noted when the blood was examined after the patients’ deaths was the increase of “white corpuscles” in the blood. This gave rise shortly afterwards to the term “leukaemia”, derived from the Greek words for “white” and “blood”. We now know that leukaemia is essentially a bone marrow disease and overspill of the abnormal cells into the blood, producing “white blood”, is not essential for this diagnosis.

Leukaemia is a neoplasm or cancer arising as a result of mutation in a precursor of myeloid or lymphoid cells. In this context, “myeloid” may include not only the precursors of granulocytes and monocytes but also cells of erythroid and megakaryocyte lineages. Leukaemia differs from many other cancers in that haemopoietic and lymphoid stem cells usually circulate in the blood stream and migrate into various tissues. Mature lymphocytes, granulocytes and monocytes also normally enter tissues. It is difficult to apply the concepts of local invasion and metastasis to populations of cells that are normally mobile. The formation of localized tumour masses is also not inevitable in leukaemia, at least not in the earlier stages of the disease. We have to look at other characteristics of this disease to understand that leukaemia is a type of cancer.

The terms “malignant” and “benign” are not usually applied to leukaemias. However there are two terms that describe the clinical behaviour of different types of leukaemia that show a greater or lesser degree of malignancy; these are “acute and “chronic”. They describe the natural history of leukaemia in the absence of effective treatment.

An acute leukaemia is one that, if untreated, has profound pathological effects and leads to death in a matter of days, weeks or months.

A chronic leukaemia is one that causes less impairment of function of normal tissues and, although it will eventually lead to death, this usually does not occur for a number of years. Leukaemias can thus be acute or chronic, lymphoid or myeloid, permitting an oversimplified classification, which will suffice for our purposes.

Table 1

	Acute	Chronic
Lymphoid	Acute lymphoblastic leukaemia	Chronic lymphocytic leukaemia
Myeloid	Acute myeloid leukaemia	Chronic myeloid leukaemia

Leukaemia results from a number of mutations occurring in a primitive cell that, as a result, has a growth or survival advantage over normal cells that have not undergone mutation. That single cell gives rise to a clone that steadily replaces normal cells. The mutations concerned are in proto-oncogenes (also known as oncogenes) and sometimes also in tumour suppressor genes.

The types of mutations in oncogenes known to contribute to leukaemias include:
(i) point mutations;
(ii) internal tandem duplication of parts of genes;
(iii) formation of fusion genes;
(iv) dysregulation when a gene comes under the control of the promoter or enhancer of another gene;
(v) somatic hypermutation subsequent to a translocation.

In addition, gene expression may be abnormal as a result of demethylation.
The types of changes that can occur in tumour suppressor genes include deletion of the gene and inactivation through mutation. The abnormal behaviour of the leukaemic clone may include growth that occurs without a dependence on growth factors, continued proliferation without maturation, and a failure to undergo normal cell death or apoptosis.

Why does leukaemia occur? Mutations occur in human genes. Mutations in germ cells may be beneficial, neutral or harmful; beneficial germline mutations permit the species to evolve. Mutations in somatic cells are rarely useful. They may be neutral or positively harmful, in the latter case sometimes the cell just dies but sometimes the mutation leads to leukaemia or other cancer. Sometimes there is an identifiable cause (Table 2) but often no specific cause can be identified and yet mutation has occurred and leukaemia has developed. This process of mutation in a somatic cell may be the result of undetected exposure to mutagens or it may be a random, spontaneous process. The older a person is the more likely it is that enough spontaneous or induced mutations to have accumulated in a single cell for the cell to expand into a clone, replacing normal cells and behaving in a ‘malignant’ manner.

Acute myeloid leukaemia that occurs in late middle and old age can often be demonstrated to be the result of multiple sequential mutations. Leukaemia may thus be, in part, the result of spontaneous mutations—an inevitable feature of our ability as a species to change and evolve—and, in part, a consequence of exposure to environmental mutagenic influences that increase the rate of mutation considerably above the natural baseline rate.

Somatic mutation starts well before birth, many cases of leukaemia in infants now being known to result from intra-uterine events. In the case of B-lineage lymphoid leukaemias, antigenic stimulation may also be relevant to leukaemogenesis. This normally leads to rearrangement of DNA so that antibodies of greater affinity are produced. If the process goes wrong, a lymphoid stem cell could acquire a malignant phenotype. There is circumstantial evidence for this in the case of B-lineage acute lymphoblastic leukaemia (and also some B-lineage lymphomas).

Table 2 Causes of leukaemia

Acute lymphoblastic	Usually unknown, sometimes mutagenic drugs or exposure to irradiation or chemicals *in utero*; possibly delayed exposure to a common pathogen or pathogens
Acute myeloid	Usually unknown, sometimes irradiation or mutagenic drugs or chemicals (benzene, cigarette smoke)
Chronic myeloid	Usually unknown, rarely irradiation or mutagenic drugs
Chronic lymphoid	Unknown but some families are predisposed

The signs and symptoms of leukaemia mainly result from:

(i) proliferation of abnormal cells, e.g. bone pain, hepatomegaly, splenomegaly and lymphadenopathy (the latter mainly in lymphoid leukaemias)

(ii) loss of function of normal tissues as a result of replacement by leukaemic cells, e.g. loss of bone marrow function leading to anaemia, thrombocytopenia, neutropenia

Obviously, it is the nature of mutations that determines whether a leukaemia is acute or chronic. Acute leukaemias often result from mutation in genes encoding transcription factors with a resultant profound abnormality in the cells ability to mature. However the cells continue to proliferate so that there is an accumulation of primitive cells referred to as blast cells, either lymphoblasts of myeloblasts, as the case may be.

In chronic myeloid leukaemias the mutations often involve constitutive activation of signalling pathways within the cell. Cells can proliferate without needing growth factors. Interaction with stroma may be abnormal and cell survival may be prolonged so that there is a steady expansion of the leukaemic clone. However maturation still occurs and, in the case of myeloid cells, mature end cells are still able to function. The impairment of normal physiological processes is therefore much less than in the acute leukaemia. The mutational events underlying chronic lymphocytic leukaemias are less well understood but they also result in the steady expansion of a clone of cells, in this case functionally useless; eventually there is impaired tissue function as the leukaemic clone replaces normal cells.

Different types of leukaemia differ in their aetiology and also in the nature of the mutational events and thus in the nature of the disturbance in maturation, proliferation or both shown by the leukaemic clone. They also differ in age of onset, clinical and haematological features and prognosis.

Acute lymphoblastic leukaemia

Acute lymphoblastic leukaemia (ALL) is particularly a disease of childhood. Lymphocytes can be B-cells or T-cells so it is not surprising that ALL can be B-lineage (about ¾ of cases) or T-lineage (about ¼ of cases). Nothing is known about the cause of T-lineage ALL. A little bit is known about the cause of B-lineage ALL. Uncommon causes are (i) irradiation or exposure to chemicals *in utero* and (ii)

mutagenic drugs. A much more common postulated mechanism is delayed exposure to an unidentified common pathogen. The evidence for this is an association with smaller family size, earlier birth order, higher socio-economic status and higher incidence in 'green-field' new towns in comparison with overspill new towns.

More is understood about the molecular mechanisms than about the causes.
The oncogenic event in the lymphoid stem cell that gives rise to the leukaemic clone can be (i) formation of a fusion gene (ii) juxtaposition to the promoter of a different gene (iii) dysregulation of a gene by juxtaposition to enhancers of T-cell receptor genes. The net result is that a clone of cells continues to proliferate but cells do not mature or die. The clone thus expands progressively, replacing normal cells.

The pathological effects of clonal expansion are:

(i) The direct effects of the proliferation of the leukaemic cells (lymphadenopathy, hepatomegaly, splenomegaly, thymic enlargement (T-lineage ALL), bone pain, renal enlargement, testicular enlargement, cranial nerve palsies (meningeal infiltration), hyperuricaemia

(ii) The indirect effect of leukaemic cell proliferation, which leads to replacement of normal bone marrow cells by leukaemic cells (causing anaemia, thrombocytopenia, neutropenia).

The clinical features are therefore fatigue, lethargy, pallor, bruising and petechiae, bone pain, abdominal enlargement, lymphadenopathy and fever as a result of infection. Chest radiographs may show gross thymic enlargement (T-lineage ALL). Abdominal imaging may show hepatosplenomegaly and sometimes gross renal enlargement.

Essential investigations include blood count and film, immunophenotyping (to confirm T or B lymphoid cells), bone marrow examination and cytogenetic analysis.
The principles of treatment are:
(i) blood products (red cells, platelets) to correct the effects of bone marrow failure;
(ii) systemic chemotherapy to kill off the cells of the leukaemic clone and permit normal cells to regenerate;
(iii) intrathecal chemotherapy to destroy small numbers of leukaemic cells in the cerebrospinal fluid;
In poor prognosis cases, bone marrow (or other haemopoietic stem cell) transplantation may be needed.

About ¾ of children with ALL can now be cured. b.bain@imperial.ac.uk

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Chest radiograph showing thymic enlargement in T-lineage ALL. Why do you think this is a feature of T-lineage but not B-lineage ALL?

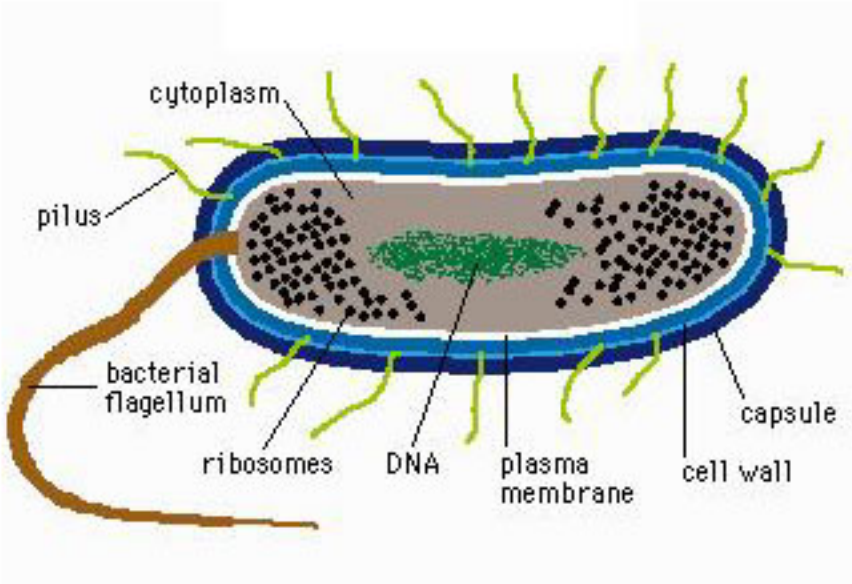
(Figure from Bain BJ, Interactive Hematology Imagebank, Blackwell Science, 1999. This is available to you on the Intranet)

MICROBIOLOGY 1
COMMUNITY INFECTIONS
Dr Angelika Gründling

In this lecture we will cover community acquired **bacterial** infections

<u>We will cover</u>

Composition of a bacterial cell
What is a Gram-positive bacterium
What is a Gram-negative bacterium



<u>We will specifically cover the following bacteria and infections caused by them:</u>

1) Respiratory tract infections
   a. *Legionella pneumophila*
   b. *Mycobacterium tuberculosis*

2) Sexually transmitted infections
   a.) *Chlamydia trachomatis*
   b.) *Neisseria gonorrhoea*
   c.) *Treponema pallidum* (syphilis)

3) Food and water borne diseases
   a) *Bacillus anthracis*
   b) *Vibrio cholera*
   c) *Listeria monocytogenes*
   d) *Shigella*
   e) *Salmonella*
   f) *Escherichia coli*

4) Vector borne diseases

a) *Yersinia pestis* (plague)

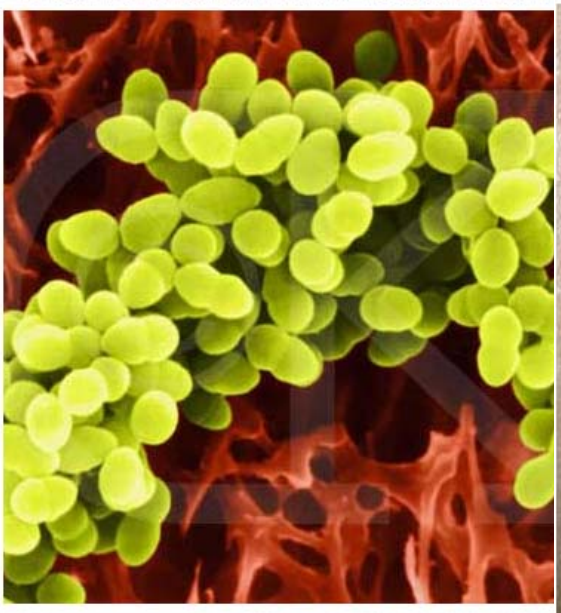
5) Vaccine preventable diseases
   a) *Corynebacterium diphtheria*
   b) *Neisseria meningitidis*
   c) *Streptococcus pneumonia*
   d) *Bordetella pertussis* (pertussis)
   e) *Clostridium tetani* (tetanus)

MICROBIOLOGY 2
HOSPITAL INFECTIONS
Dr Angelika Gründling

In this lecture we will cover hospital acquired **bacterial** infections and define what is considered as hospital acquired infection

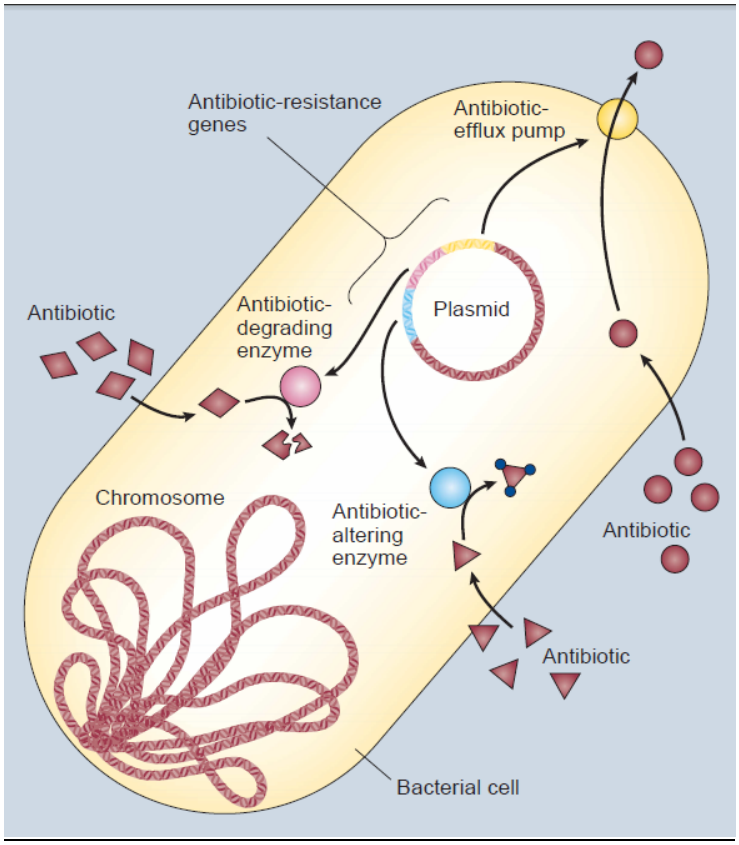
<u>We will specifically cover the following bacteria and infections caused by them</u>

a. *Staphylococcus aureus*
b. *Klebsiella pneumonia*
c. *Pseudomonas aeruginosa*
d. *Enterococcus*
e. *Escherichia coli*
f. *Clostridium difficile*
g. *Acinetobacter baumanii*



We will cover antimicrobial resistance in health care associated infection

Infection in Hospital with resistant organisms is associated with:
- longer hospital stays
- increased healthcare costs
- increased mortality



The Infamous Five for antibiotic resistant infections in the UK

Gram negative
…….Hospital acquired pneumonia, UTI, particularly affects immune compromised hosts (e.g. chemotherapy, individuals with cystic fibrosis). Survives on abiotic surfaces
……..Extended spectrum *b*-lactamase producers. *E. coli, Klebsiella*
…….. ITU infections, Survives on abiotic surfaces

Gram positive
…… colonises skin and nasopharynx, Causes line associated sepsis, urinary tract infections, bloodstream infections, disseminated spread
……commensal of gastrointestinal tract. Causes line and urinary tract infection

Insert: *Enterococcus faecium*, Methicillin Resistant *Staphylococcus aureus*, ESBL, *Pseudomonas aeruginosa, Acinetobacter baumanii)*

MICROBIOLOGY 3

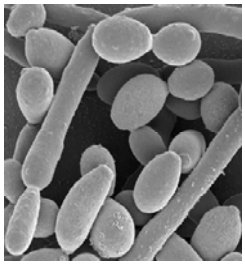
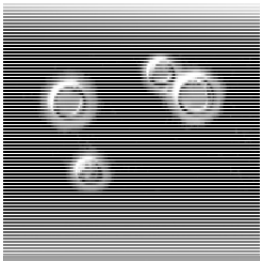
IMMUNITY TO FUNGAL INFECTIONS

Dr Elaine Bignell

Learning objectives:

Following this lecture you should be able to:

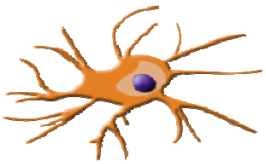
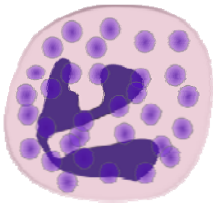
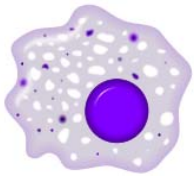
1) Name the major antifungal immune effector cells
2) Describe 2 mechanisms of cell-mediated antifungal defence
3) Summarise the sequence of events leading to effective clearance of infectious fungal cells
4) Understand how immune status determines risk of fungal infection

Questions to consider:

How does the healthy host defend against:

a) Airborne fungal pathogens?
b) Fungal commensals?

Which host cells are important for defence against fungal infection?

How do innate immune responses protect against *Aspergillus fumigatus* infection?

How do innate immune responses protect against *Candida albicans* infection?

How do innate immune responses protect against *Cryptococcus neoformans* infection?

How do the following risk factors promote susceptibility to infection:

a) Immunocompromise
b) Surgery
c) Hereditary defects

What antifungal functions are performed by:

a) Macrophages
b) Neutrophils
c) Dendritic cells

How is adaptive immunity to fungal pathogens orchestrated?

How does adaptive immunity impact the host-commensal relationship?

MICROBIOLOGY 4

VIRAL EVASION OF HOST IMMUNITY

Professor Wendy Barclay

The outcome of virus infection is the result of a 'battle' between the virus's ability to replicate and the host immune response that attempts to control and eliminate virus.

Outline, using examples from named viruses, how viruses escape innate immunity by subversion of host innate immune responses

Innate immunity comprises:

1 Barriers: epithelial surfaces are major points of entry for virus into host. They are covered with protective secretions that contain mucus and collectins which can act as decoy receptors.

Influenza virus neuraminidase can enzymatically remove the decoy receptors from mucus allowing virus to gain access to the 'real' receptor at the cell surface.

2. Interferon response: All cells can make and respond to type I interferons, IFN alpha and IFN beta. These are soluble cytokines that are secreted when the cell detects a foreign pattern. IFN lambda acts at epithelial cells in the same way.

Detection of the virus or other invading pathogens is by pattern recognition receptors, **PRR**s. These can be at the cell surface, for example Toll Like receptors **TLR**s

Or they can be intracellular, for example RIG-I.

The PRRs detect unusual nucleic acids that are nonself because they are different to those made by the host or they are in the wrong place. These are the pathogen associated molecular patterns or **PAMP**s.

When the PAMP is detected, a signaling cascade is set in motion that results in the transcription of the IFN genes.

Newly transcribed and translated IFN is secreted from the infected cells and acts on specific receptors on the surface of the infected cell or neighbouring cells and signals the synthesis of hundreds of new genes that have antiviral effects.

For example:

PKR will shut down protein translation, a host function on which viruses are absolutely dependent.

Mx will bind and nullify incoming virus genomes.

IFITM3 will prevent the entry of enveloped viruses.

Tetherin will prevent the budding of many enveloped viruses.

In addition IFN will recruit other arms of the immune response such as dendritic cells and NK cells.

Because this IFN response is so powerful, many viruses have had to evolve strategies to antagonize it.

For example:

Hepatitis C virus encodes a protease that targets and destroys MAVS, a key protein in the detection pathway.

Influenza A virus NS1 protein binds to RIG-I and stops it seeing the PAMP.

Poxviruses secrete soluble cytokine receptors, vaccinia virus B18, that mop up IFN and stop it from reaching its own receptor.

Interferon can be used as an antiviral treatment. However it stimulates so many aspects of a cytokine and chemokine response that it is associated with side effects like fever and aching. Pegylated IFN is used as a treatment for hepatitis C virus infection.

Describe, with named examples of viruses, how antigenic variation may lead to viral evasion of host immunity

Viruses that cause acute infections are susceptible to **neutralization** by virus specific antibodies which protect against reinfection. Many such viruses escape antibody recognition for example:

Human rhinoviruses that cause the common cold exist as multiple antigenically distinct serotypes
HIV exists as multiple clades
Influenza viruses mutate and evolve to change year on year, **antigenic drift**

Describe with named examples how viruses escape host cellular immune responses

Infected cells can be recognized and destroyed by antigen specific T cells when viral peptides are processed and presented by MHC class I. To avoid this many viruses encode products that intervene with **MHC processing and presentation**. For example

Herpes Simplex Virus HSV ICP47 prevents the loading of peptides in to the transporter protein complex **TAP**.
Human cytomegalovirus US3 binds to MHC class I and prevents it transport to teh cell surface
HCMV US2, US11 and adenovirus E3 gp19 proteins stimulate the MHC to recycle from the cell surface to the cytosol

Although stopping MHC getting to the cell surface is good, it creates another problem for the virus because cells that lack MHC expression are targets for **NK** cell killing.
HCMV encodes an MHC mimic UL18, to stop this.

When viruses lose the proteins that normally control the host immune response, either by passage through cell culture many times (for example strains of HCMV or poxviruses like MVA) or by deliberate genetic engineering (for examples GM strains of influenza virus that lack NS1 protein) they become attenuated and thus suitable for use as vaccines.

MICROBIOLOGY 5 & 6

PARASITIC INFECTIONS
Dr Ingrid Müller

Strictly speaking a parasite is an organism that lives in (or on) (infects) the host and is dependent on it for nutrition, often causing damage/disease. Thus whilst **protozoal and helminthic** infections are commonly referred to as '**parasitic'** infections, viruses, bacteria and fungi are equally so.

Protozoa are single celled organisms and unlike prokaryotes are eukaryotic in that their genome is contained within a nucleus and their cytoplasm contains complex organelles. The pathogenesis (mechanisms by which they cause disease) is varied and many are carried by insect vectors. They **do not** produce eosinophilia in the host. By contrast, helminths are complex multicellular organisms (metazoa) can be free living, transmitted by vectors and their life cycle often involves an intermediate host. Humans are the definitive host. Adult worms lay eggs or produce microfilariae but cannot themselves multiply in man- therefore a cause of morbidity rather than mortality. Forms which invade the blood often cause an eosinophilia.

PROTOZOA

These can be divided into:

1. Amoebae e.g. *Entamoeba histolytica* can cause a bloody form of diarrhoea (dysentery) when it infects the colon and also can lead to abscess formation in the liver. These amoebae can ingest red cells. Other amoebae e.g. *Entamoeba dispar* are non pathogenic.

2. Coccidia
Plasmodium species e.g. *P. falciparum, vivax, ovale* and *malariae (* and most recently *knowlesi)* cause malaria. The life cycle involves the invertebrate (*Anopheles* mosquito) and vertebrate host (man). In man there is both a liver and a blood component to the cycle.
The major disease forms are a febrile illness, cerebral malaria (alteration in conscious level), respiratory distress and severe anaemia.

Toxoplasma is another coccidian protozoa which can cause an illness (toxoplasmosis) in the newborn especially involving the retina in the eye, and in immunosuppressed patients (e.g. HIV infected) affecting the brain. Infection is commonly from animals e.g. kitten faeces or undercooked meat.

Cryptosporidium, another coccidian parasite can infect the small bowel and produces severe diarrhoea. Infection is commonly waterborne. Occurs in the UK.

3. Flagellates
Giardia infects the upper small bowel (jejunum) and leads to giardiasis, a troublesome prolonged diarrhoeal illness, sometimes causing malabsorption. The organism has a ventral sucker by which it attaches to the bowel wall and flagellae which provide motility.

Trypanosoma a flagellated protozoa, transmitted by tsetse flies (often in the vicinity of animals e.g. game parks), lead to a febrile illness with lymphadenopathy and ultimately invades the nervous system leading to alterations in consciousness (sleeping sickness). *Leishmania* are transmitted by sandflies and can lead to a skin or organ (liver, spleen and bone marrow) form of disease (cutaneous and visceral leishmaniasis respectively). Apart

from the tropics, the disease also occurs around the Mediterranean and is associated with HIV infection.

Trichomonas is the commonest protozoal in the UK causing a vaginitis.

4.Ciliates

Balantidium is an unusual pathogen in man causing diarrhoea.

METAZOA (Helminths; worms)

Helminths have developed immune evasion mechanisms which allow them to cause chronic illness. Invasive forms lead to eosinophilia (an increase in eosinophil count- one of the granulocytic white blood cells found in the blood).

1. Round worms (Nematodes)

Ascaris leads to ascariasis in the bowel. Worms can cause obstruction especially of bile duct or if sufficient numbers, the bowel. No intermediate host.

Filaria can cause a variety of diseases. Lymphatic filariasis (elephantiasis), cutaneous filariasis (onchocerciasis) leading to blindness and loaiasis (eyeworm). Transmitted by *Aedes* mosquitoes, blackflies and mango flies, respectively.

Strongyloides is an important worm infestation because the eggs produced by the adults can hatch within the host and invade human tissue leading to severe illness especially when the host is immunocompromised. It is cause of diarrhoeal illness in the tropics.

2. Flat worms (Cestodes)

Taenia infestation (tapeworm) can be from pork (*Taenia solium*) or beef (*Taenia saginata*). The importance of the distinction is that the larvae from the eggs of the pig tapeworm can invade the host tissues especially the brain leading to a condition called cysticercosis- with fits and focal neurological signs (eg weakness).

3. Flukes (Trematodes)

Schistosoma infection leading to bilharzia is the best example. Infection is acquired by contact with freshwater in which the invasive form (cercaria) penetrate the skin. The adult worms live in either the urinary bladder (*S. haematobium*) or bowel (*S. mansoni*) where they lay eggs. The eggs may spread to the liver where they cause an inflammatory reaction leading to fibrosis and obstruction to the portal vein draining the bowel which results in oesophageal varices (dilatation of the veins around the oesophagus) and later haemoptysis (the vomiting of blood).

Conclusion: Parasitic infections are indeed a fascinating and interesting field. They infect much of the world's population and are an increasing problem in travellers (especially malaria and bilharzia). Where the infectious load is low, clinical presentations may be quite different from those depicted in classical textbooks of medicine. Some have a high mortality e.g. malaria whilst others have high morbidity (e.g. hookworm infection is the commonest cause of an iron deficient anaemia worldwide). These diseases inflict a huge economic burden on those who can afford it least i.e. in developing countries. Sadly they are amongst the greatly neglected diseases of mankind.

ROTATIONS

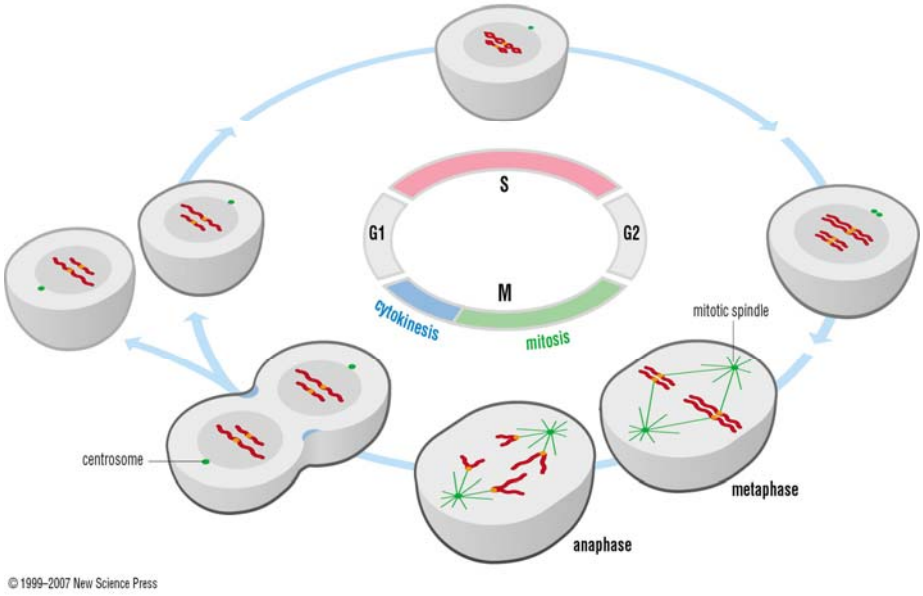
Tutorial 1 – The Cell Cycle and Cancer

Liver as an exemplar

The eukaryotic cell cycle is an exquisitely regulated machinery that mediates cell division and ensures that the replication of DNA is maintained with high fidelity from generation to generation. The cell faces more risk of serious genetic damage during cell division and DNA replication that at any other time. Thus, multiple mechanisms have been established to:

- control entry into and progression through the cell cycle
- monitor the correct sequence and timing of events
- pause the cell cycle if necessary to allow repair of defects
- trigger cell cycle arrest or programmed cell death if the stringent criteria for successful replication and division are not met

From **The Cell Cycle: Principles of Control** by David O Morgan



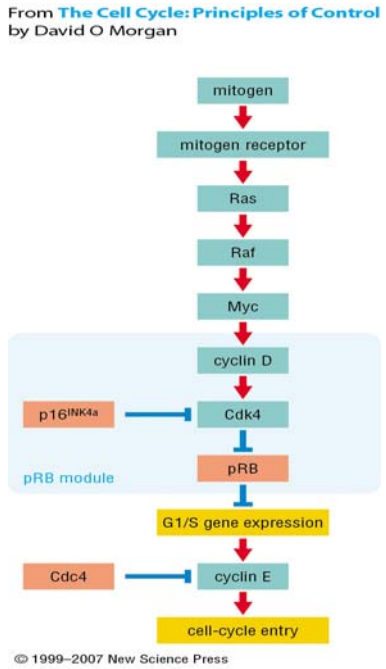
Most cells in the adult human body are not cycling but are in a quiescent state Go. This does not mean they are dormant – they are often highly metabolically active, producing proteins and metabolites that are needed by the cell or elsewhere in the body. However, when they are required to re-enter the cell cycle and multiply, perhaps in response to tissue damage, they are triggered to restart the cell cycle in the G1 phase. An example of this is the proliferation of liver hepatocytes after damage by alcohol, hepatitis virus infection or partial hepatectomy. In this typical case, entry into the cell cycle is stimulated by the presence of growth factors (e.g. hepatocyte growth factor (HGF) and EGF) that activate tyrosine kinase receptors.

Question: How do tyrosine kinase-mediated signals trigger cell cycle entry from Go in mammalian cells, e.g. regenerating liver?

Our understanding of cell cycle regulation has come from studies in many diverse systems such as yeast, sea urchin embryos, flies and mammalian cells. The cell cycle is an almost infinitely variable and controllable system. Perhaps the most obvious difference is between

mitosis in somatic cells and meiosis in germ cells, but even in somatic cells the length of the various phases of the cycle (G1, S, G2, M) can vary enormously. The progression through these phases is controlled by the cyclical activation of the Cdk protein kinases. Cdks activate gene transcription partly via the product of the retinoblastoma gene, pRb.

Question: How is the cyclical activation of the Cdks via pRb proteins achieved?



Another key pathway that controls the cell cycle and cell survival involves PI-3-kinase and its antagonistic phosphatase PTEN; components of this pathway are frequently mutated in hepatocellular cancer. At many points throughout the cell cycle its progression is under the control of multiple pathways acting in concert or opposing each other. Subtle regulation and fine-tuning of many processes in biology is achieved by the balance between opposing activities. In the cell cycle the forward drive provided by Cdk action is opposed by inhibitors such as CKIs (Cip/Kip and INK4) which enable this fine control.

Question: How does activation of the PI-3-kinase pathway occur and how does it affect the cell cycle?

Question: How do cell cycle inhibitors such as Cip/Kip and INK4 control Cdk activity?

The cell cycle is paused at certain key “checkpoints” to allow the monitoring of correct progression, e.g. the correct replication of DNA or the assembly of the spindle pole ready for mitosis. A key checkpoint monitors the fidelity of DNA replication using the p53 protein, a key target in hepatocellular cancer which is functionally attenuated by hepatitis B virus X protein and is a mutational target of the hepatotoxin aflatoxin B1. The same system monitors DNA for damage induced by external agents such as mutagens.

Question: How do cells check for DNA damage and what do they do if they detect it?

Because of the key role of the cell cycle in regulating cell growth components of the machinery are frequently targets of mutation in human cancers. Genes encoding proteins that promote cell growth or survival are common targets of activating mutation in cancers – oncogenes. In contrast, genes encoding proteins that restrain cell growth are frequently mutated to reduce their activity, thus favouring growth – tumour suppressors.

Question: Give examples of proteins controlling the cell cycle that are commonly mutated in cancers.

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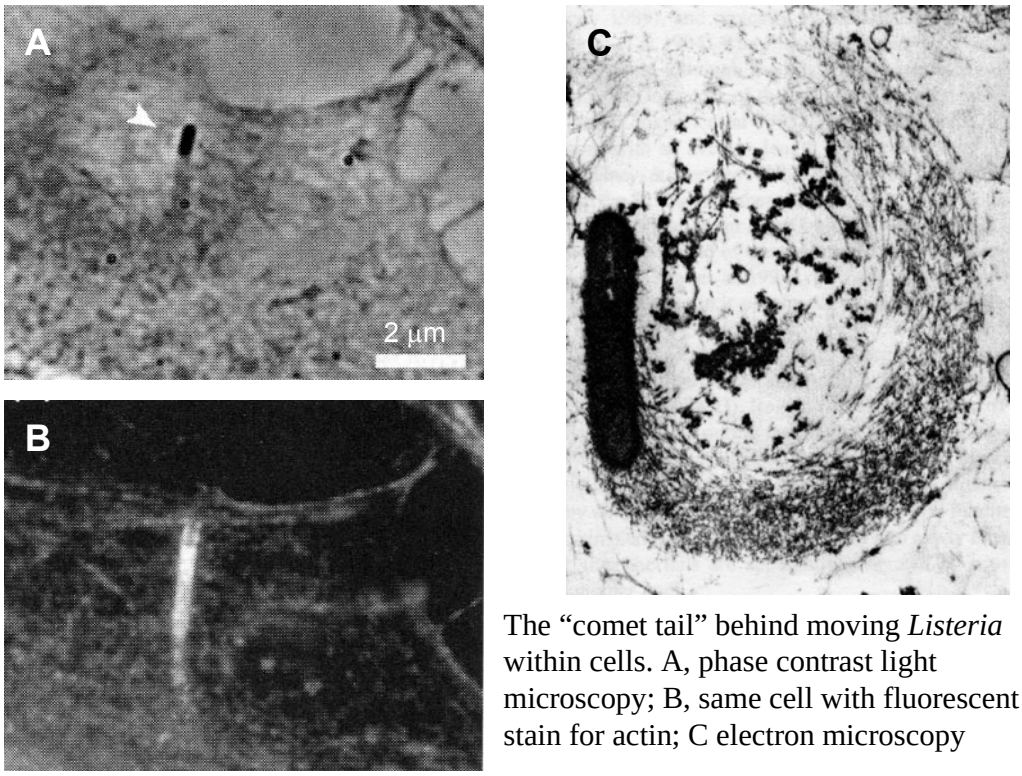
Tutorial 2: Exploitation of the actin cytoskeleton by *Listeria*

Listeria monocytogenes is a bacterium which can contaminate certain foods. In a healthy individual infection is virtually symptomless and quickly contained by the immune system. However in pregnant women infection can lead to problems for the fetus and often miscarriage.

To affect the fetus, *Listeria* must be able to gain access to the blood stream and cross the placenta. Normally bacteria cannot cross epithelial barriers. For example, most bacteria causing food poisoning remain contained within the gut lumen although their toxins can spread elsewhere. In the placenta the lining of the maternal blood space is the syncytiotrophoblast where there are not even clefts between cells, so the only way a bacterium can cross to the fetal side is through the cytoplasm.

This property requires a mechanism for movement within cytoplasm. Without motility the bacteria cannot spread through the infected cell efficiently and cannot exit the cell or invade neighbouring cells. *Listeria* have developed a means of using elements of the host cell cytoskeleton for this purpose.

Individual moving *Listeria* within cells can be seen to have "comet tails" which have been shown by immunofluorescence to contain actin (see figure). Mutant studies have shown that motility is dependent on a bacterial protein called ActA. Mutants in which ActA has been inactivated form microcolonies within individual cells but are unable to spread from cell to cell and are not virulent in animal tests.



The “comet tail” behind moving *Listeria* within cells. A, phase contrast light microscopy; B, same cell with fluorescent stain for actin; C electron microscopy

Sequence analysis of ActA shows that it contains 4 proline-rich repeats flanked by acidic amino acids (aspartate, glutamate) which are homologous to those seen in zyxin, an endogenous protein in host cells. It also contains a region similar (25% homology) with the actin-binding region of vinculin, a protein mediating attachment of actin filaments to the plasma membrane.

Listeria ActA	--SerAspPheProProProProThrAspGluGluLeuArg--
	--PheGluPheProProProProThrAspGluGluLeuArg--
	--PheGluPheProProProProThrGluAspGluLeuGlu--
	--SerAspPheProProLeuProThrGluGluGluLeuAsn--
Human zyxin	--GluAspPheProLeuProProProProLeuAlaGlyAsp--
	--GlyAlaPheProProProProProProLeuGluGluSer--
	--GluSerPheProProAlaProLeuGluGluGluLeuPhe--
	--GluLeuPheProSerProProProProProGluGluGlu—

Zyxin is found in focal contacts of cells where it is associated with actin stress fibres. It binds a protein called VASP (for vasodilator-stimulated phosphoprotein) which in turn binds profilin and Arp complex.

Questions

Where in the "comet tail" do you predict actin monomers are incorporated?

The actin crosslinking protein α-actinin has been found to be present throughout the tail, and experiments have shown it is crucial for bacterial movements. Why do you think this is?

Outline a possible mechanism for *Listeria*'s motility

Where does the energy for movement come from?

What might be the effect on *Listeria* motility of the drug cytochalasin which stabilises monomeric actin?

Background: actin polymerisation dynamics

Nucleation - at least 3 G-actin monomers (each binding ATP or ADP) come together in the right orientation to form a filament nucleus for further elongation. It is slow unless the nucleus is stabilised, e.g. by a capping protein or Arp complex

Elongation - actin monomers add to preexisting filament at either end. Equilibrium differs for the 2 filament ends and according to whether actin binds ATP (favours addition) and ADP (favours loss). ATP hydrolyses to ADP within the filament.

Preferred end for monomer addition (+) Nonpreferred end for monomer addition (–)

Effect of actin binding proteins - proteins binding free monomer (e.g. β-thymosin) decrease polymerisation. Some (e.g. profilin) enhance the normal rate of replacing ADP with ATP on monomers and favour polymerisation. Capping proteins bind to one or other filament end and prevent polymerisation there. Severing proteins cause filaments to split, exposing 2 new ends. Crosslinking proteins bind filaments together, either at right angles (e.g. filamin) or in parallel (e.g. α-actinin)

Tutorial 3 – Prostate cancer

Case scenario
Mr Harold Smith, aged 78, visits his GP at the insistence of his wife. She is fed up of being woken several times every night as he frequently has to get up to go to the lavatory. He admits that once up he has difficulty urinating. He insists he has no other complaints beyond what would be "normal at his age": some back pain and a bit of trouble with his hip.

The GP sends off a blood sample for PSA testing (see below). The result comes back as 15ng PSA/ml serum, indicating possible prostate cancer, and Mr. Smith is referred to an oncology clinic for further tests. On PR (per rectal) examination his prostate is enlarged and irregular. 6 biopsies are taken transrectally and of these, one shows evidence of malignant change and the histopathology report reads: “adenocarcinoma in 20% of the core, Gleason grade 3 + 3, 6”. A bone scan is carried out and no bone lesions are found.

Mr. and Mrs. Smith are keen for him to have surgery. They say an American friend of theirs was diagnosed with prostate cancer aged only 62 and had his prostate removed: since then, he has shown no further sign of the disease. However Mr. Smith is put onto hormone therapy consisting of leuprolide, an LHRH agonist, and flutamide, a steroidal anti-androgen. After a couple of months PSA tests reveal his levels have reduced to 1ng/ml and his urinary symptoms have disappeared. Routine PSA tests are carried out every 4-6 weeks and remain low until a year later, when the PSA levels have risen to 8ng/ml although Mr. Smith has no recurrence of his previous symptoms.

Background

- The prostate is a gland immediately inferior to the bladder in males, responsible for making some of the fluid contents of semen. It completely surrounds the urethra thus hyperplasia for any reason constricts the urethra leading to problems in urination.
- The prostate is androgen-dependent. It requires testosterone for growth, with the main sources being the testis (which in turn is under pituitary control) and to a smaller extent the adrenal glands.
- Prostate cancer is the most common male-specific cancer cancer in the West, affecting 1 in 12 British men. The prevalence of both latent and clinically detected prostate cancer increases dramatically with age: 20% to 30% of men older than 50 years and, perhaps, 50% of men older than 80 years may harbor latent prostate cancer.
- Prostate tumours appear to be heterogenous with respect to oncogenes and tumour suppressors. Two main approaches have been used: analysis of familial forms of the cancer, and identification of chromosomal abnormalities in tumour biopsies. These have indicated a number of chromosomal loci of cancer susceptibility but have not led to an association of the disease with particular oncogenes or tumour suppressors. The clearest relationship is with the androgen signalling pathway. Late stage aggressive prostate cancer frequently becomes androgen-independent.
- Prostate specific antigen (PSA) is a kallikrein protein that is secreted by the epithelial cells of the prostate into the lumen of the duct (see diagram). From here, it joins the seminal fluid and is eventually discharged via the urethra. Under normal circumstances, PSA is prevented by the basement membrane of the gland from leaving the cells by any other route, hence will not be detected in the blood. However, damage to the prostate gland results in PSA leaking into the bloodstream. Here, the concentration can be measured by a simple immunoassay on the serum. A level above 4ng/ml indicates possible prostate disease and PSA can rise to 2000 ng/ml and above in aggressive metastatic disease. Other conditions that result in prostate damage can also cause slight increase in serum PSA levels.

- The Gleason grading system is commonly used in histopathological reporting of prostate cancer. The two largest areas of tumour found are scored 1-5 (1 is least aggressive, 5 is most), and the two scores are quoted plus their sum. Sums in the range 2-4 are considered low grade, 5-7 intermediate, and 8-10 high grade.
- Treatments for prostate cancer range from "watchful waiting" to radical prostatectomy, and also include radiotherapy, chemotherapy and hormone therapy.

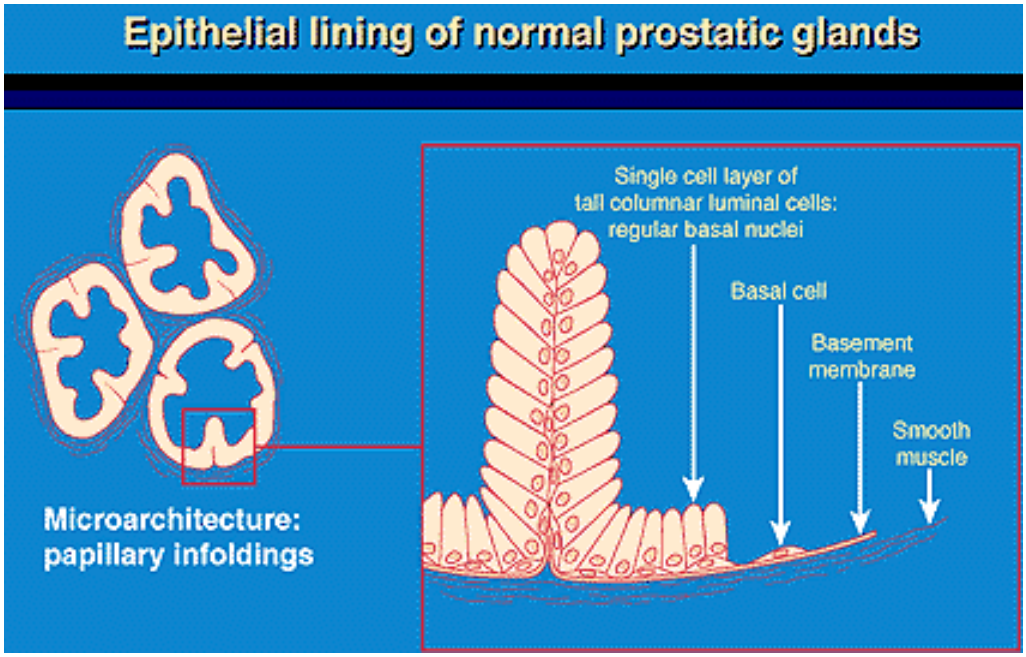


Figure reproduced from http://www.uronet.org/visual/apr97.asp .
The normal prostate is composed of secretory glands embedded in the fibromuscular stroma of the gland. The epithelial surface of these glands consists of tall columnar epithelium beneath which lies a layer of basal cells. These in turn are surrounded by a contiguous basement membrane.

Questions/Discussion points

- Why does the GP send Mr. Smith's blood for PSA testing?
- What could a PSA test result of 15ng/ml indicate?
- Why do you think the oncologist puts Mr. Smith on a course of hormone therapy, rather than recommending surgery? Consider the treatment options for various stages of prostate cancer.
- What is the rationale behind the combination of leuprolide and flutamide given to Mr. Smith?
- What mechanisms could be contributing to the recurrence of Mr. Smith's disease in spite of continued anti-androgen treatment?
- Discuss how we can go about searching for markers of aggressive versus latent prostate cancer.
- There is constant debate about routine PSA testing for men over a certain age to detect early prostate cancer. Can you envisage any potential problems of such a screening programme?

Practical 1

Cancer - Cellular pathology of cancer

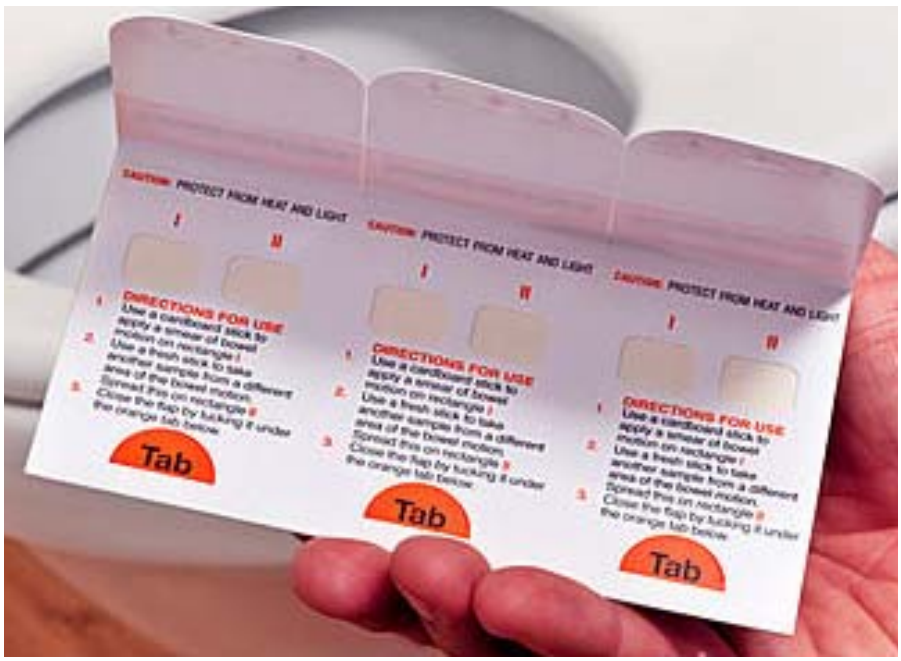
Dr Marjorie Walker

Aim
To show the value of histology to determine **dysplasia**, low grade and high grade (in situ cancer), invasive **cancer** (of different types and **grade**) – adenocarcinoma, squamous carcinoma and demonstrate cancer **staging** – lymph node **metastases,** by examples of cancer that will be discussed in the cancer lectures

Case 1

Mr CC aged 62 has undergone a screening test for bowel cancer, which tested positive.

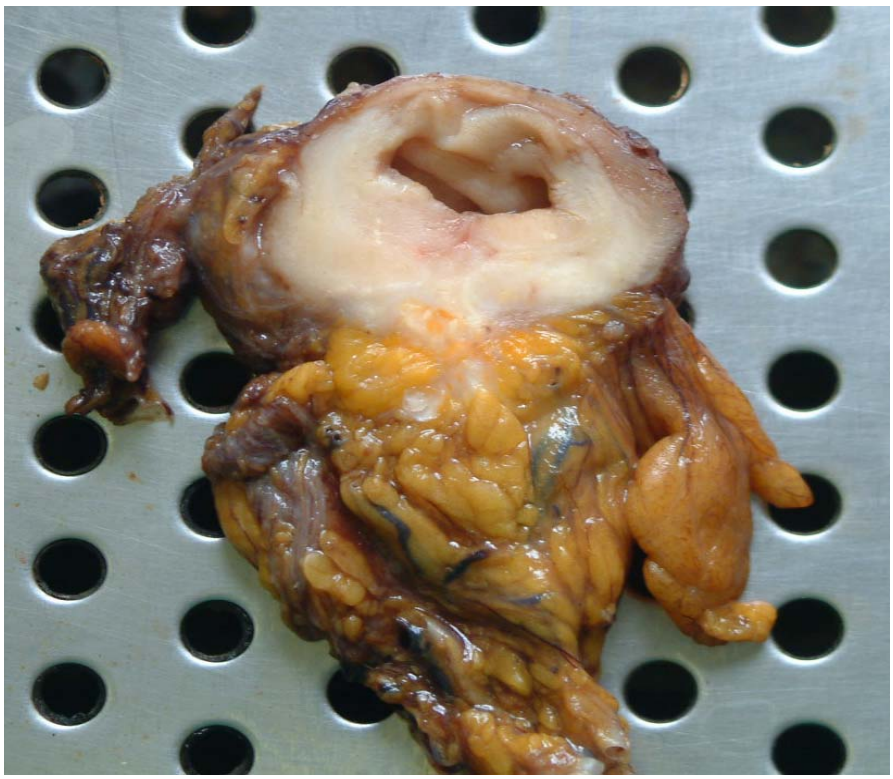
What is the screening test used to detect bowel cancer?



http://www.sciencephoto.com/media/412140/

If bowel cancer is detected at the earliest stage, there is over a 90% chance of survival (Cancer Research UK, 2005. Cancerstats).

His test is positive and he undergoes a colonoscopy which detects a polyp and a cancer. He undergoes a colectomy for removal of the cancer, this is a section of the colon to include the cancer. How far has the tumour invaded through the bowel wall?



Case 1 slide 1
Section of a colonic polyp

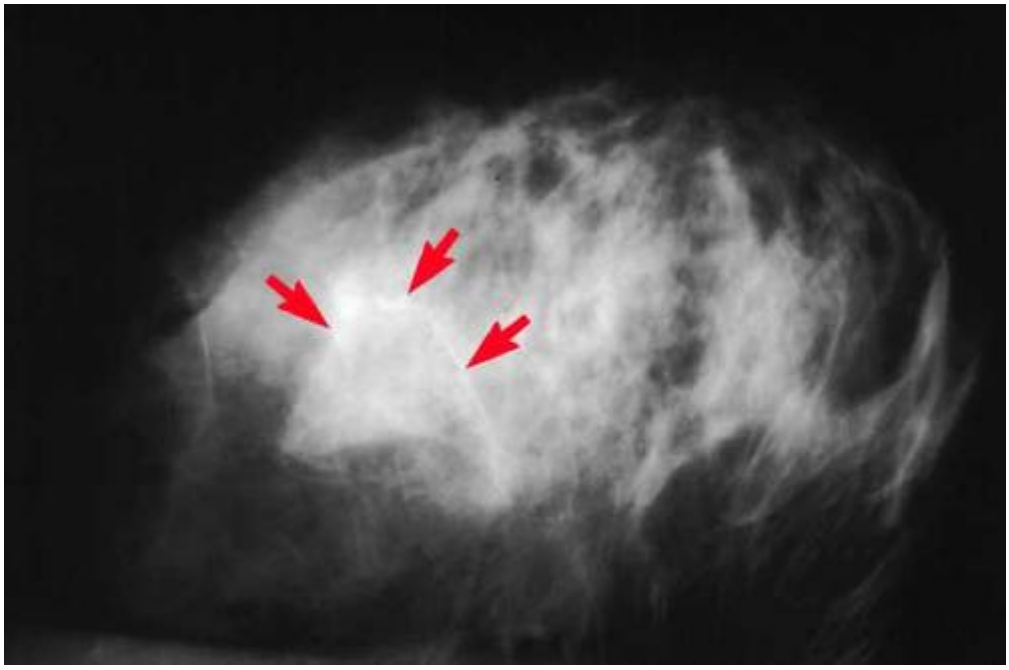
What would the report on this polyp state?

Case 1 slide 2
Section of colon cancer

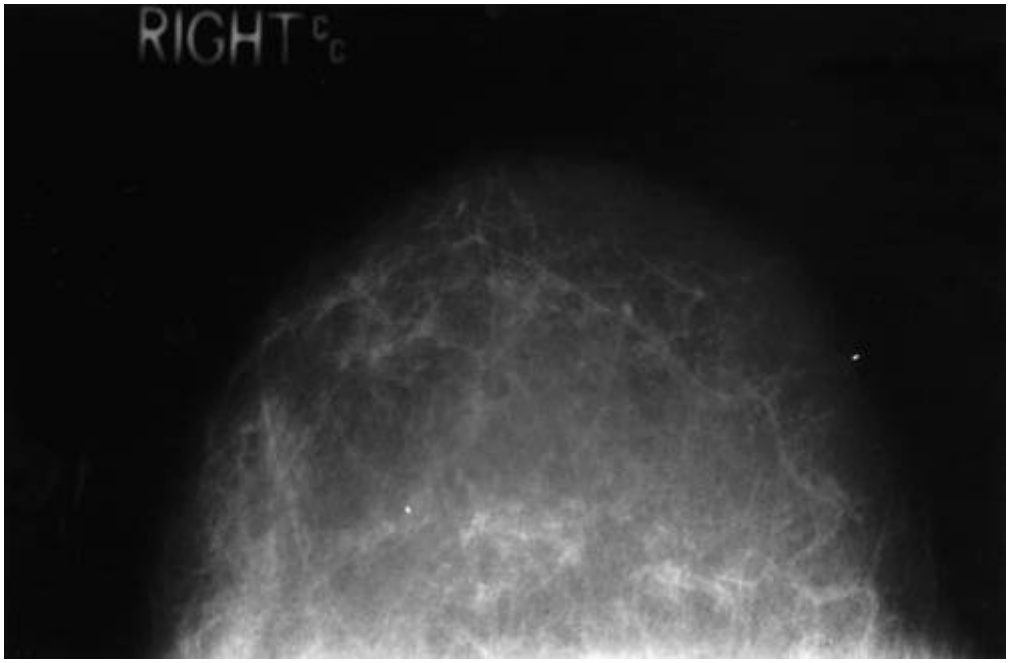
What is the grade of differentiation of this tumour? What is the Dukes' stage of this cancer from this slide?

Case 2

Mrs DC is 56 years old. She has attended for a mammogram in breast screening and this shows calcification as marked by the arrows



This is a normal mammogram



breastcancer.about.com/.../Breast-Tumor.htm

A core biopsy is performed which shows ductal carcinoma in situ, following this, she has wide local excision of this area at surgery. This is an illustration of the specimen which shows a firm area with the consistency of a “gritty pear” from which sections are taken.

http://en.wikipedia.org/wiki/File:Breast_cancer_gross_appearance.jpg

Case 2 Slide 1
This is a section showing cancer confined to the ducts, not invading the stroma.
How would you define this cancer stage?

Case 2 Slide 2
In another area, cancer invades the stroma.

Case 2 Slide 3
Cancer is present in the lymph node.

What would the histology summary state and what is the staging of this cancer?

Case 3

Mr SCC, aged 60 years, noticed a rough patch on the back of his hand. He has worked as a farm labourer outdoors all his life, in all weathers.

www.assh.org/.../Skin-Cancer-of-the-Hand.aspx

A punch biopsy shows squamous cell cancer. What are the other types of common skin cancers?

Case 3 Slide 1

This section shows cancer in situ in the skin. What is the histological term used for this condition?

Case 3 slide 2

Squamous cell carcinoma of skin. What is the grade and stage of this cancer?

Practical 2

Microbiology - Diagnosis of bacterial infections using PCR

Dr Angelika Gründling

A detailed worksheet for this practical will be put on the Intranet in advance of the practical, and printed copies will be provided at the practical on the day.

Practical 3 – Haematology on 15th March 2012

- This session is a combination of microscopy, computer-assisted learning (CAL) and a seminar.
- These activities are carried out in rotation as shown below, according to your tutorial group.
- For all groups it will take place in SAFB.

NOTE: The seminar will serve as revision and it is important that prior to this session you have read through all the handouts and lecture notes for the haematology component of the course from LAST term.

Worksheets will be provided on the day.

TIME	PRACTICAL IN 2MDL	CAL IN COMPUTER LAB G29	SEMINAR IN 1MDL/D	SEMINAR IN 119
9.30	E1-E5	E6-E7 & F6-F7	F1-F3a	F3b-F5
10.30	F1-F5	E1-EA5	E6-E7	F6-F7
11.30	F6-F7 & E6-E7	F1-F5	E1-E3a	E3b-E5
12.30	Lunch	Lunch	Lunch	Lunch
13.00	G1-G5	G6-G7 & H6-H7	H1-H3a	H3b-H5
14.00	H1-H5	G1-G5	G6-G7	H6-H7
15.00	G6-G7 & H6-H7	H1-H5	G1-G3a	G3b-G5

Thursday 15th March	**Tutors**
a.m. (Seminar)	TBA
p.m. (Seminar)	TBA
All day (CAL)	TBA
All day (Practicals)	TBA