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Tumour suppressor genes and oncogenes

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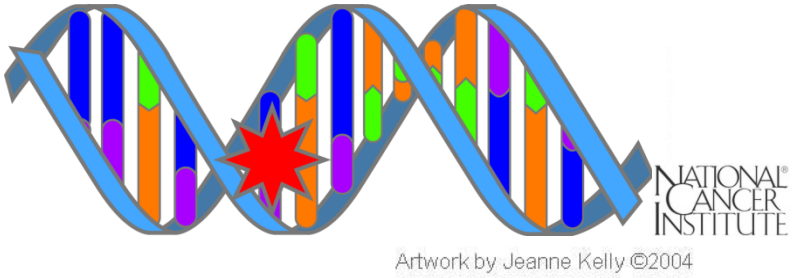
BSc Gastroenterology (2011-2012)
Module 2 - November 18, 2011

What we will discuss

- Different type of mutations
- Proto-oncogenes and oncogenes
- Tumor suppressor genes
- Inheritance of certain tumours

Mutations

A mutation is a change in the normal base pair sequence of DNA



These changes may occur in either coding or noncoding regions. Mutations may be silent and have no effect on the resulting protein. This is especially true if they occur in non-coding regions of the DNA. But even base pair changes in the coding region may be silent because of the redundancy of the code. For example, a mutation within a codon may occur, yet still call for the same amino acid as was called for earlier.

Mutations may involve a single base change--called a point mutation--or may involve larger sections of DNA through deletions, insertions, or translocations.

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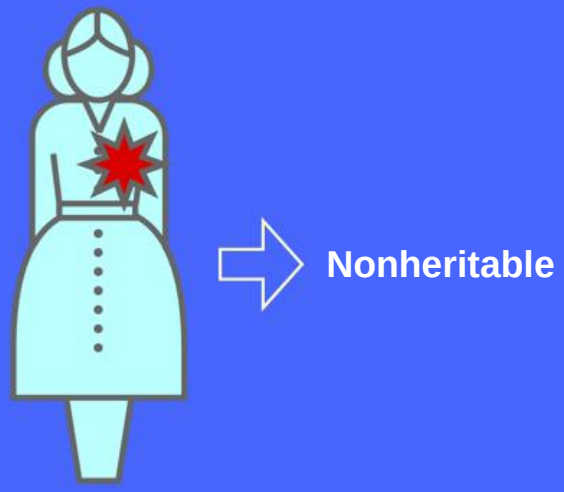
Cancer development: current view

Cancer arises through a multistage process that involve inherited and somatic mutations of cellular genes.

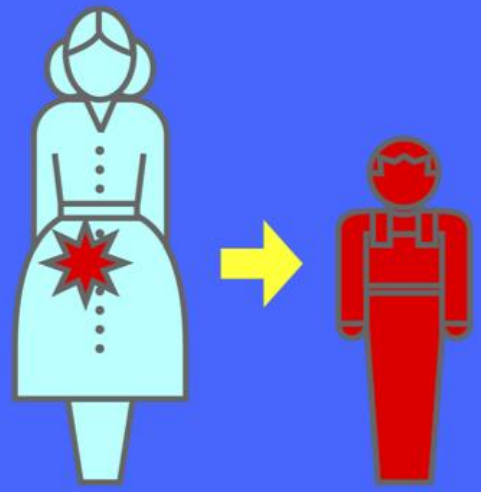
Somatic mutations

- Occur frequently (about 1 mutation/cell division)
- Normally inconsequential (Not passed on to next generation)
- Dangerous if occur in specific genes



Germline mutations

- Occurs rarely
- Present in all somatic cells of affected individual
- Cancer develops only in specific tissue/organ
- Increased risk of cancer passed on to future generation



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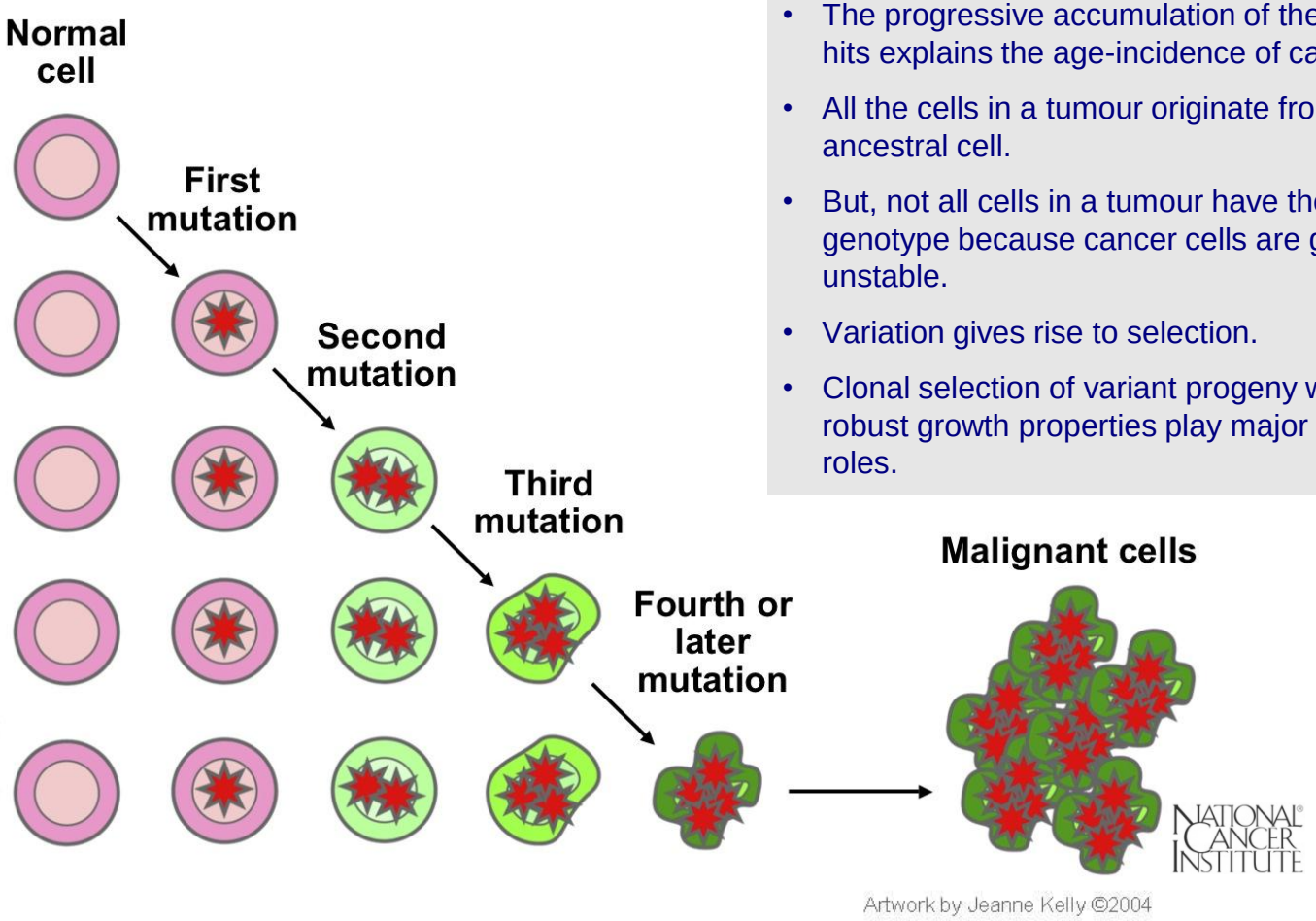
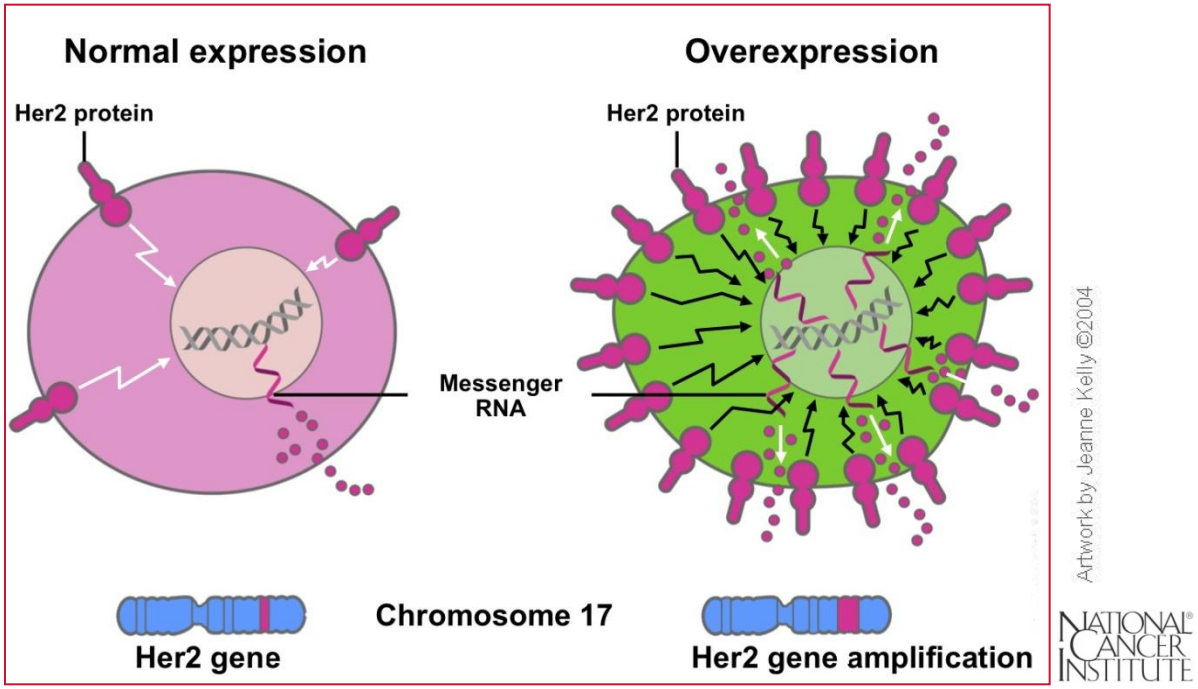
Cancers are clonal



- Many mutations in several distinct genes are required for a normal cells to become cancerous.
- The progressive accumulation of these multiple hits explains the age-incidence of cancer.
- All the cells in a tumour originate from a single ancestral cell.
- But, not all cells in a tumour have the same genotype because cancer cells are genetically unstable.
- Variation gives rise to selection.
- Clonal selection of variant progeny with the most robust growth properties play major contributing roles.

Regulatory mutations



Although mutations in the noncoding region are generally silent, that is not always the case. Some of the most important regulatory regions are in the 5′ non-coding flanking region of the gene. Promoter sequences that regulate the gene are located there. Also, enhancer sequences that regulate the rate of gene activity are in non-coding regions a considerable distance from the gene. And gene repressor regions, which negatively regulate gene activity, also exist. Mutations in any of these regions can change the rate of protein production.

Her2 protein expression is a good example of how gene amplification can have a regulatory impact upon a tumor's growth. In breast cancer, overexpression of Her2 protein results from gene amplification in chromosome 17. This increase in production of growth-signaling molecules speeds up the rate of the cancer's progress.

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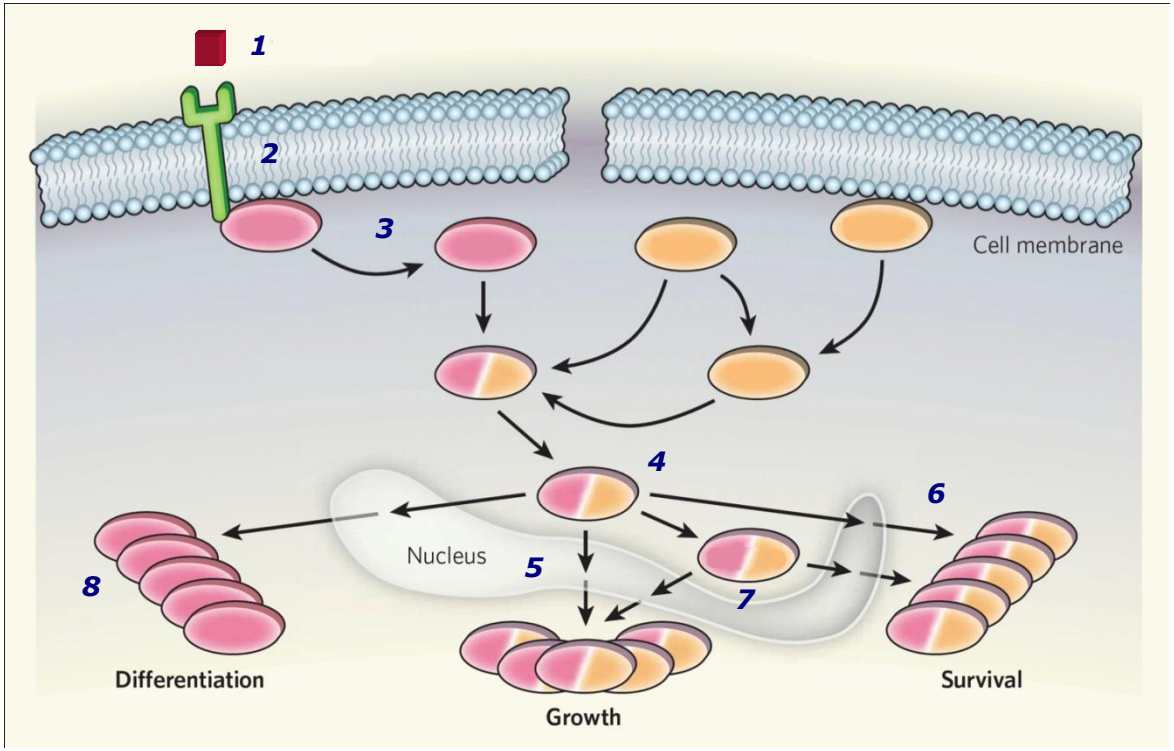
Cancer-associated mutations



Modified from: Huang PH & Richard Marais R. (2009). Nature 459, 336-337

- Growth factors, cytokines (1)
- Receptors genes (2)
- Cell signalling genes (3)
- Transcription factors genes (4)
- Cell cycle control genes (5)
- Cell death and survival genes (6)
- DNA repair genes (7)
- Cellular differentiation genes (8)

Cancer-associated mutations, whether somatic or germline, whether point mutations or large deletions, alter key proteins and their functions in the human biosystem. A wide variety of mutations seems to be involved. Even mutations in non-coding regions, such as in promoters, enhancers, or negative regulatory regions, can result in under- or over-expression of proteins needed for normalcy. Other mutations may cause production of important checkpoint proteins to malfunction. Collectively, these mutations conspire to change a genome from normal to cancerous.

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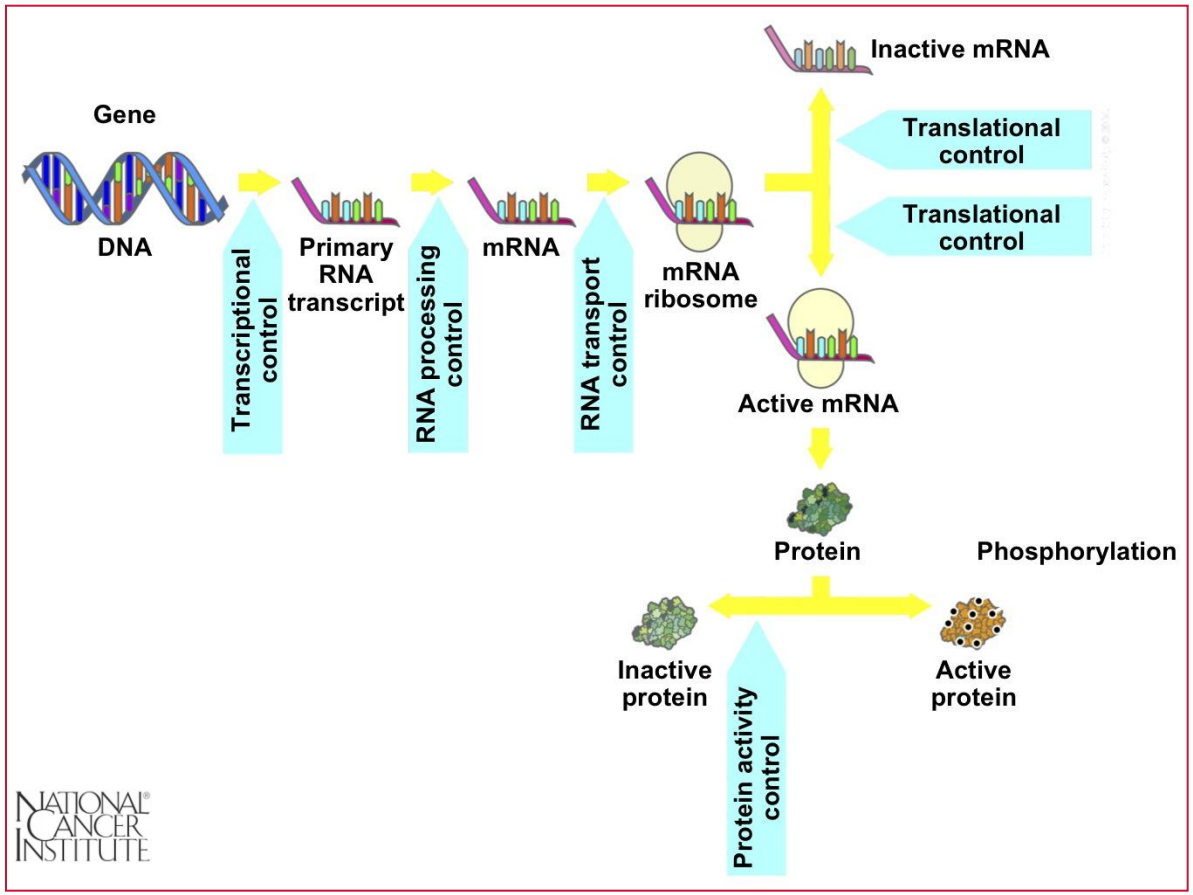
Cancer depends also on epigenetic factors



Epigenetic factors are mechanisms outside the gene such as a cell's exposure to carcinogens or hormones, or genetic variations that modify a gene or its protein by methylation, demethylation, phosphorylation, or dephosphorylation. These factors can alter what is ultimately expressed; they can change a phenotype. In cancer, both the genotype and the phenotype change over time. Epigenetic factors play a key role in these changes.

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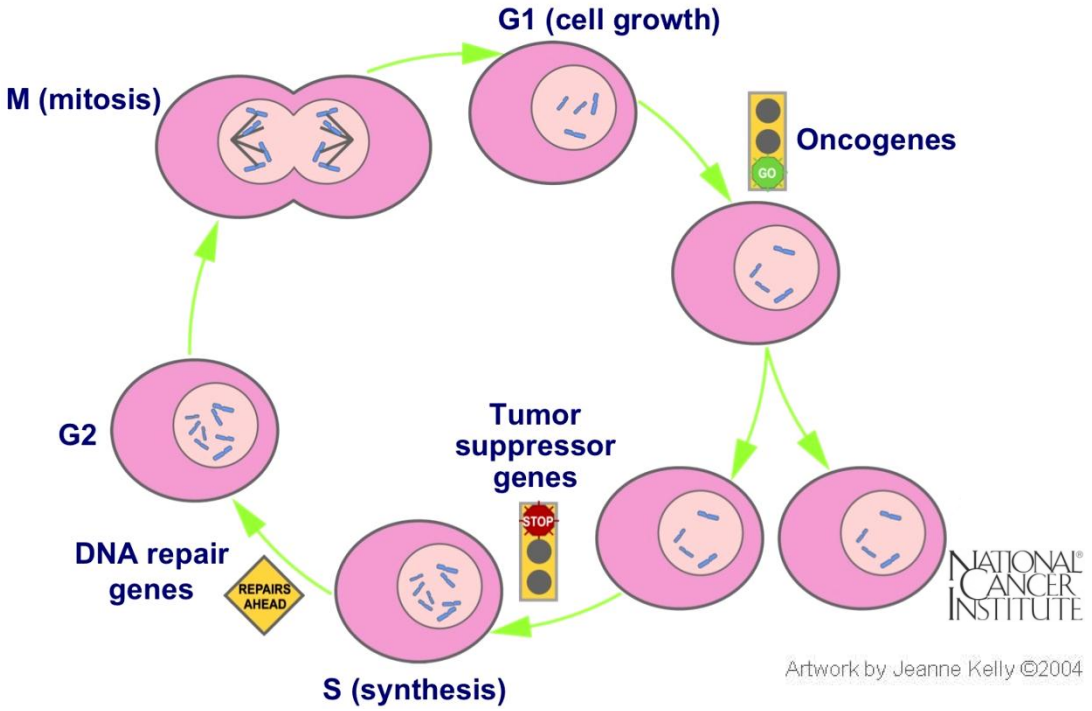
Normal cell growth



The cell cycle is a critical process that a cell undergoes in order to copy itself exactly. Most cancers have mutations in the signals that regulate the cell's cycle of growth and division. Normal cell division is required for the generation of new cells during development and for the replacement of old cells as they die.

Most cells remain in interphase, the period between cell divisions, for at least 90 percent of the cell cycle. The first part of the interphase is called G1 (for first gap), followed by the S phase (for DNA synthesis), then G2 (for second gap). During G1, there is rapid *growth* and metabolic activity, including synthesis of RNA and proteins. Cell growth continues during the S phase, and DNA is replicated. In G2, the cell continues to grow and prepares for cell division. Cell *division* (mitosis) is referred to as the M phase. Cells that do not divide for long periods do not replicate their DNA and are considered to be in G0. In normal cells, **tumor suppressor genes** act as braking signals during G1 to stop or slow the cell cycle before S phase. DNA repair genes are active throughout the cell cycle, particularly during G2 after DNA replication and before the chromosomes prepare for mitosis.

Oncogenes and tumor-suppressor genes: definitions

In cancer, two classes of cellular genes are targets for mutations:

- **Proto-oncogenes**
  - A **proto-oncogene** is a normal gene that can become an oncogene, either after mutation or increased expression. Proto-oncogenes code for proteins that help to regulate cell growth and differentiation. Proto-oncogenes are often involved in signal transduction and execution of mitogenic signals. Upon *activation*, a proto-oncogene becomes a tumour-inducing agent, which is named oncogene.
- **Tumour suppressor genes**
  - A **tumor suppressor gene** is a gene that protects a cell from uncontrolled cell growth. When this gene is mutated to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes.

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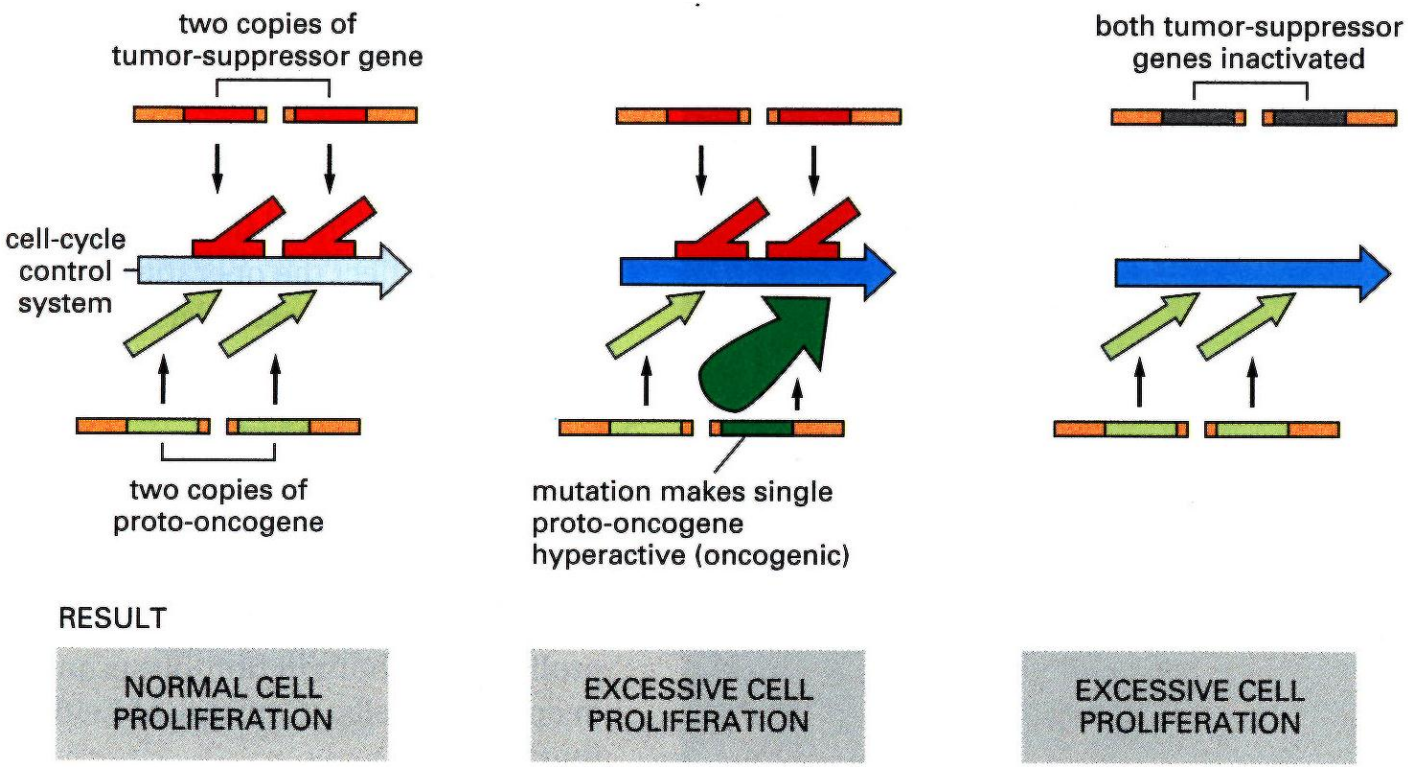
Cell growth defects caused by alterations in proto-oncogenes and tumour suppressor genes



Molecular Biology of the Cell, 2002 © Garland Science

Most cancers have mutations in proto-oncogenes, the normal genes involved in the regulation of controlled cell growth. These genes encode proteins that function as growth factors, growth factor receptors, signal-relaying molecules, and nuclear transcription factors (proteins that bind to genes to start transcription). When the proto-oncogene is mutated or overregulated, it is called an oncogene and results in unregulated cell growth and transformation. At the cellular level, only one mutation in a single allele is enough to trigger an oncogenic role in cancer development. The chance that such a mutation will occur increases as a person ages.

On the contrary, both copies of a tumor suppressor gene must be lost or mutated for cancer to occur. A person who carries a germline mutation in a tumor suppressor gene has only one functional copy of the gene in all cells. For this person, loss or mutation of the second copy of the gene in any of these cells can lead to cancer.

Examples of oncogenes

A proto-oncogene is a normal gene that can become an oncogene due to mutations or increased expression. The resultant protein may be termed an oncoprotein. Proto-oncogenes are often involved in signal transduction and execution of mitogenic signals, usually through their protein products. Examples of proto-oncogenes include:

- **RAS** - activated in many cancers (e.g., Colon cancer)
  - The Ras subfamily is a protein subfamily of small GTPases that are involved in cellular signal transduction. Activation of Ras signalling causes cell growth, differentiation and survival. Ras is the prototypical member of the Ras superfamily of proteins which are all related in structure and regulate diverse cell behaviours.
- **c-MYC** - over-expressed in colon cancer (amplified in lung, and rearraged in lymphoma)
  - Myc gene encodes for a transcription factor that regulate expression of 15% of all genes, through binding on Enhancer Box sequences (E-boxes) and recruiting histone acetyltransferases (HATs). Hence, in addition to its role as a classical transcription factor, Myc also functions to regulate global chromatin structure by regulating histone acetylation both in gene-rich regions and at sites far from any known gene.
- **CDK4** - Familial melanoma
- **MET** - Hereditary papillary renal cancer
- **BCR/ABL** - Chronic myelogenic leukemia
- **BCL2** - Follicular lymphoma

Tumour suppressor genes

- Act as negative regulators of cancer growth:
  - » inhibit proliferation
  - » induce apoptosis,
  - » inhibit angiogenesis
  - » induce cell adhesion
- Act to maintain chromosome integrity
- Example includes:
  - » Rb
  - » p53
  - » APC

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Retinoblastoma (Rb)

- Retinoblastoma is a paediatric intraocular tumour accounting for 5% of childhood blindness. It occurs as an inherited disease with autosomal dominant transmission and 90% penetrance.
- Sporadic cases are also known, but these differ from the typical hereditary disease.
- Several modes of treatment exist, including surgery and radiotherapy, and these are usually curative and preserve vision. However, significant mortality still occurs after successful treatment of hereditary cases due to the increased incidence of subsequent primary tumours of various types.

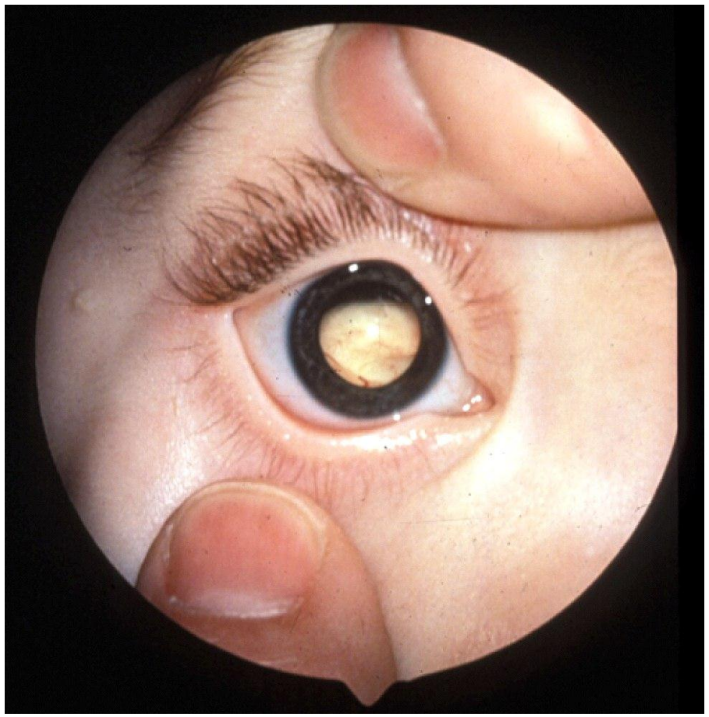

Figure 7-4c The Biology of Cancer (© Garland Science 2007)

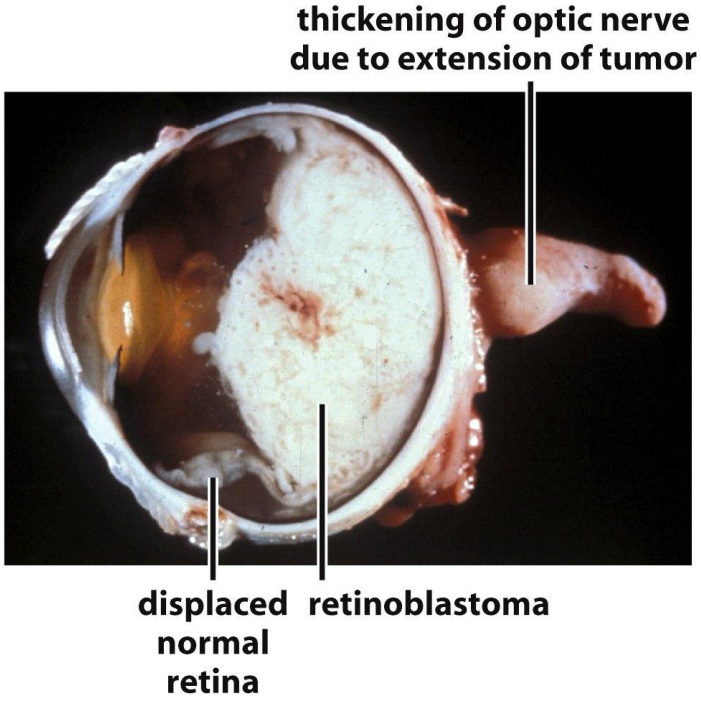

Figure 7-4b The Biology of Cancer (© Garland Science 2007)

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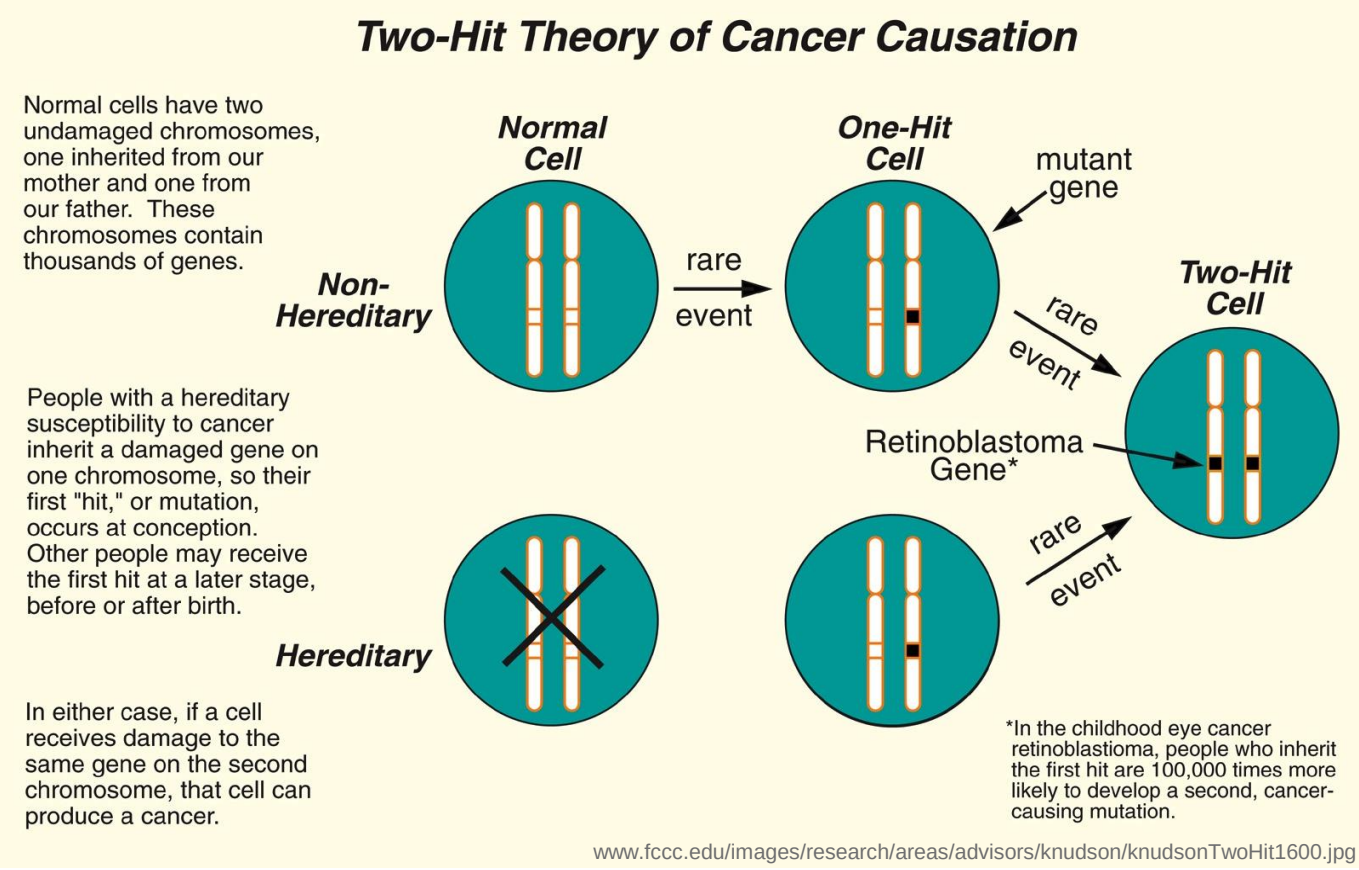
Retinoblastoma: a paradigm for the 'Two-Hit model'



www.fccc.edu/images/research/areas/advisors/knudson/knudsonTwoHit1600.jpg

Multiple "hits" to DNA are necessary to cause cancer. In the children with inherited retinoblastoma, the first insult is inherited in the DNA, and any second insult would rapidly lead to cancer. In non-inherited retinoblastoma, two "hits" had to take place before a tumor could develop, explaining the age difference.

The development of malignancy) depended both on the activation of proto-oncogenes (genes that stimulate cell proliferation) and deactivation of tumor suppressor genes (genes that keep proliferation in check). A first "hit" in an oncogene would not necessarily lead to cancer, as normally functioning tumor suppressor genes (TSGs) would still counterbalance this impetus; only damage to TSGs would lead to unchecked proliferation. Conversely, a damaged TSG (such as the Rb1 gene in retinoblastoma) would not lead to cancer unless there is a growth impetus from an activated oncogene.

Function of Rb protein

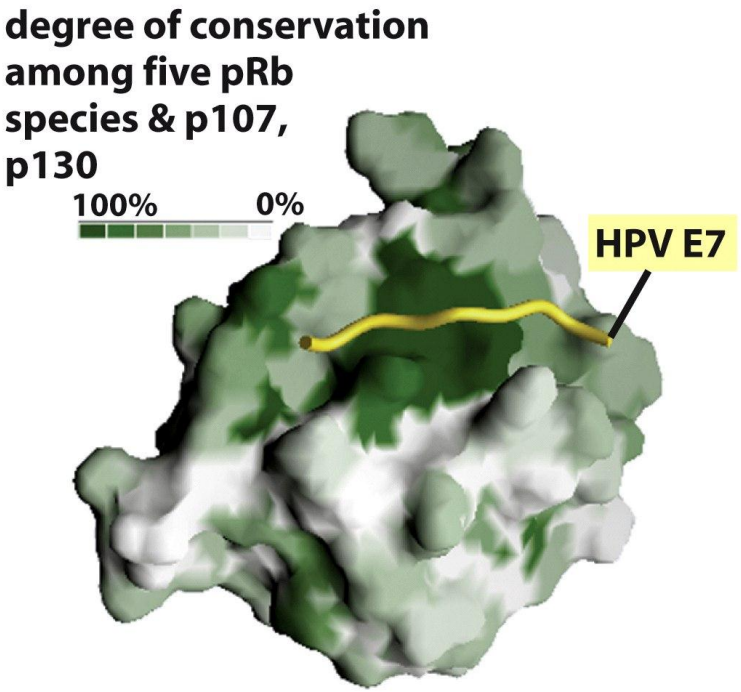


Figure 8-21c The Biology of Cancer (© Garland Science 2007)

- ~ 105 kDa, known as pRb
- Member of the 'pocket protein family', which consists of three proteins:
  - pRB – Retinoblastoma protein
  - p107 – Retinoblastoma-like protein 1
  - p130 – Retinoblastoma-like protein 2
- They play crucial roles in the cell cycle through interaction with members of the E2F transcription factors family.
- pRB, p107 and p130 contain a "pocket" which interacts with oncogenic DNA viral proteins such as HPV16 E7 and other proteins including the E2F transcription factor
- p107 and p130 are not classical tumour suppressors – their functions may be redundant.

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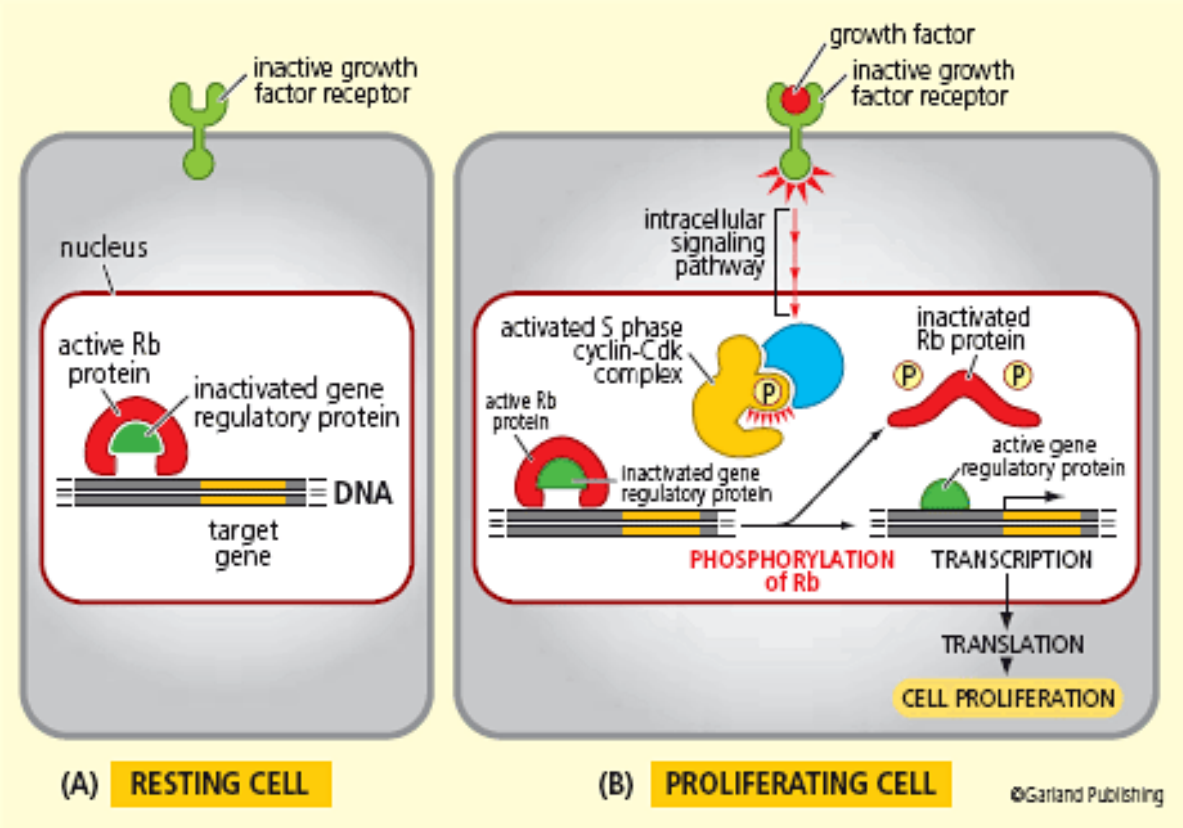
pRb acts as a brake of phase-G1 of the cell cycle



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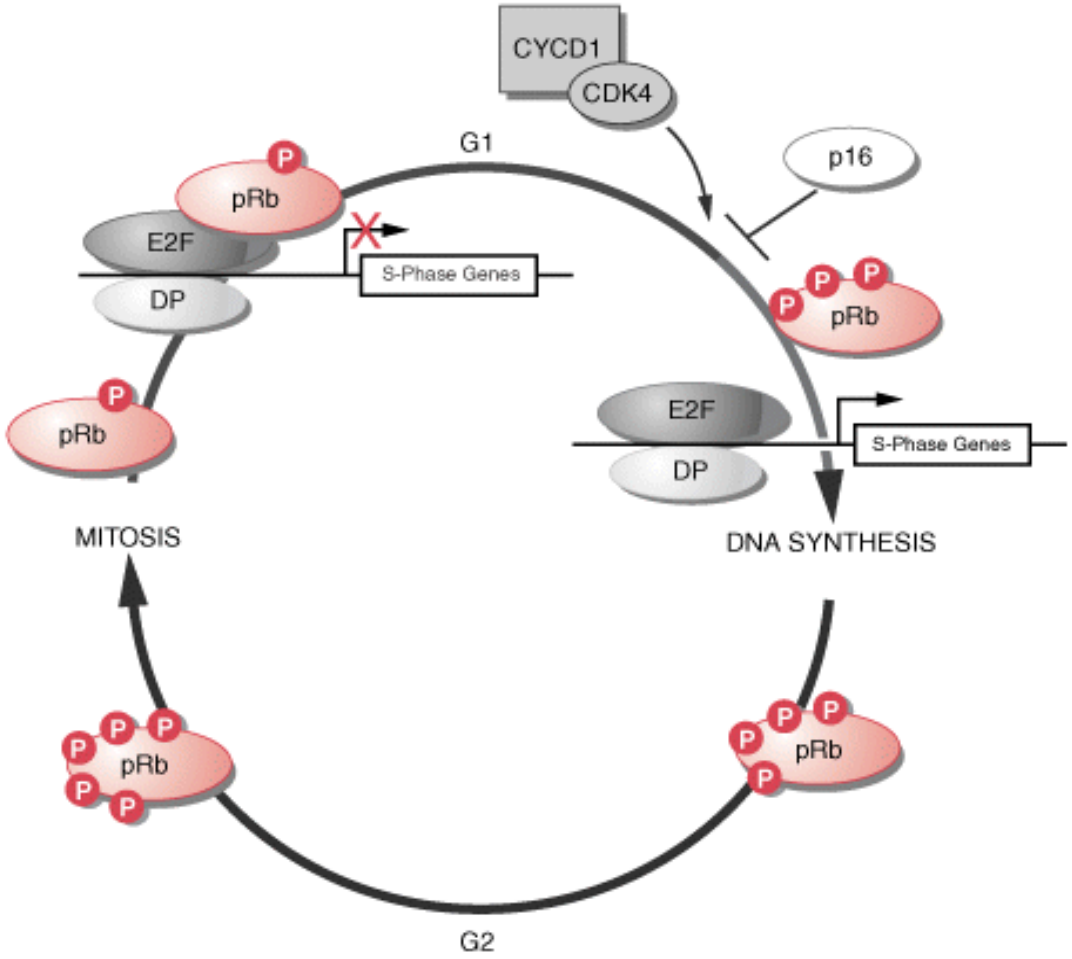
The Rb tumour suppressor protein (pRb) binds to the E2F-1 transcription factor preventing it from interacting with the cell's transcription machinery. In the absence of pRb, E2F-1 (along with its binding partner DP-1) mediates the trans-activation of E2F-1 target genes that facilitate the G1/S transition and S-phase.

G1-Cdk activity (cyclin D-Cdk4) initiates Rb phosphorylation. This inactivates Rb, freeing E2F to activate the transcription of S-phase genes, including cyclin E and cyclin A.

Rb activity is regulated by phosphorylation



When Rb is phosphorylated, transcription factors like E2F are free

↓

Transcription of target genes

↓

Entry into S phase

The p53 tumour suppressor gene

- The p53 gene, like the Rb gene, is a tumor suppressor gene.
- If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. This condition is rare, and is known as Li-Fraumeni syndrome.
- Mutations in p53 are found in approximately 50% of cancers, and so contribute to the complex network of molecular events leading to tumor formation.

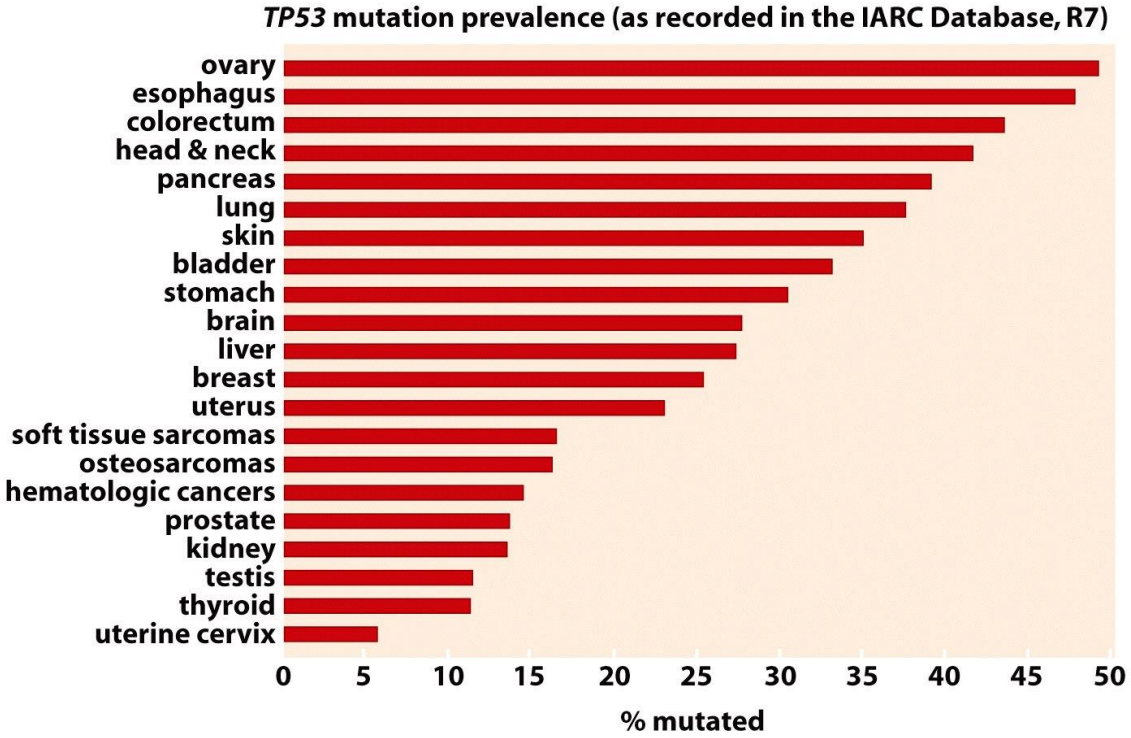


Figure 9-4 The Biology of Cancer (© Garland Science 2007)

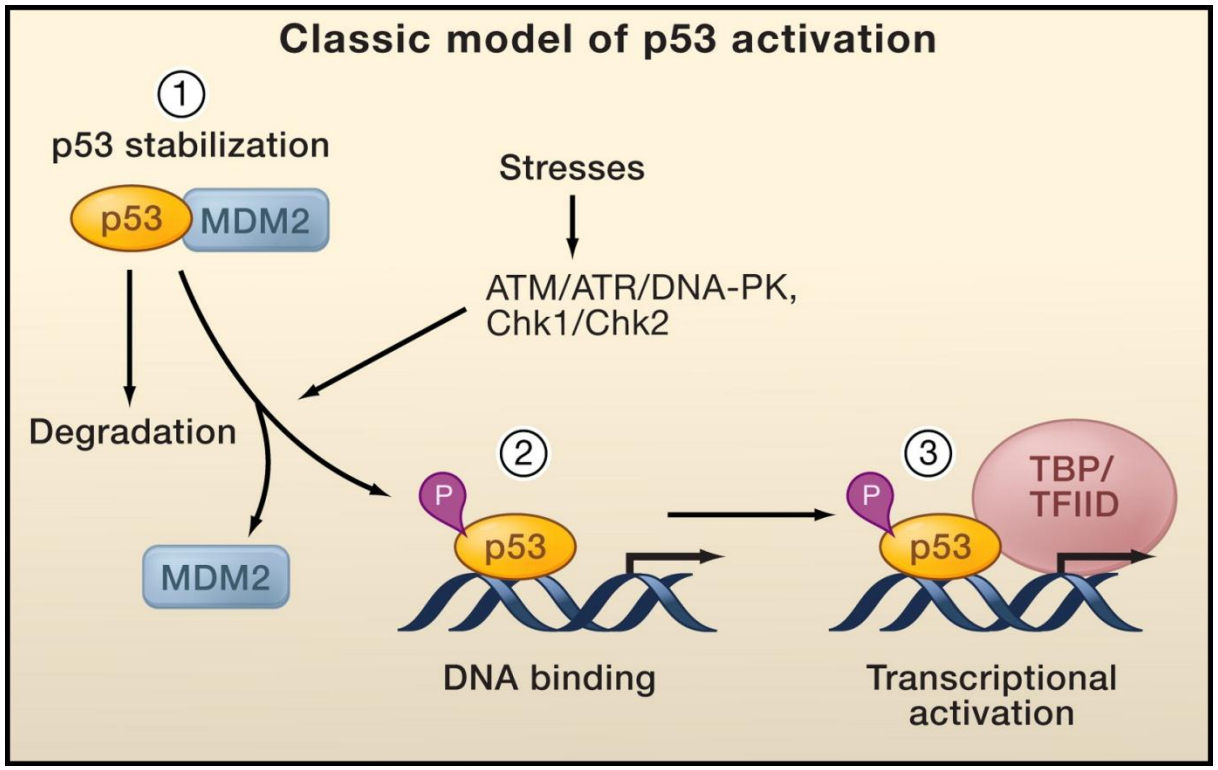
p53: general overview

- The p53 gene has been mapped to chromosome 17.
- p53 act not only as a transcription factor, but also as regulatory protein.
- Human p53 consists of 393 amino acids long and, like other transcription factors, has three domains:
  - » An N-terminal transcription-activation domain **(TAD)**, which activates transcription factors
  - » A central DNA-binding core domain **(DBD)**. Contains zinc molecules and arginine amino acid residues.
  - » A C-terminal homo-oligomerisation domain **(OD)**. Tetramerization greatly increases the activity of p53 *in vivo*.
- Mutations that deactivate p53 in cancer usually occur in the **DBD**.
  - » Most of these mutations destroy the ability of the protein to bind to its target DNA sequences, and prevent transcriptional activation of these genes.
  - » Mutations in the DBD are recessive loss-of-function mutations.
- Molecules of p53 with mutations in the **OD** dimerise with wild-type p53, and prevent them from activating transcription. Therefore OD mutations have a dominant negative effect on the function of p53.

p53 function

- p53 regulates the expression of stress response genes and mediates a variety of anti-proliferative processes.
- p53 mediates its activities through the activation of genes regulating:
  - cell cycle checkpoints,
  - DNA damage and repair,
  - and apoptosis.
- For apoptosis specifically, p53 enhances the expression of Bcl-2 family members including Bax, BID, PUMA, and Noxa.
- It is also known to regulate APAF-1, a co-activator of the apoptosis initiator Caspase-9.
- Although its role as a mediator of transcription is well established, some studies appear to suggest that p53 might affect apoptosis via novel transcription-independent pathways.
  - For instance, apoptosis can still occur in the presence of inhibitors of protein synthesis, or when p53 mutants incapable of acting as transcription factors are ectopically expressed.

Classical model of p53 activation



From: Kruse JP, Gu W. (2009) Cell 137: 609-622.

p53 activation generally consists of three sequential activating steps: (1) stress-induced stabilization mediated by phosphorylation (P), (2) DNA binding, and (3) recruitment of the general transcriptional machinery. During normal homeostasis, p53 is degraded after Mdm2-mediated ubiquitination (left), while stress signal-induced p53 phosphorylation by ATM, ATR, and other kinases stabilizes p53 and promotes DNA binding. DNA-bound p53 then recruits the transcriptional machinery to activate transcription of p53 target genes.

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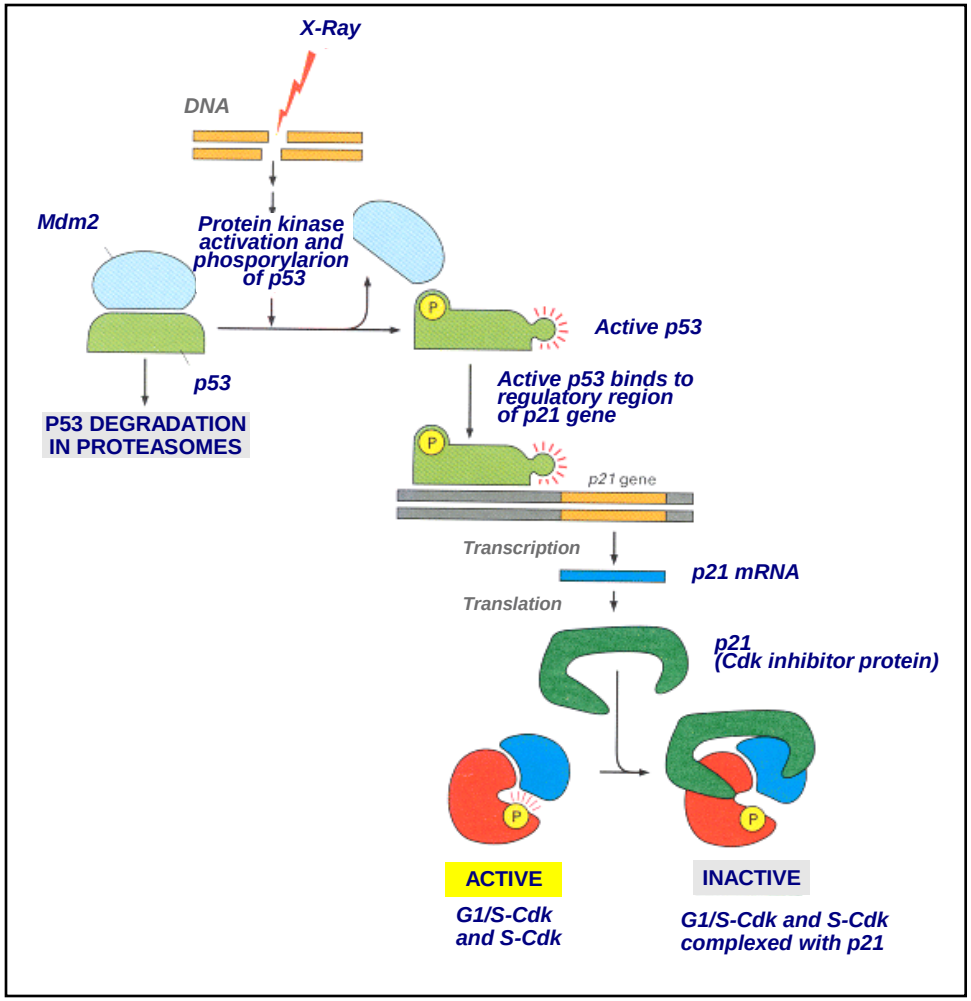
Induction of p53 leads to activation of cell-cycle checkpoints

DNA damage activates p53 by an indirect mechanism. In undamaged cells, p53 is highly unstable and is present at very low concentrations. This is because it interacts with another protein, Mdm2, that acts as a ubiquitin ligase that targets p53 for destruction by proteasomes. DNA damage activates protein kinases that phosphorylate p53 and thereby reduce its binding to Mdm2. This decreases p53 degradation, which results in a marked increase in p53 concentration in the cell. In addition, the decreased binding to Mdm2 enhances the ability of p53 to stimulate gene transcription. One of these genes encodes a CKI protein called p21, which binds to G1/S-Cdk and S-Cdk and inhibits their activities, thereby helping to block entry into S phase.



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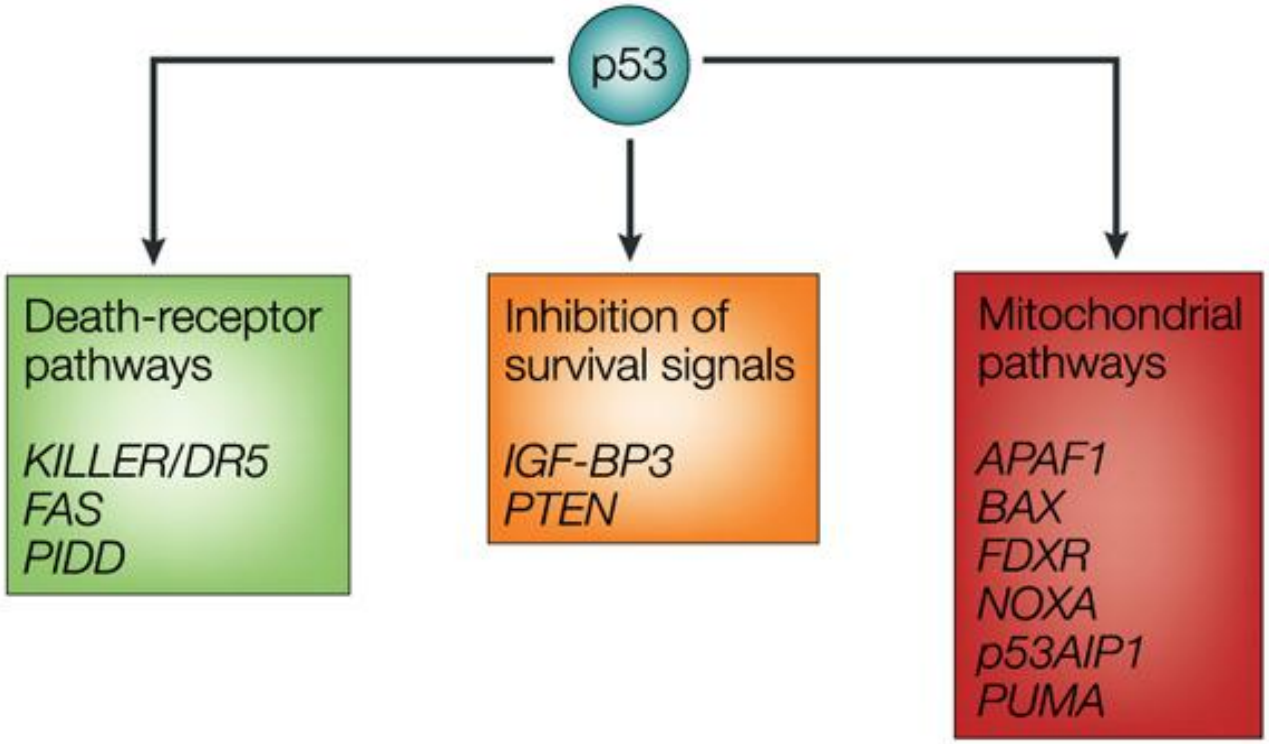
p53 regulates different apoptotic pathways



From Vousden and Lu (2002) Nature Reviews Cancer 2:594-604

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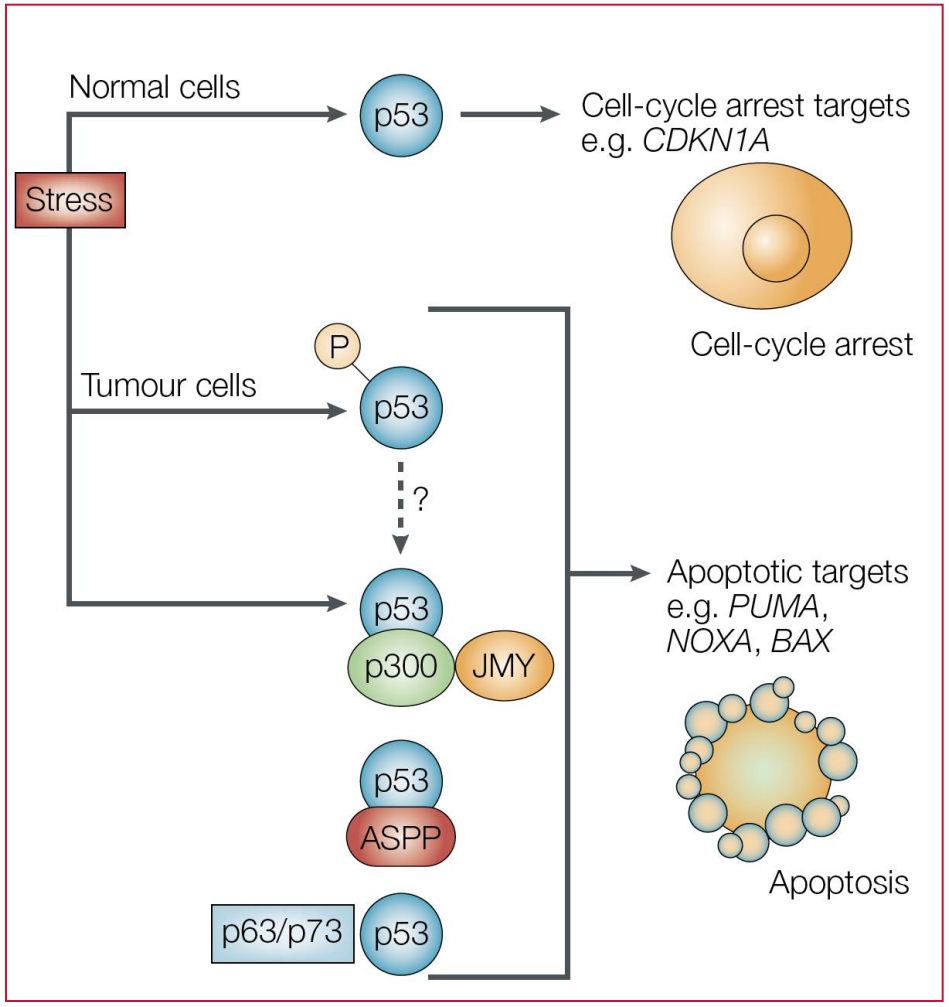
Model for the regulation of the choice of response to p53



From Vousden and Lu (2002) Nature Reviews Cancer 2:594-604

The choice of response to p53 activation is determined, in part, by differential regulation of p53 activity in normal and tumour cells. In this model, activation of p53 in normal cells leads to the selective expression of cell-cycle-arrest target genes (such as CDKN1A, which encodes WAF1), resulting in a reversible or permanent inhibition of cell proliferation.

In tumour cells, phosphorylation of p53 at Ser46 (through activation of kinases, expression of co-activators such as p53DINP1 or repression of phosphatases such as WIP1) and/or functional interaction with apoptotic cofactors (such as ASPP, JMY and p63/p73) allows for the activation of apoptotic target genes. These cofactors can bind p53 (directly or indirectly) as shown for ASPP and JMY or — as shown for p63 and p73 — assist p53 DNA binding by directly interacting with p53-responsive promoters. Although not proven, it is possible that phosphorylation alters the conformation of p53 to either enhance the interaction with apoptotic cofactors, or allow binding to apoptotic target promoters.

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Refined model for p53 activation

Promoter-specific p53 activation consists of three key steps:

(1) p53 stabilization

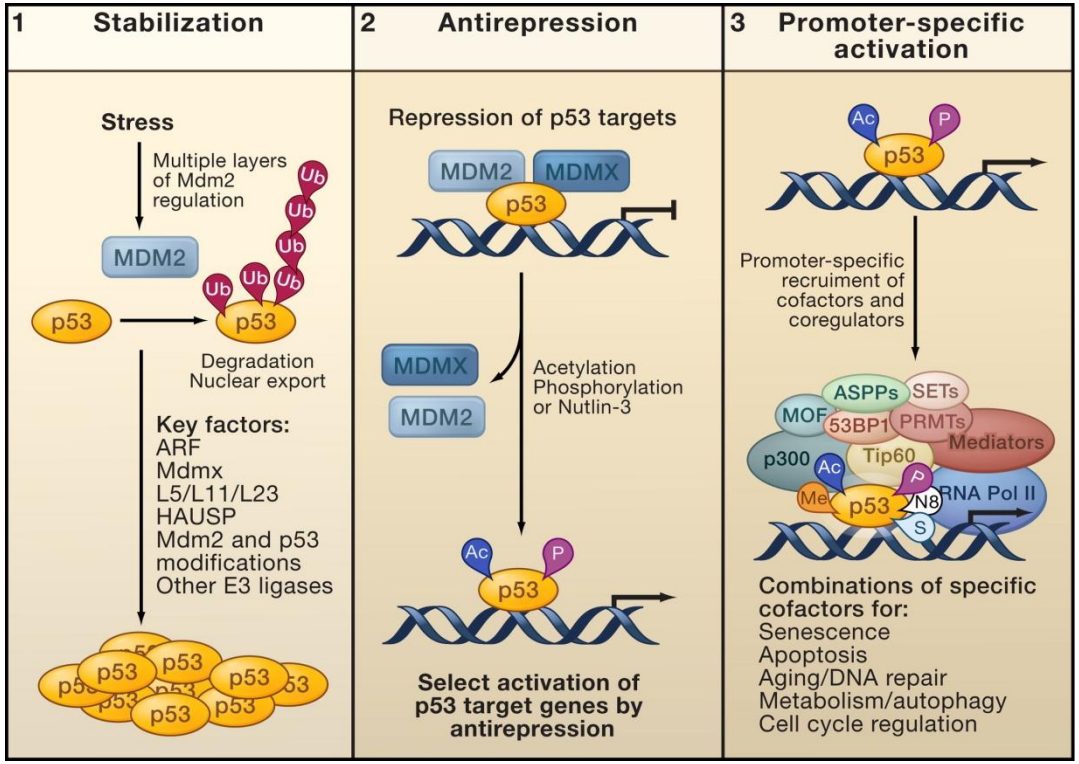
- Occurs through many different mechanisms, many of which act by affecting the ability of Mdm2 to ubiquitinate p53.

(2) Anti-repression

- Consists of the release of p53 from the repression mediated by Mdm2 and MdmX. This step requires the acetylation of p53 at key lysine residues and facilitates the activation of specific subsets of p53 targets.

(3) Promoter-specific activation

- For full activation of specific promoters, p53 recruits and interacts with numerous cofactors. These act by modifying p53, the surrounding histones, or other transcription factors. Regulating the activation of specific groups of p53 targets for apoptosis, senescence, cell cycle control, DNA repair, autophagy, metabolism, or aging may require exact combinations of cofactors and posttranslational modifications.



From: Kruse JP, Gu W. (2009) Cell 137: 609-622.

The APC tumour suppressor gene

- **APC (Adenomous Polyposis Coli) is inactivated in FAP (Familial Adenomous polyposis)**
- **APC is also mutated in the vast majority of colorectal cancer.**
- **APC gene encodes a multifunctional protein that might participate in several cellular processes:**
  - » cell adhesion and migration
  - » signal transduction
  - » microtubule assembly
  - » chromosome segregation.

Note: Despite the fact that each of these roles is potentially linked with cancer, it seems that the main tumour-suppressing function of APC resides in its capacity to properly regulate intracellular β-catenin levels.

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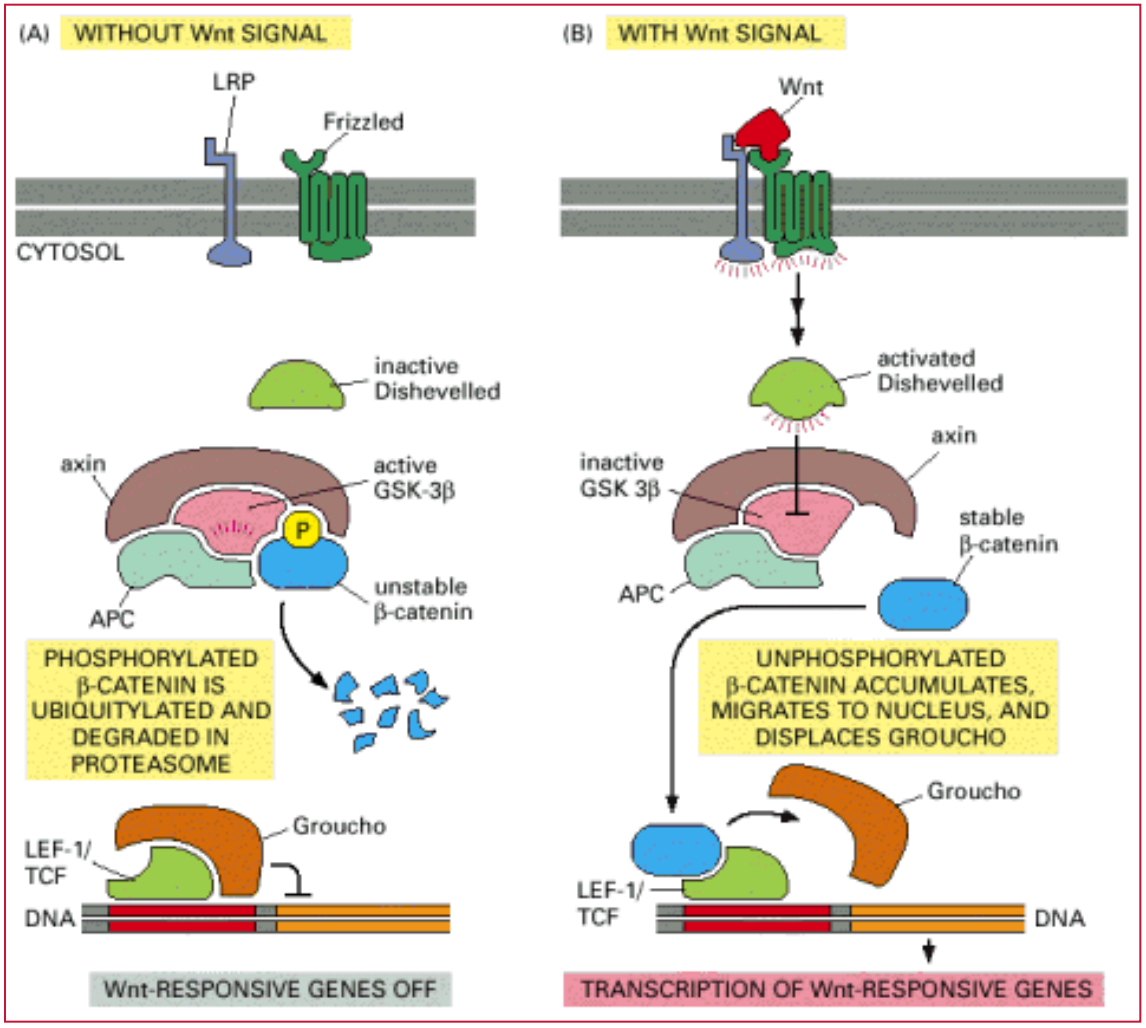
APC function in the Wnt signalling pathway



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In the absence of a WNT signal, APC protein normally binds to glycogen-syntase kinase 3-beta (GSK3-β) and Axin.

GSK3β phosphorylates β-catenin, allowing it to be recognized by an SCF complex containing the F-box protein β-TrCP.

Other proteins in the SCF complex catalyse the addition of a polyubiquitin chain to β-catenin, allowing β-catenin to be recognized and degraded by the proteasome. Consequently, β-catenin cannot reach the nucleus, and cannot co-activate TCF-responsive genes.

Groucho, a corepressor, also prevents the activation of TCF-responsive genes in the absence of βcatenin.

In the presence of WNT, its receptor, Frizzled, in complex with LRP6, is activated. This leads to a poorly understood signalling cascade in which Dishevelled activates GBP, an inhibitor of GSK3β.

Consequently, β-catenin cannot be targeted for destruction and is free to diffuse into the nucleus, where it acts as a co-activator for TCF-responsive genes.

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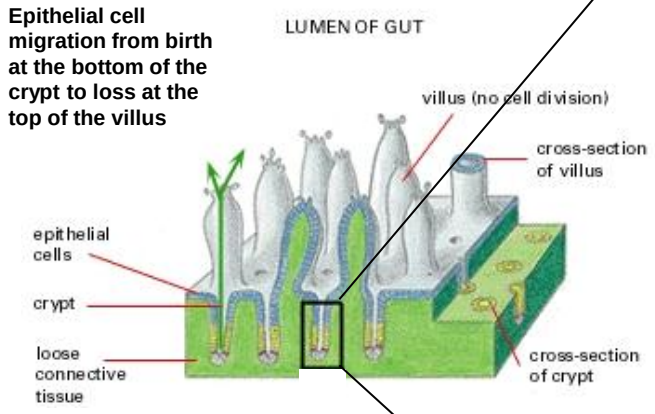
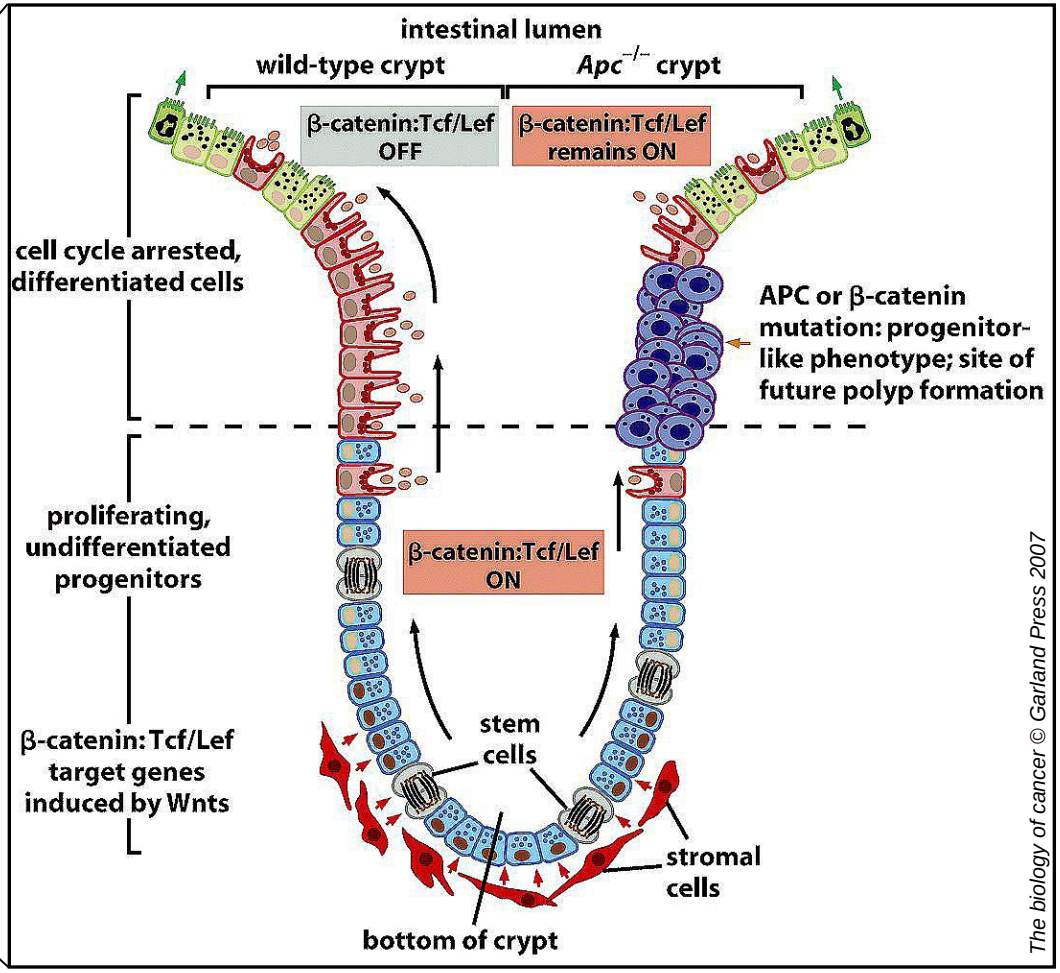
Loss of APC function promotes aberrant cell migration

Epithelial cell migration from birth at the bottom of the crypt to loss at the top of the villus





Inactivation of APC prevents migration of the affected enterocytes out of the colonic crypt. The mutant cells are therefore retained rather than being lost through emigration and apoptosis.

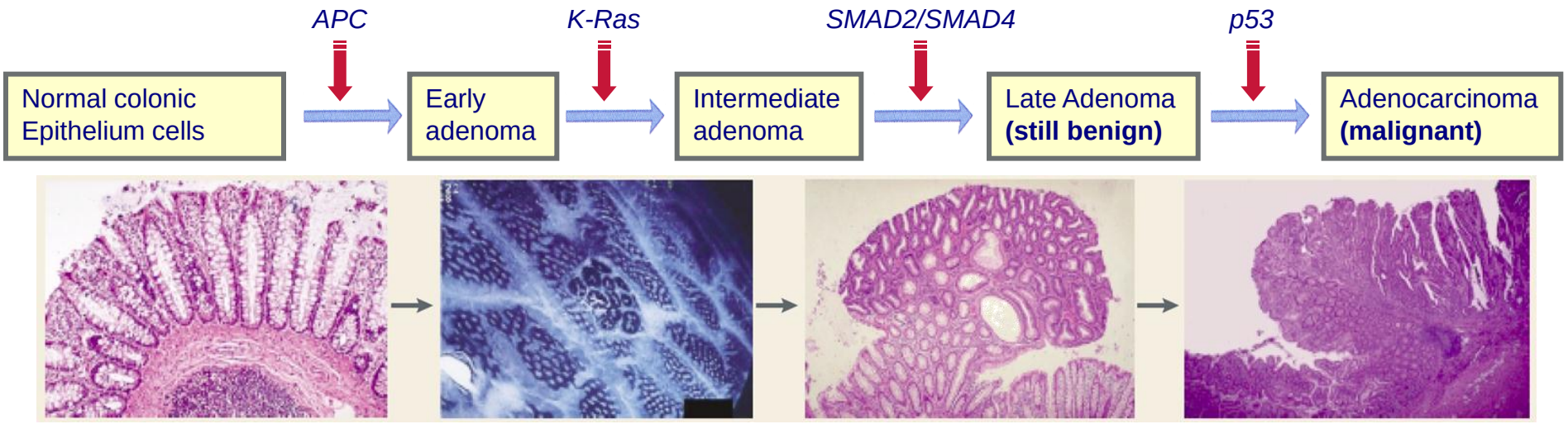
Loss of APC as initiator of colon carcinogenesis

- Aberrant cell migration
  - mutant cells fail to migrate upwards towards the gut lumen and are retained in the crypt.
- Increased cell proliferation through stabilisation of β-catenin.
- Decreased cell differentiation through effects on Wnt signalling.
- Chromosomal instability through chromosome mis-segregation during mitosis leading to aneuploidy.

Further reading: Narayan S and Roy D (2003) Molecular Cancer 2: 41-55.

Imperial College London

Colon tumour progression: multi-step process



Modified from: Fodde R et al., (2001). Nature Reviews Cancer 1, 55-67.

Conclusions

- The development of cancer depended both on the activation of proto-oncogenes (genes that stimulate cell proliferation) and deactivation of tumor suppressor genes (genes that keep proliferation in check).

- Tumour suppression genes controls key steps in various cellular process like:
  - Control of cell division (Rb, p53)
  - Responses to stress (p53)
  - Control of signalling pathways (APC)
  - DNA damage repair genes

Learning outcomes

At the end of this class you will be able to:

- Give example and definition of distinct type of mutations, oncogenes and tumour suppressor genes
- Understanding the differences between oncogenes and tumour suppressor genes
- Understand the principles that govern inheritance of certain tumours: retinoblastoma, familial adenomatous polyposis.
- Understand and describe the normal role of tumour suppressor genes (e.g. p53, Rb and APC) in control of cell growth.

References

Reviews:

- Vousden KH and Lu X (2002). Live or let die: the cell's response to p53. ***Nature Reviews Cancer*** 2: 594-604.
- Kruse JP, Gu W. (2009) Modes of p53 regulation. ***Cell*** 137: 609-622.
- Narayan S and Roy D (2003). Role of APC and DNA mismatch repair genes in the development of colorectal cancers. ***Molecular Cancer*** 2: 41-55.
- Fodde R et al., (2001). APC, Signal transduction and genetic instability in colorectal cancer. ***Nature Reviews Cancer*** 1: 55-67.

Books:

- Robert A. Weinberg (2006). **The Biology of Cancer**, Garland Sciences.
- Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2002). **Molecular Biology of the Cell**, 4th edition, Garland Sciences.