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Cancer cell regulation

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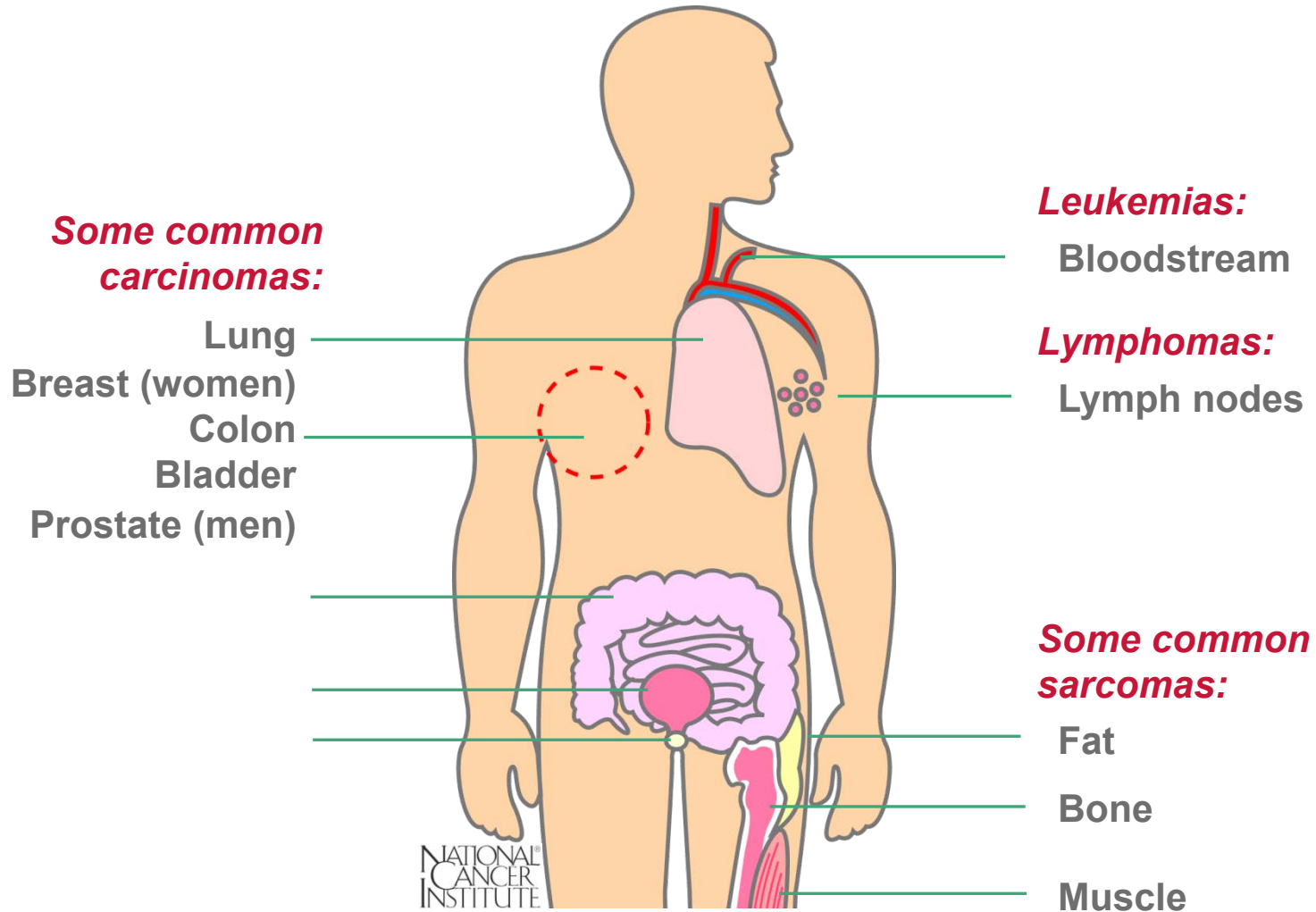
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BSc Gastroenterology (2012-2013)
Module 2 - November 16, 2012

What we will discuss

- **Cancer: definition, different kinds of cancer,**
- **From where cancer arise**
- **Hallmarks of Apoptosis and Necrosis**
- **Transcription process and Transcription Factors**
- **NF- κ B, I κ B, and IKK Protein Families**
- **Molecular mechanisms by which NF- κ B blocks cell death**
- **New therapies prospects**

Different kinds of cancer

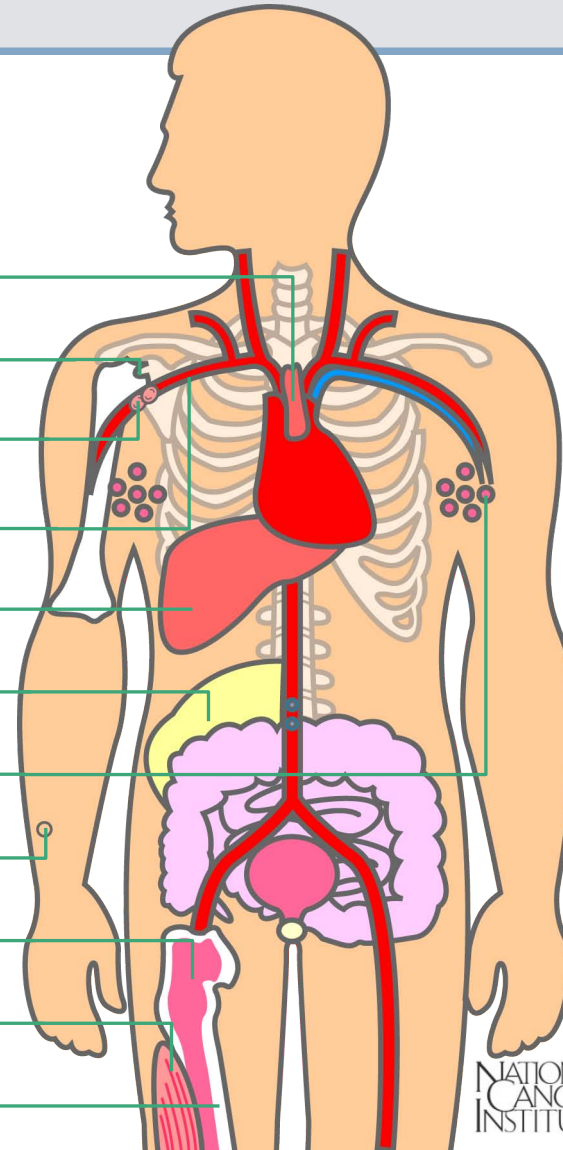


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Naming cancer

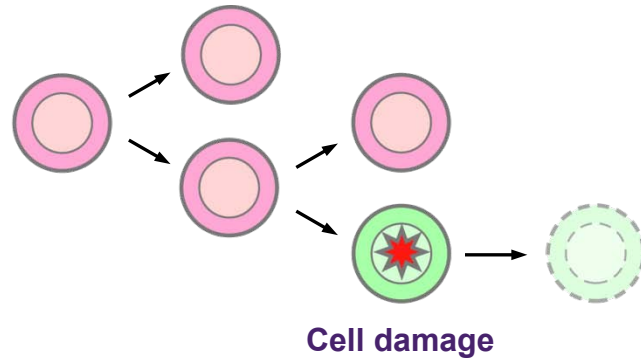
<i>Prefix</i>	<i>Meaning</i>
adeno-	gland
chondro-	cartilage
erythro-	red blood cell
hemangio-	blood vessels
hepato-	liver
lipo-	fat
lympho-	lymphocyte
melano-	pigment cell
myelo-	bone marrow
myo-	muscle
osteo-	bone



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Normal cell growth

Normal
cell division

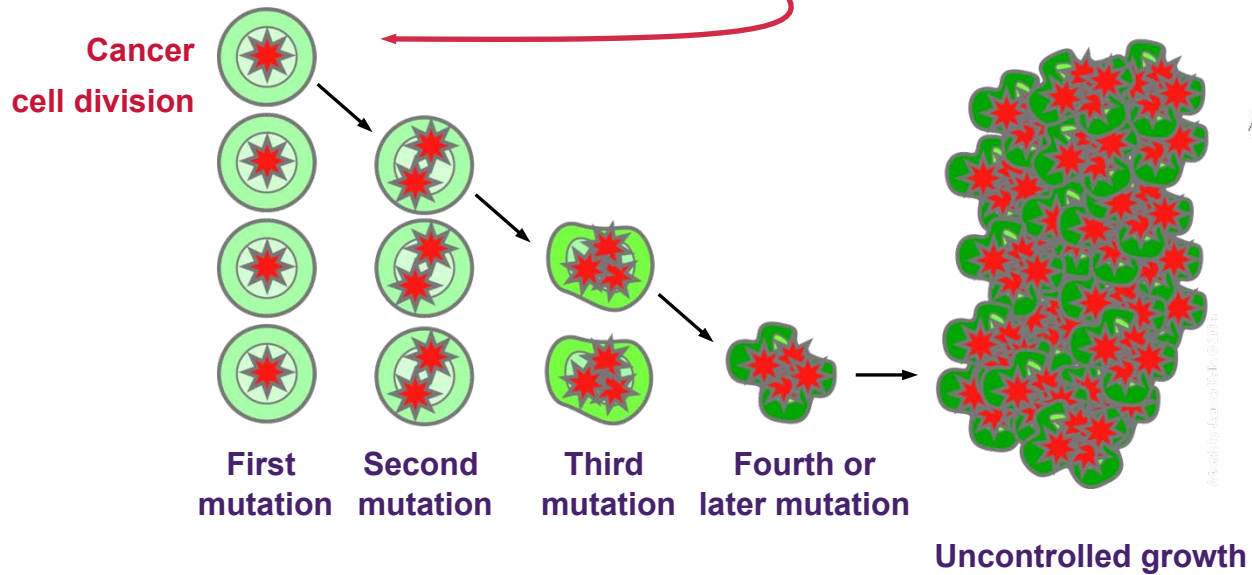
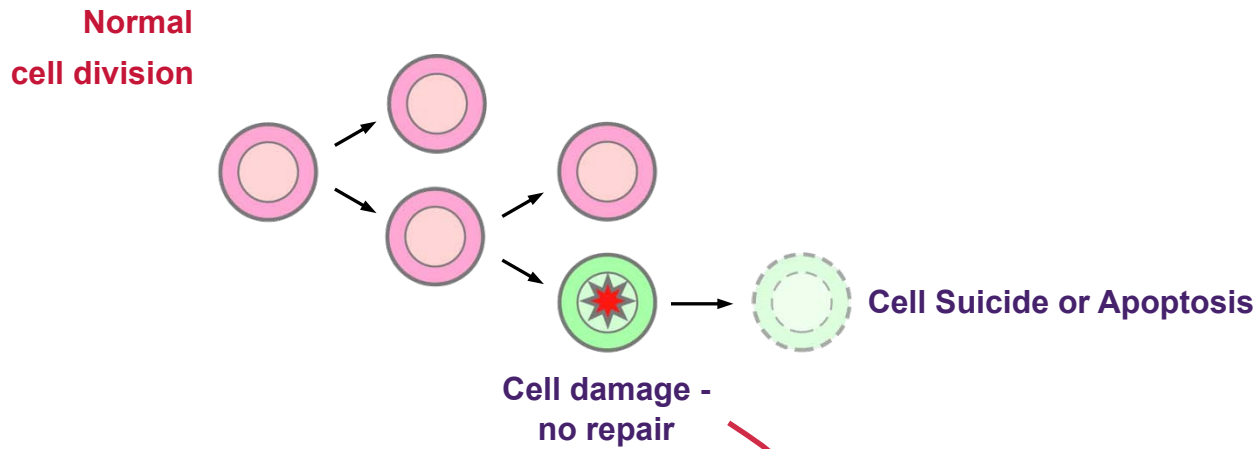


Regulated by:

- » Growth Factors
- » Cell contacts
- » Control check-point molecules

Cell Suicide or Apoptosis

Loss of normal cell growth

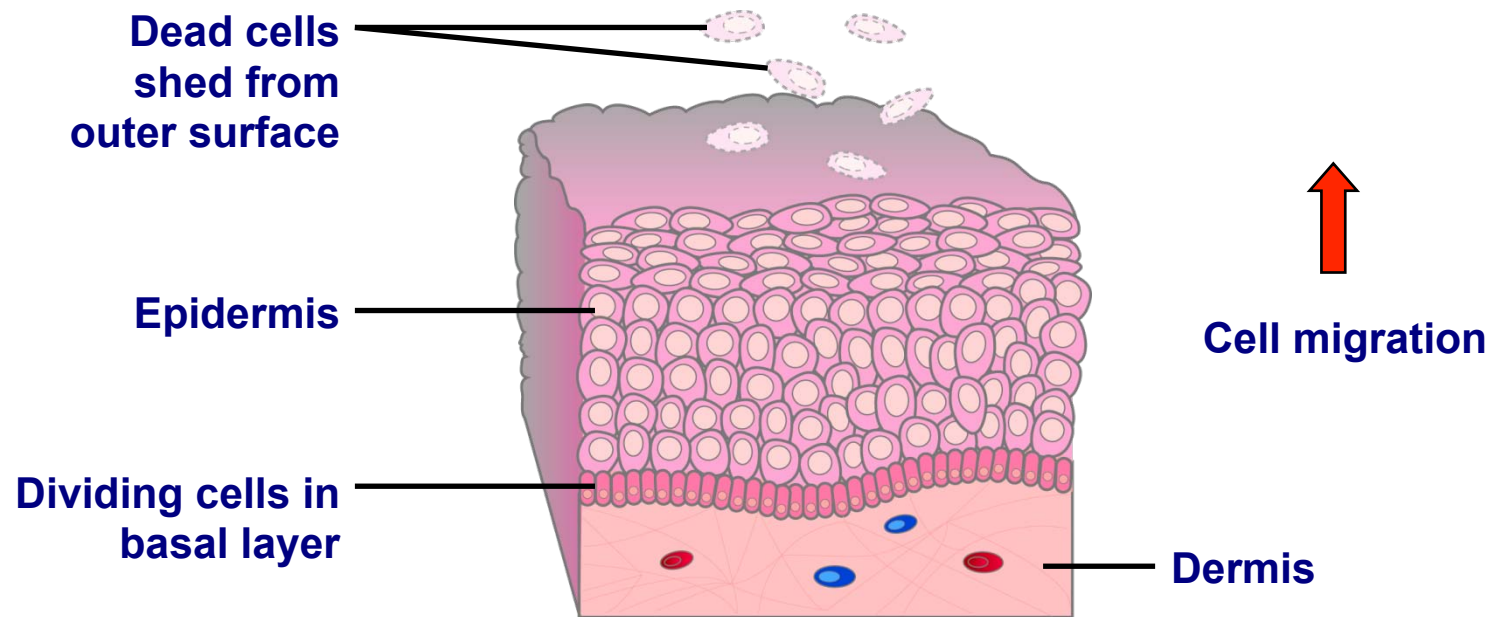


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Example of normal growth



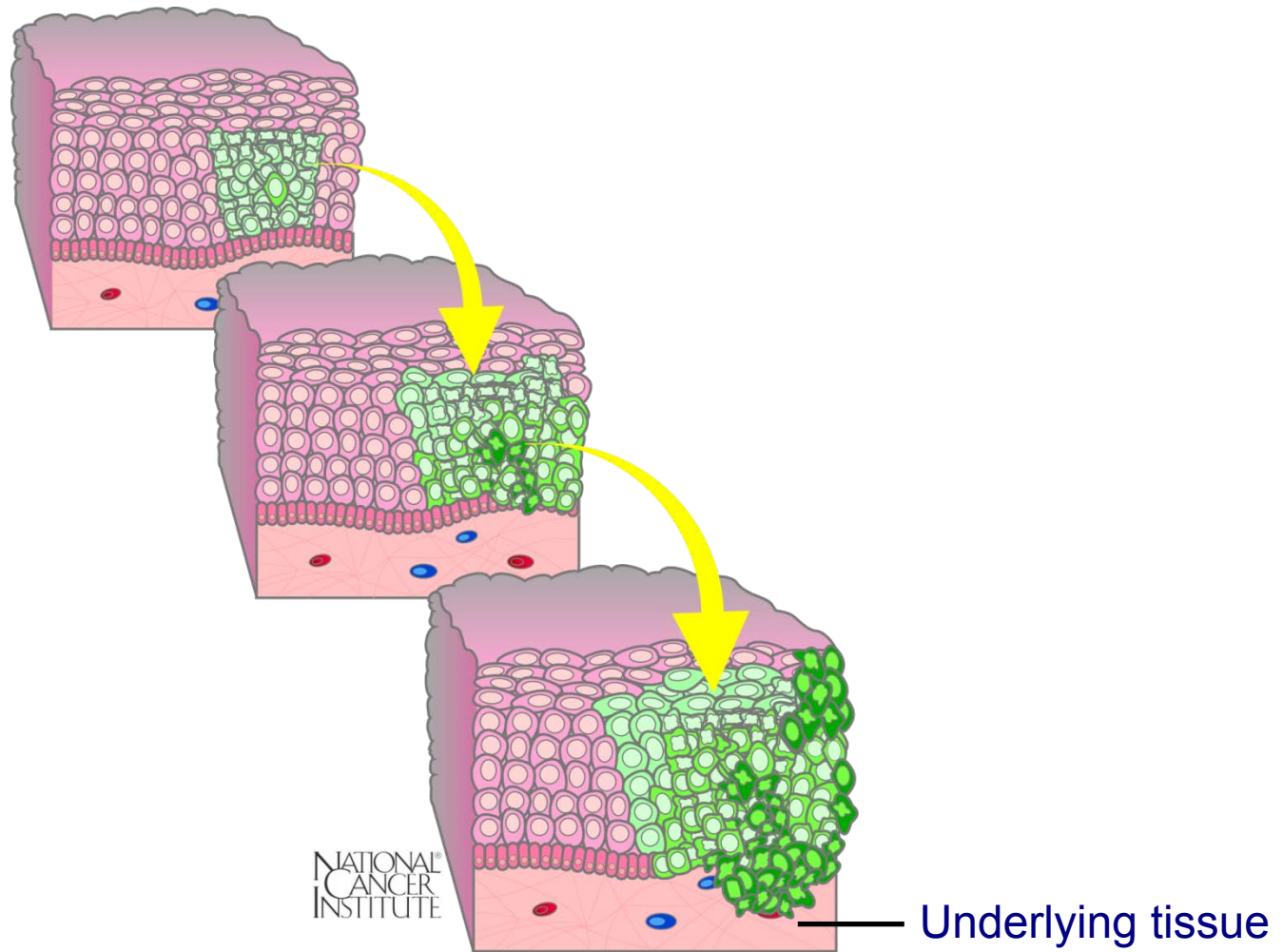
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Number of dividing cells in the basal layer = Number of dead cells that are lost from the surface

Tumors

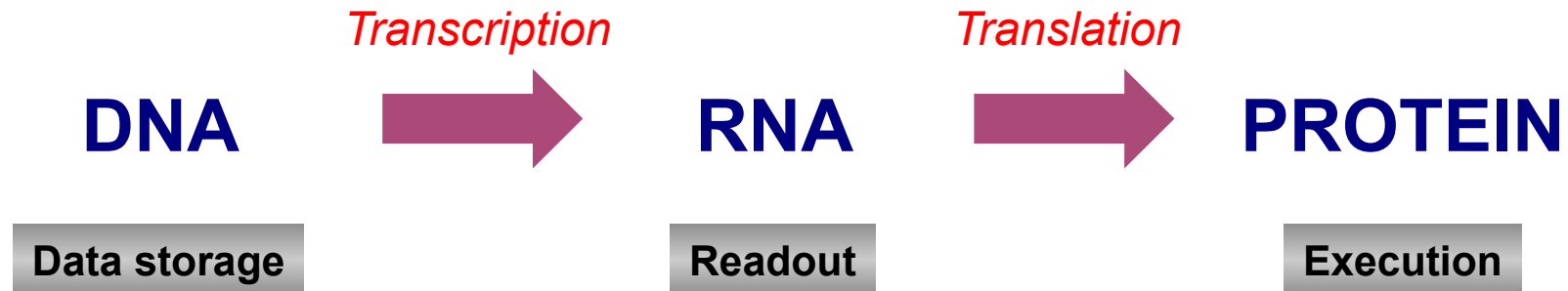


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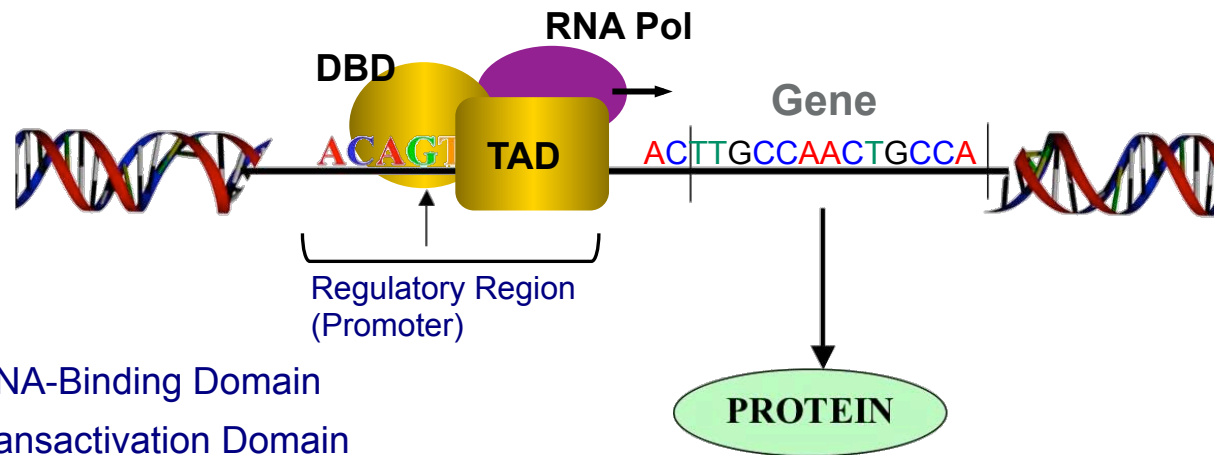
The central dogma of Molecular Biology



Transcription Factors: definition

- **Transcription factors are proteins involved in the regulation of gene expression that bind to the regulatory regions upstream of genes and either facilitate or inhibit transcription.**
- **Transcription factors are composed of two essential functional regions:**
 - The DNA-binding domain consists of amino acids that recognize specific DNA bases near the start of transcription.
 - The activator domains of transcription factors interact with the components of the transcriptional apparatus (RNA Polymerase) and with other regulatory proteins, thereby affecting the efficiency of DNA binding

Transcription factor: how it works?



DBD = DNA-Binding Domain
TAD = Transactivation Domain

1986: discovery of the Nuclear Transcription Factor, NF- κ B

Cell, Vol. 47, 921–928, December 26, 1986, Copyright © 1986 by Cell Press

Inducibility of κ Immunoglobulin Enhancer-Binding Protein NF- κ B by a Posttranslational Mechanism

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Whitehead Institute for Biomedical Research
Cambridge, Massachusetts 02142
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

NF- κ B is a nuclear protein, found only in cells that transcribe immunoglobulin light chain genes, that interacts with a defined site in the κ immunoglobulin enhancer. This protein can be induced in pre-B cells by stimulation with bacterial lipopolysaccharide (LPS). The induction involves a posttranslational activation, and the combined action of LPS and cycloheximide causes a superinduction. An active phorbol ester also induces this factor, and with kinetics more rapid than

Ephrussi et al. (1985) and Church et al. (1985) have reported in vivo footprinting analysis of a putative B-cell-specific enhancer-binding protein that interacts with the immunoglobulin heavy chain enhancer, and in vitro (Sassone-Corsi et al., 1985) and in vivo experiments (Mercola et al., 1985) have shown that enhancer effects may be competed away by the presence of large excesses of the enhancer sequence.

As a step toward understanding the mechanism of tissue-specific enhancer function, we have recently reported the identification of five factors that interact with the B-cell-specific heavy chain and κ light chain enhancers (Sen and Baltimore, 1986; Staudt et al., 1986; Weinberger et al., 1986; Singh et al., 1986). The most interesting of these factors, NF- κ B, is one that interacts only with the κ enhancer and appears to be stage-specific within the lymphoid lineage, being expressed in mature B cells and plasma cells but not in pre-B cells or T cells (Sen and Baltimore, 1986). This distribution corresponds to

40,871 articles on the subject NF- κ B... and counting: Why then?

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NFKB1 (NFKAPPAB) nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 [Homo sapiens]

► [nfkappab](#) in [Homo sapiens](#) | [Mus musculus](#) | [Xenopus laevis](#) | [All 3 Gene records](#)

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2. Kotsakis P, Wang Z, Collighan RJ, Griffin M.
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5. Fan JT, Su J, Peng YM, Li Y, Li J, Zhou YB, Zeng GZ, Yan H, Tan NH.
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Is **NF-kappaB** a good target for cancer therapy? Hopes and pitfalls. [Nat Rev Drug Discov. 2009]

SIRT6 links histone H3 lysine 9 deacetylation to **NF-kappaB**-dependent gene express [Cell. 2009]

NF-kappaB signaling, liver disease and hepatoprotective agents. [Oncogene. 2008]

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Sphingosine-1-phosphate receptor-2 deficiency leads to inhibition of macroph [J Clin Invest. 2010]

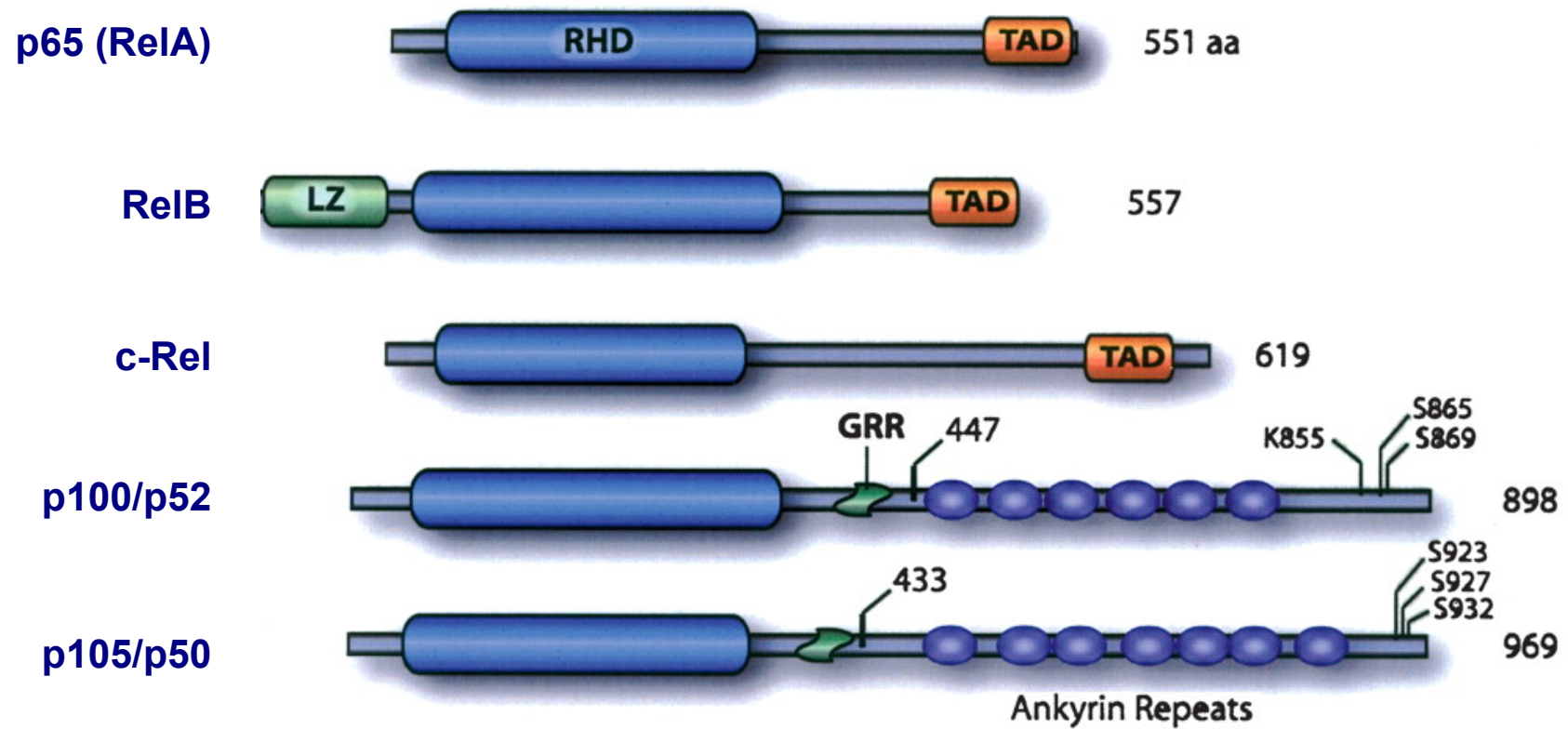
Gene network analysis of bone marrow mononuclear cells reveals activ [PLoS One. 2010]

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Database:

Nuclear Factor- κ B (NF- κ B) protein family



NF- κ B: assembling details

The term NF- κ B refers to a family of inducible dimeric transcription factors that recognize a common sequence motif, the “ κ B site”

The possible dimeric combinations are:

p50:p50

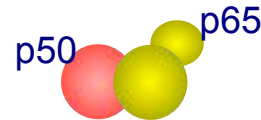
p65:p65

RelB:RelB

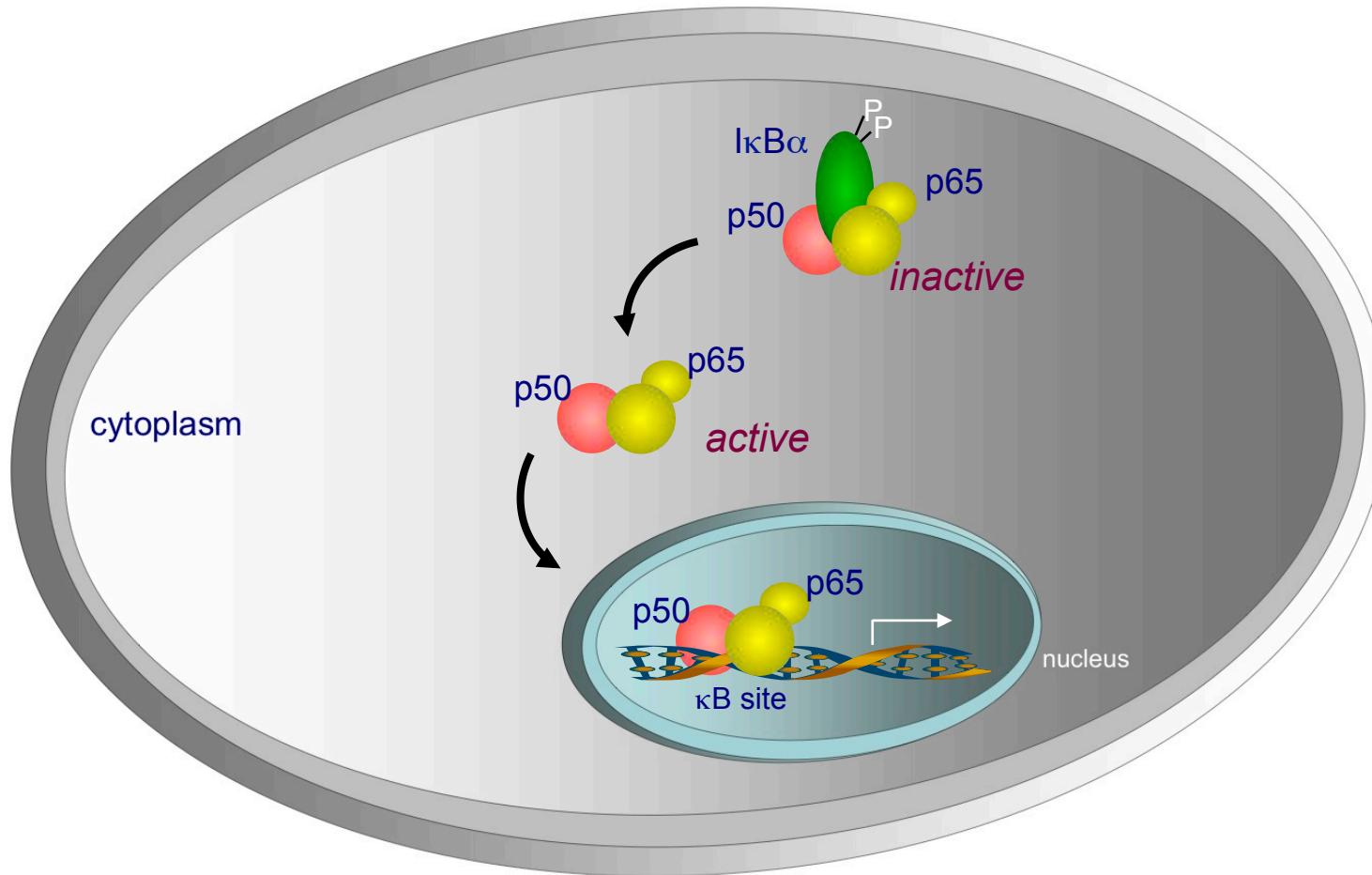
p50:p65

p50:RelB

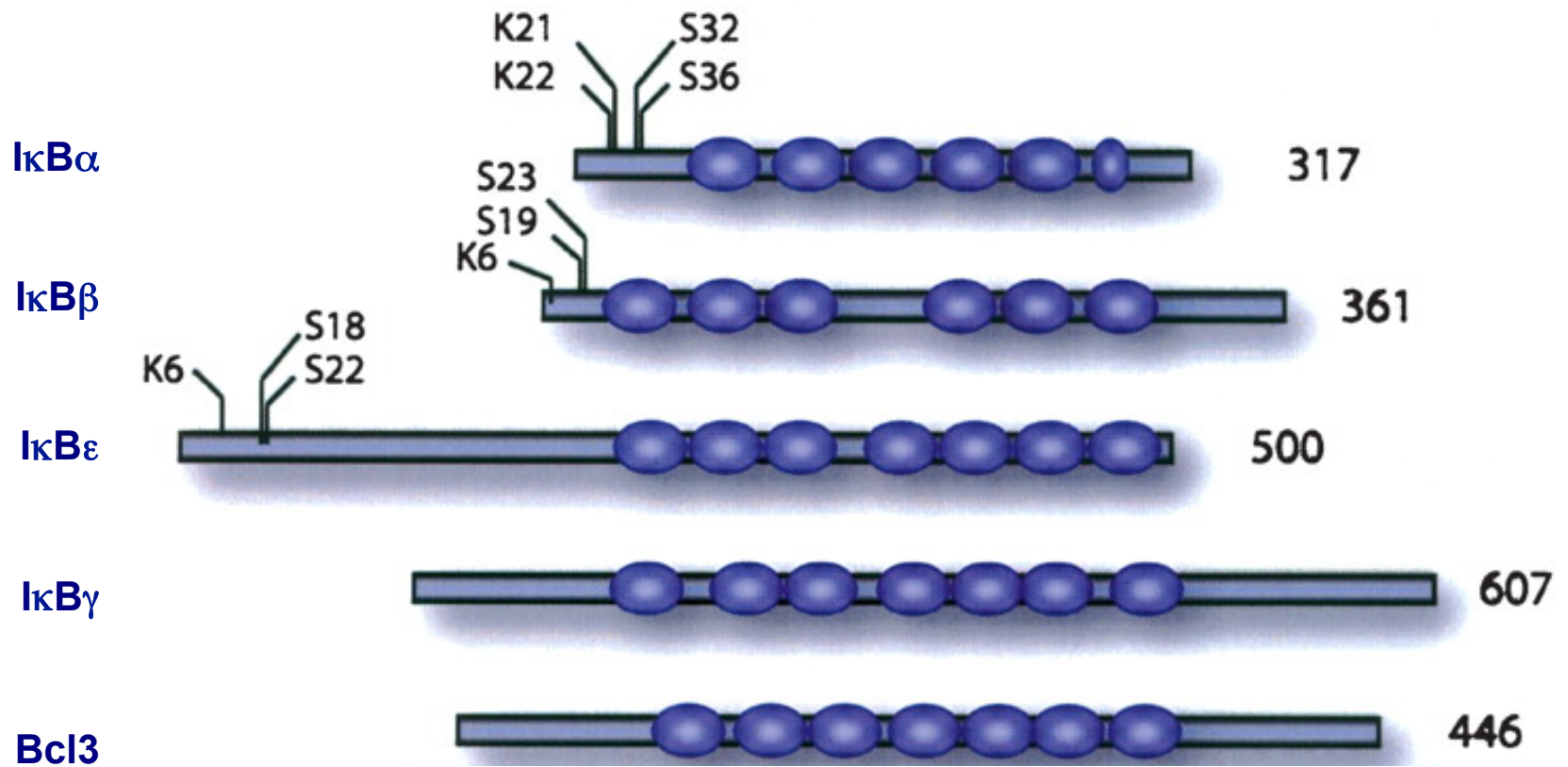
p52:RelB



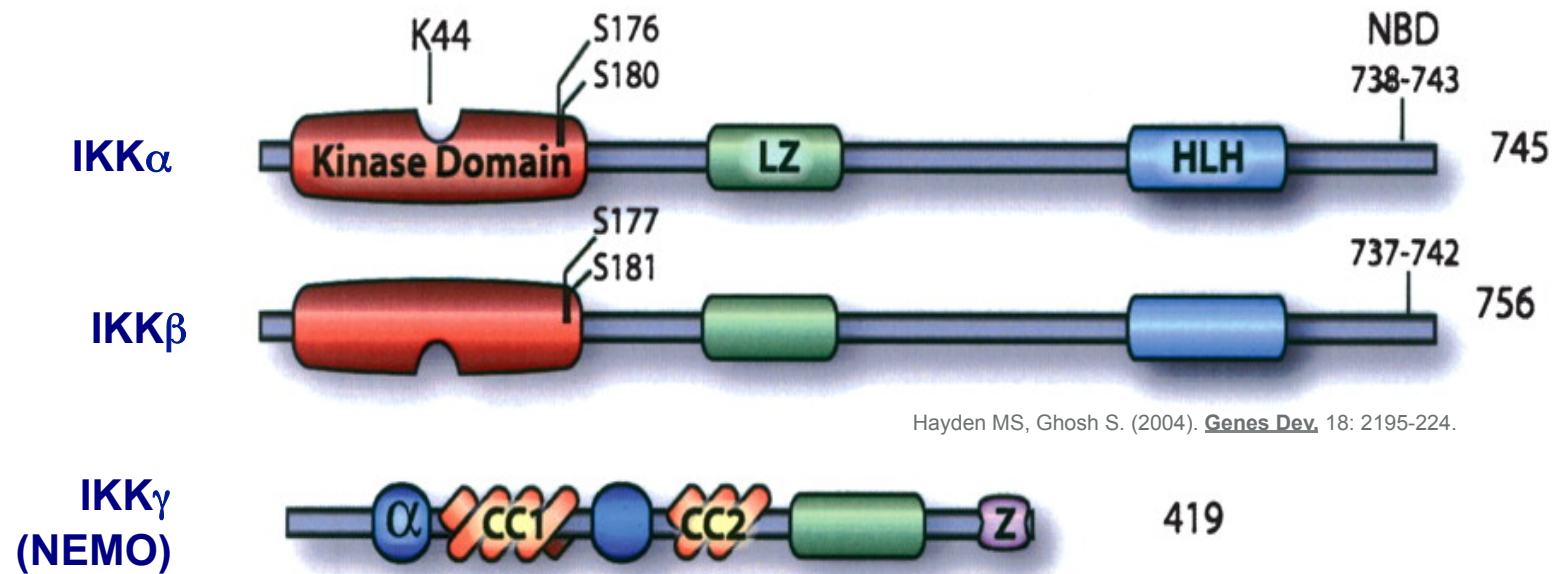
NF- κ B: assembling details



Inhibitor- κ B ($I_{\kappa}B$) proteins family

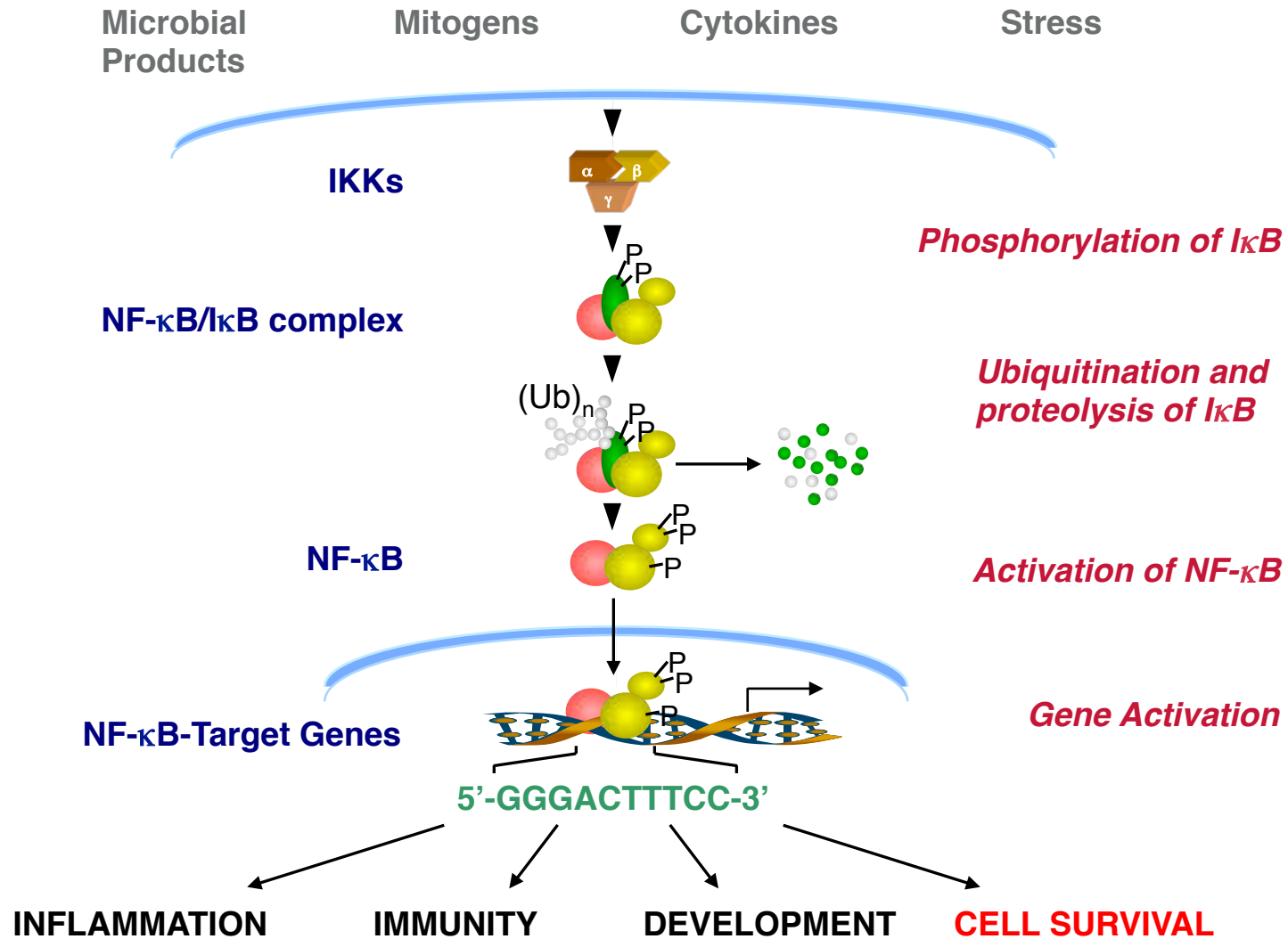


Inhibitor- κ B kinase (IKK) proteins family

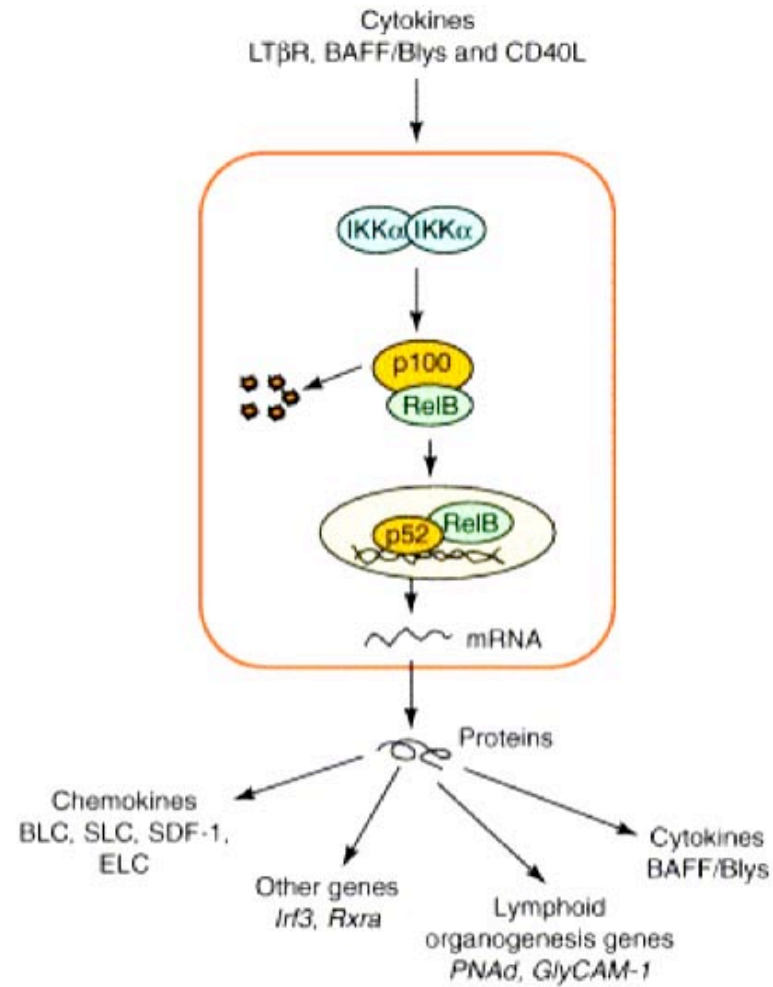


Hayden MS, Ghosh S. (2004). *Genes Dev.* 18: 2195-224.

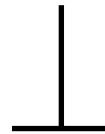
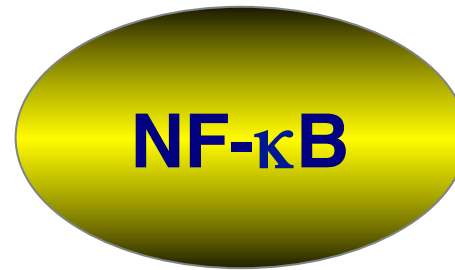
The classical NF- κ B pathway



The alternative NF- κ B pathway



The pro-survival activity of NF- κ B



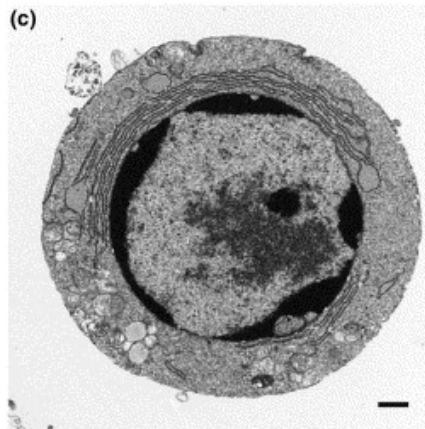
*Programmed
Cell Death*

What is Programmed Cell Death (PCD)?

An important mechanism in both development and homeostasis in adult tissue for removal of either superfluous, infected, transformed or damaged cells by activation of an intrinsic suicide program

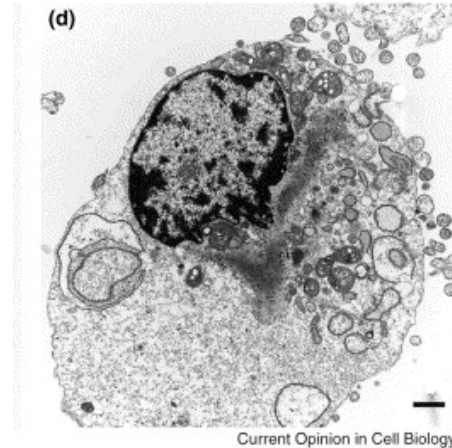
Programmed Cell Death

Apoptosis



versus

Necrosis



- Energy-dependent Process
- Suppression of Inflammation
- Plasma membrane integrity maintained
- Order DNA fragmentation
- Cell first shrink, nuclei condense formation of '*apoptotic bodies*'

- Energy-independent Process
- Induction of Inflammation
- Plasma membrane integrity lost
- Random DNA fragmentation
- Cell swells, ruptures during demise Releasing of cellular contents

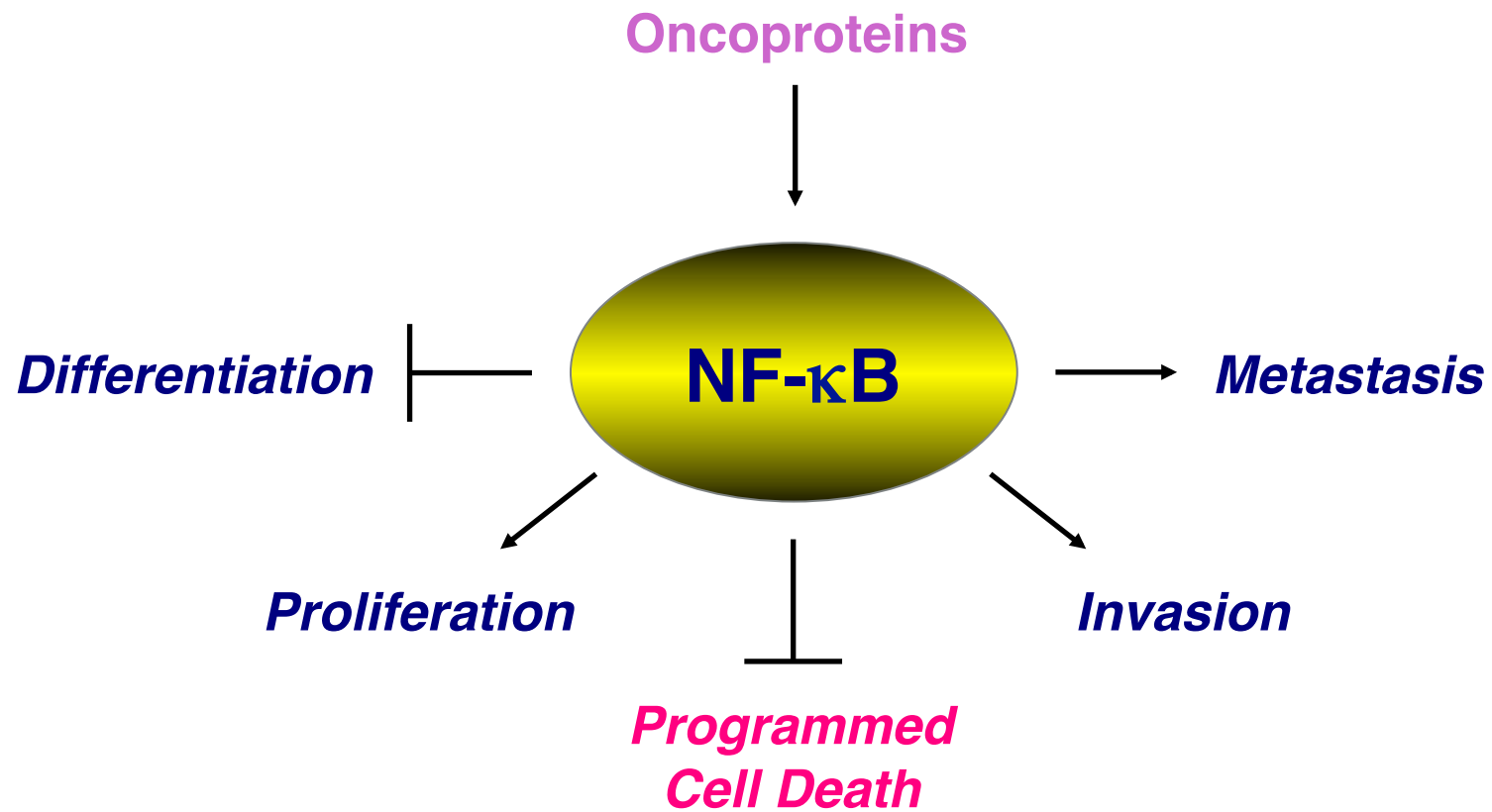
Biological roles of the pro-survival activity of NF- κ B

- Cellular Responses to the Triggering of
- TNF-Rs, TRAIL-Rs and Fas
- B Lymphopoiesis
- Bone Morphogenesis
- B- and T-Cell Costimulation (CD40, CD28, etc.)
- Liver Development

The pro-survival activity of NF- κ B in disease

- Cellular Responses to the Triggering of
 - TNF-Rs, TRAIL-Rs and Fas
 - B Lymphopoiesis
 - Bone Morphogenesis
 - B- and T-Cell Costimulation (CD40, CD28, etc.)
 - Liver Development
-
- **Cancer**
 - **Cancer chemo- and radio-resistance**
 - **Chronic inflammatory disease (IBD, RA)**
 - **Metabolic & vascular disorder (atherosclerosis)**

NF- κ B in cancer



Role of the NF- κ B pro-survival activity in cancer

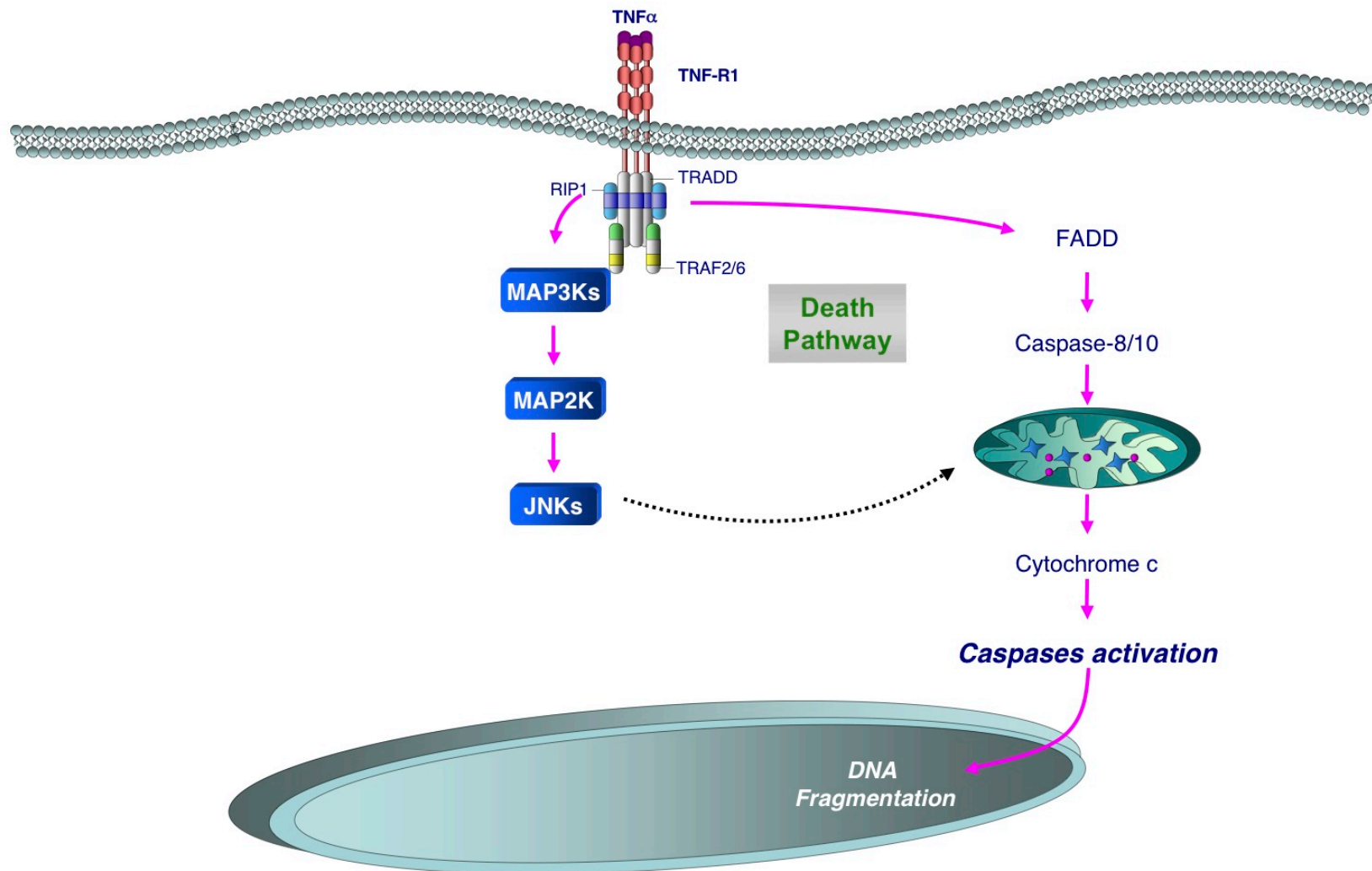
- ***Suppression of Transformation***
 - ***Associated Apoptosis:***
 - oncogenic Ras, Bcr-Abl, etc.
- ***Survival of Late Stage Tumors:***
 - Hodgkin's Lymphoma
 - Breast Cancer
 - Diffuse Large B Cell Lymphoma
 - Multiple Myeloma
 - Colon Cancer
 - Prostate Cancer
 - Burkitt's Lymphoma
- ***Resistance to Anti-Cancer Therapy:***
 - Ionizing Radiation
 - Topoisomerase Inhibitors
 - Cisplatin

Summary: part 1

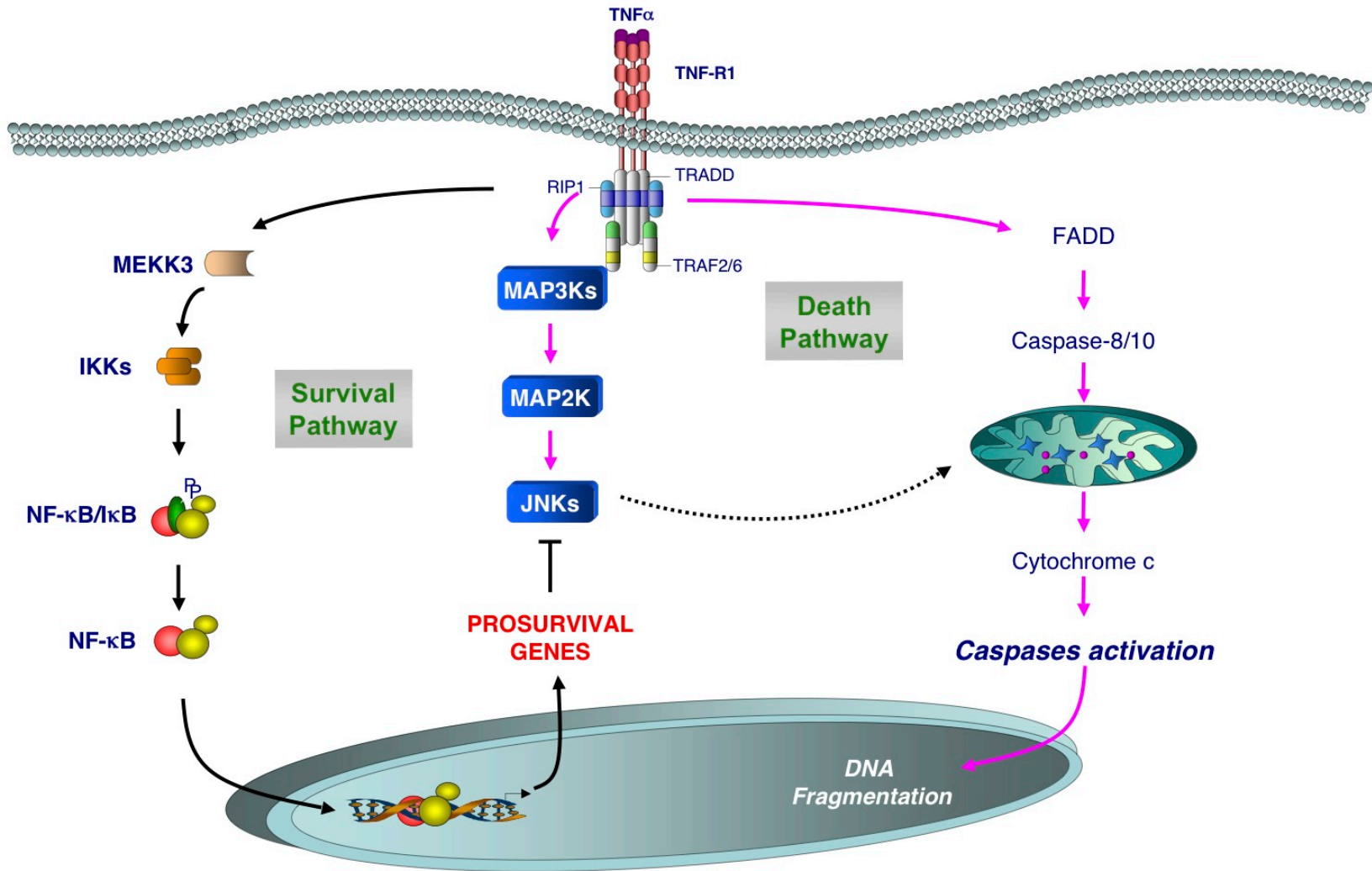
- **NF- κ B** are transcription factors that block Programmed Cell Death (PCD)
- Apoptosis and necrosis are two forms of PCD
- Deregulation of the ability of NF- κ B to control PCD leads to oncogenesis

**What is the molecular mechanism by which
NF- κ B control Programmed Cell Death?**

TNF-R1 signalling



TNF-R1 signalling



Protein kinase: definition

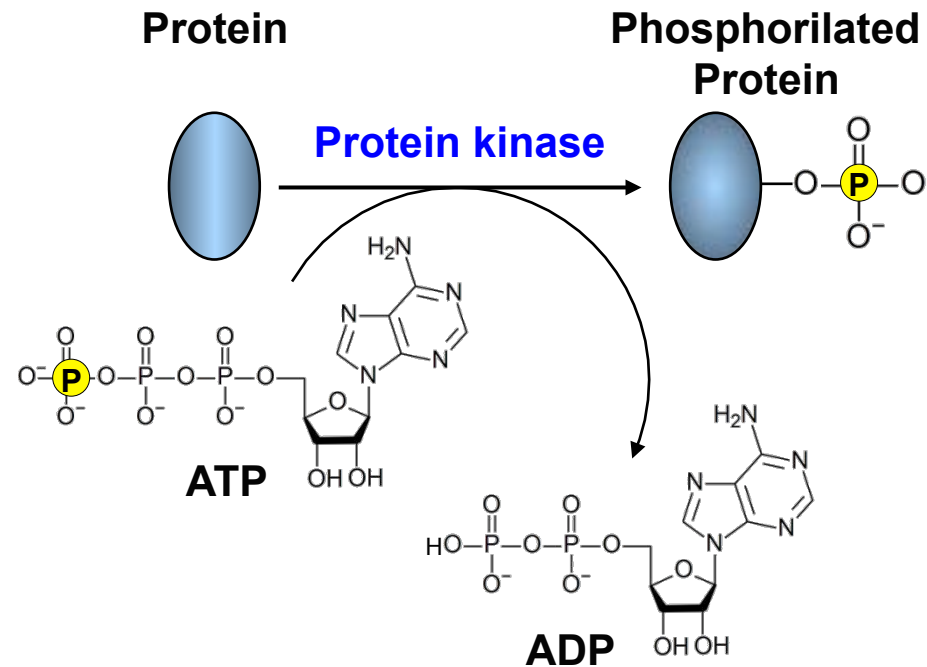
A **kinase** is an enzyme that modifies other proteins by adding phosphate groups (phosphorylation).

Phosphorylation usually results in a functional change of the target protein (substrate).

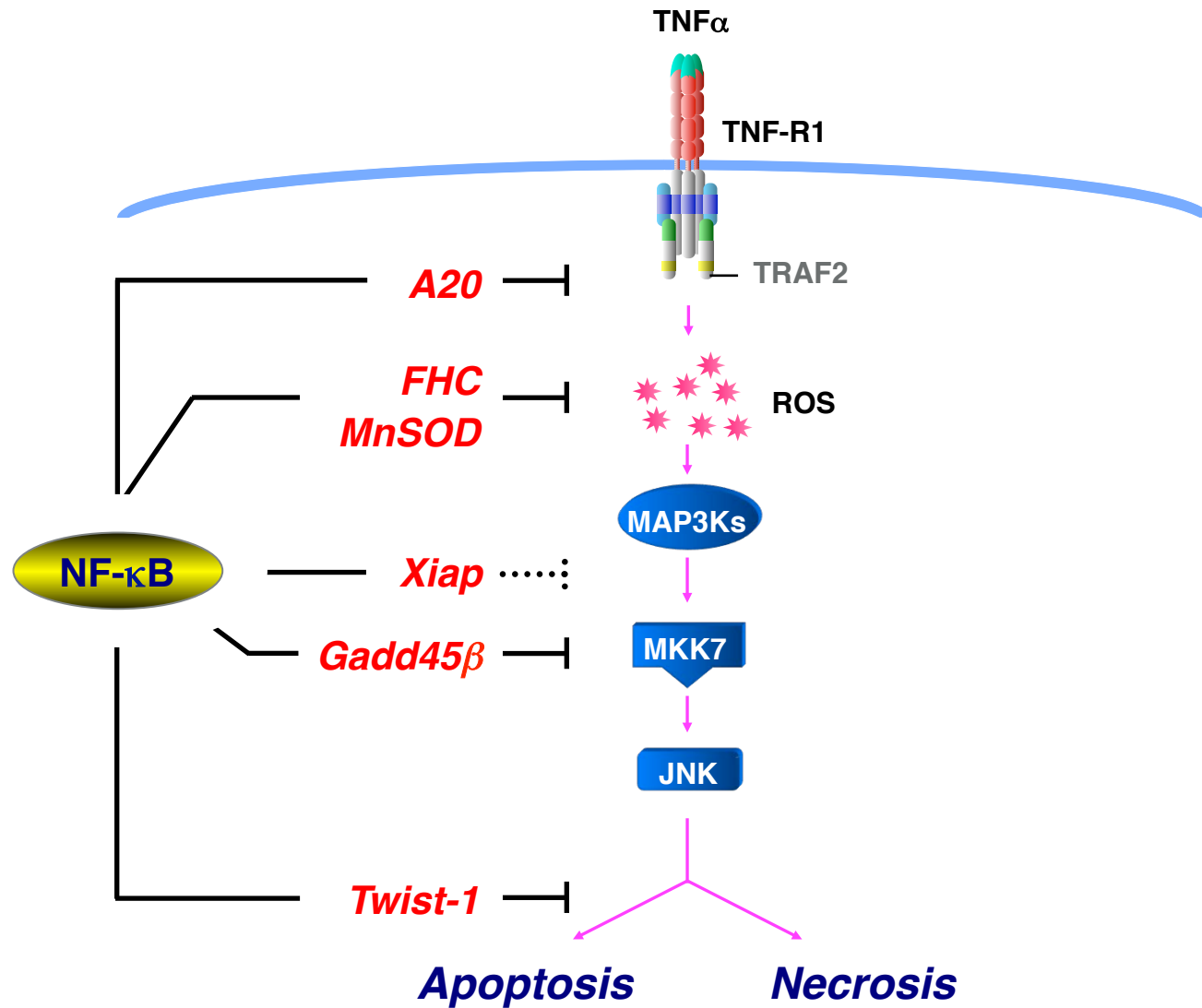
The activity of a kinase involves removing a phosphate group from ATP and covalently attaching it to an amino acids that have a free hydroxyl group.

Most kinases act on both serine and threonine, others act on tyrosine.

By contrast, **phosphatase** is an enzyme that removes a phosphate group from its substrate.



Distinct mechanisms of PCD inhibition



Gadd45 β /Myd118

- ~21 kDa
- Predominantly Nuclear
- Member of the Gadd45 Family of Inducible Factors,
- Also Including Gadd45 α and Gadd45 γ
- Proposed Functions of Gadd45 β :
- Specific inhibitor of JNK cascade
- Ectopic expression of Gadd45 β inhibits the
- JNK-mediated PCD downstream of TNFR

Gadd45 β interact with MKK7

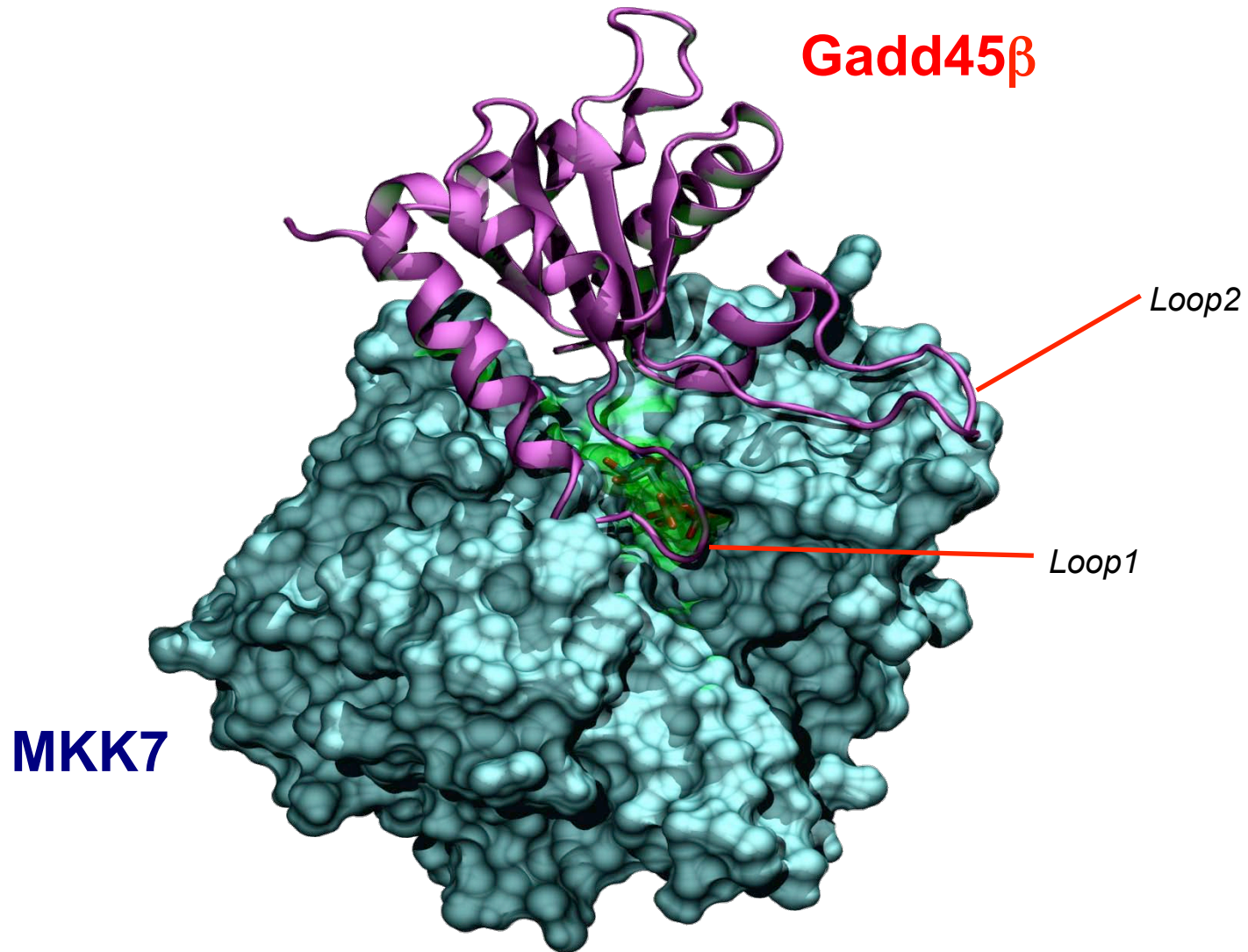
Active MKK7



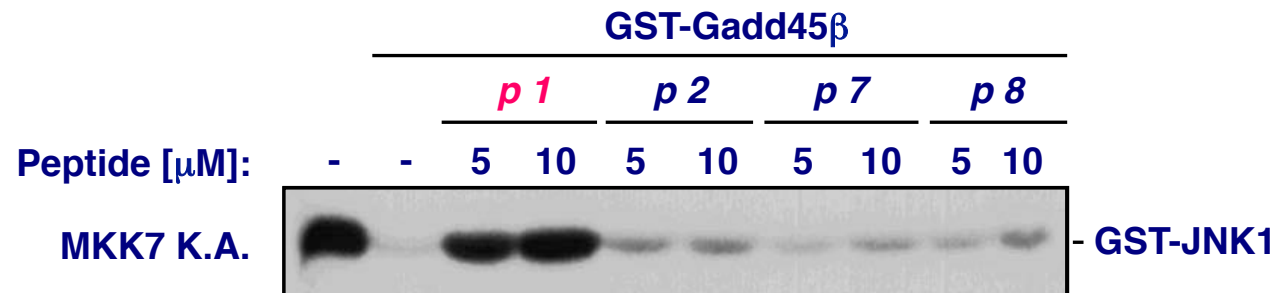
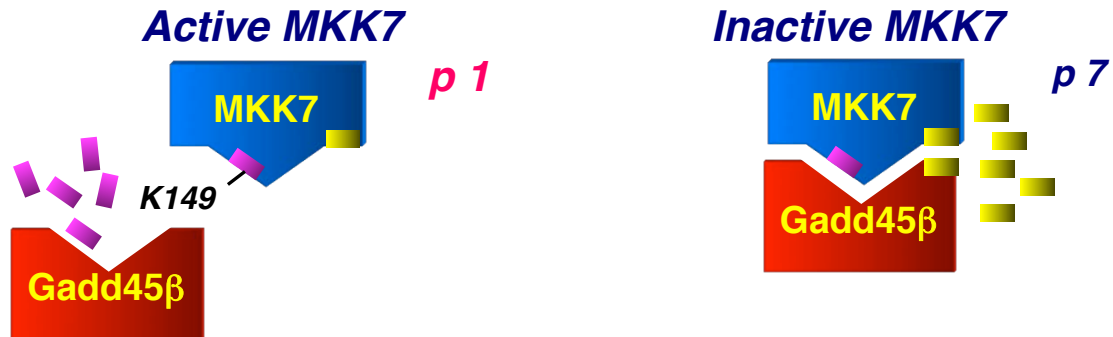
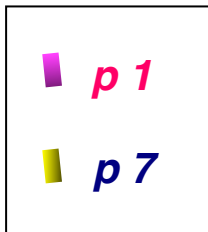
Inactive MKK7



Gadd45 β bind the ATP-binding site of MKK7

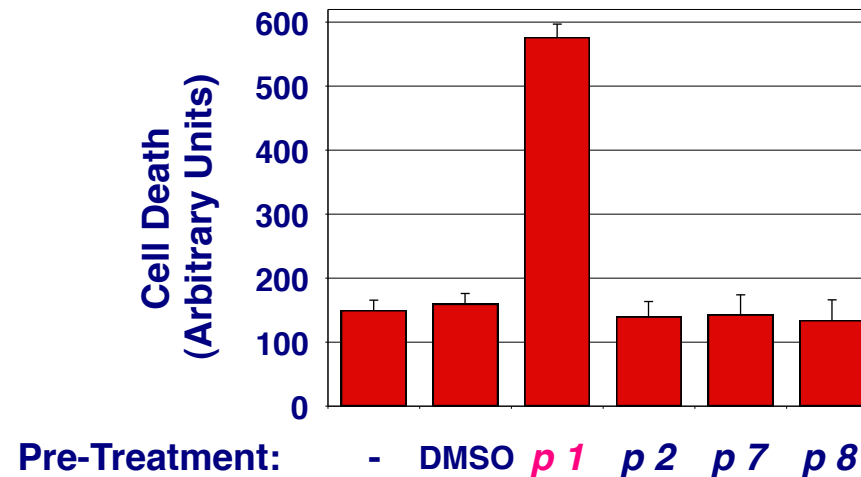


Peptides (P1) disrupting Gadd45 β /MKK7 interaction restores MKK7 activity



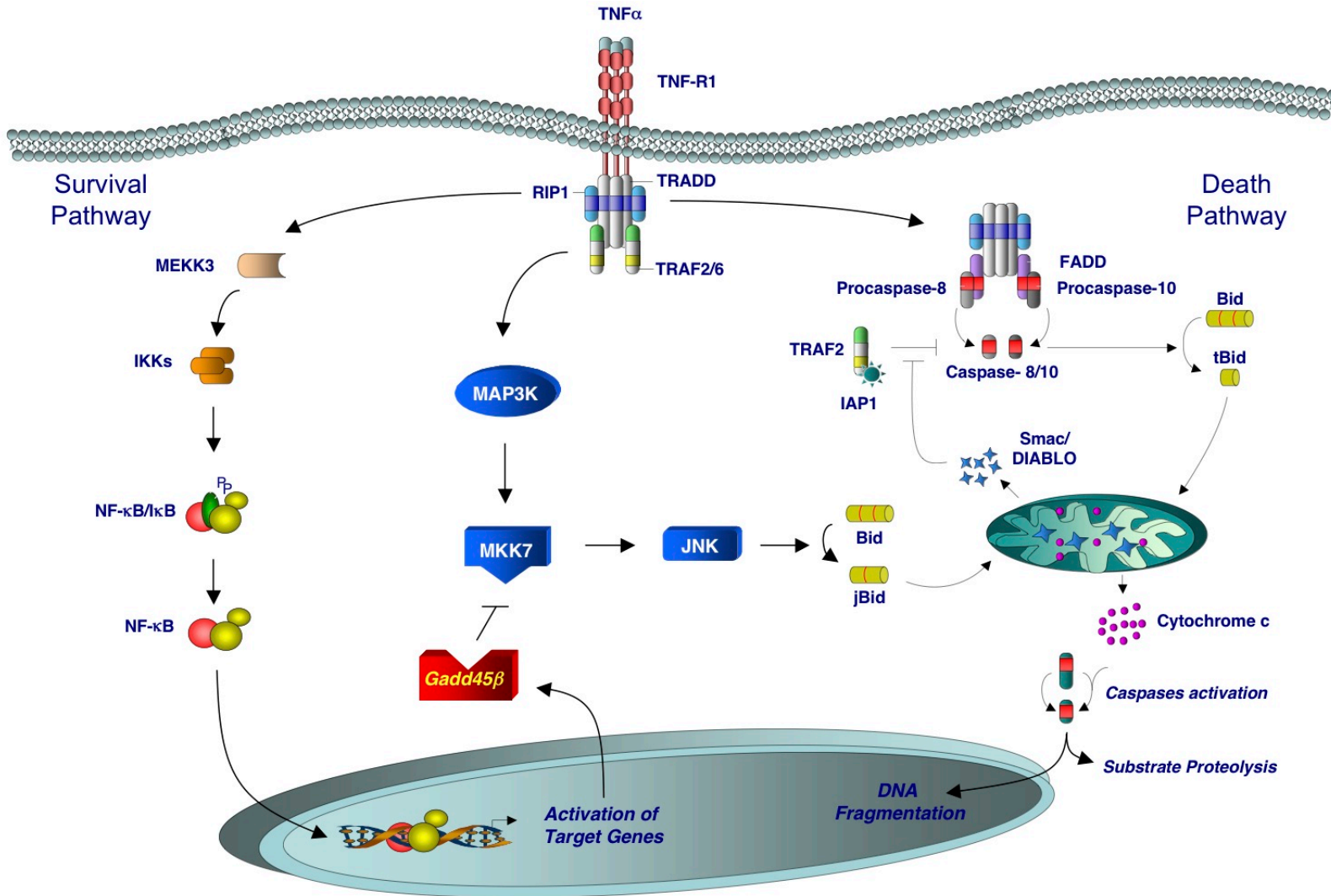
Cell-permeable Peptide 1 (P1) prevents inhibition of $\text{TNF}\alpha$ -induced apoptosis

NF- κ B-null cells over-expressing Gadd45 β



NB: Cell treatment with Peptide 1 induce death in cells otherwise resistant to death-signal

Summary: part 2

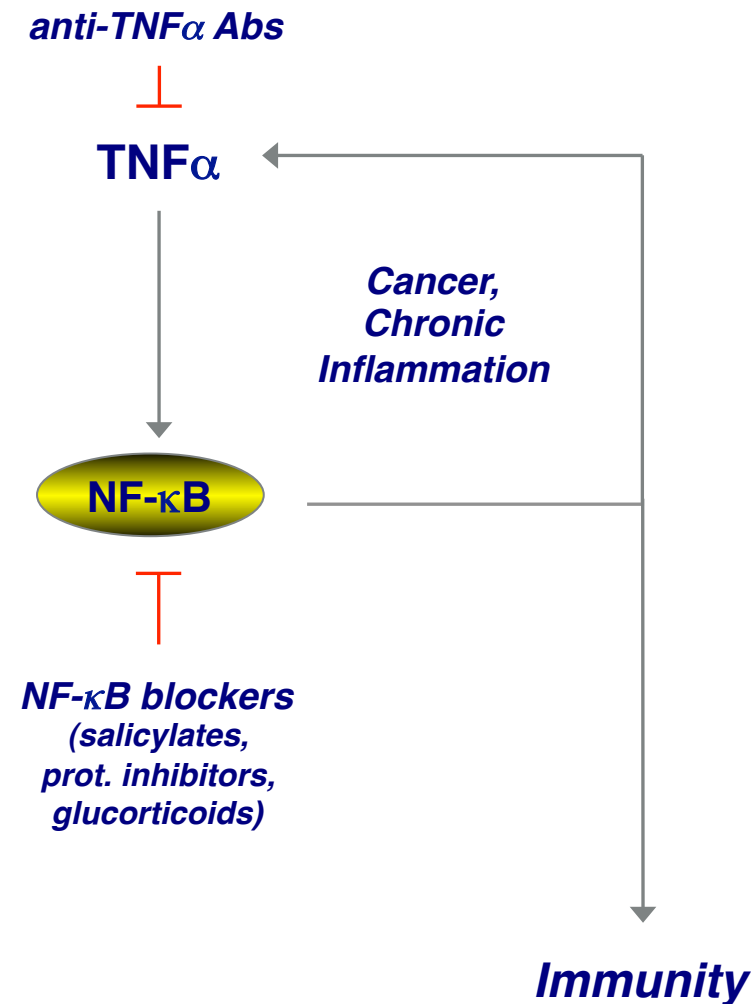


**Why study molecular mechanisms by which NF- κ B
blocks PCD are important ???**

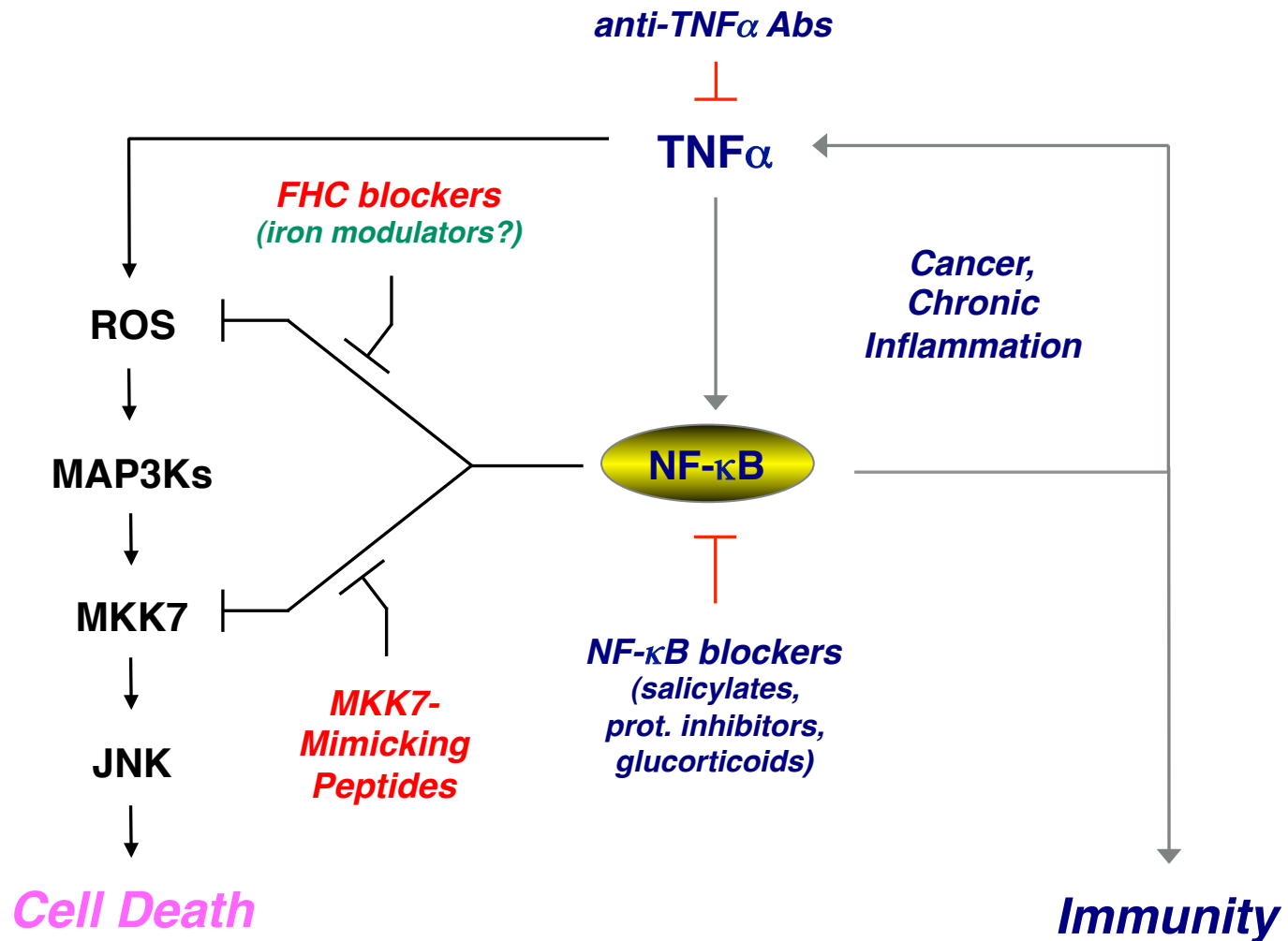
NF- κ B blockers as pharmacological compound

Inhibition of NF- κ B by either glucocorticoids or proteasome inhibitors is beneficial in certain malignancies, including HL and MM.

However, current compounds can only achieve partial inhibition of NF- κ B and have considerable side effects, which limit their use in humans.



Future therapies: Gadd45 β and FHC may represent new targets



Conclusions

- **NF- κ B** are transcription factors that block Programmed Cell Death (PCD)
- Apoptosis and necrosis are two forms of PCD
- Deregulation of the ability of NF- κ B to control PCD leads to oncogenesis
- Downstream TNFR1, Gadd45 β is up-regulated by NF- κ B and inhibits JNK pathway by blocking MKK7
- New anticancer therapy can be developed based on inhibitors of Gadd45 β -MKK7 interaction (?)

Learning outcomes

- **Describe the NF- κ B signalling**
 - *Defined protein families*
 - *The key molecular events and molecules involved in its activation*
- **Compare the morphological changes and the different host response to the two forms PCD**
- **List the genes that are induced by NF- κ B in response to TNFa**
- **Describe one of the molecular mechanism by which NF- κ B blocks PCD**
 - *Draw the JNK pathway*
- **Relate the survival activity of NF- κ B to the therapeutic potential of its inhibition in cancer and the possible complications**

References

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