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Cancer cell regulation

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

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-κB, IκB, and IKK Protein Families
- Molecular mechanisms by which NF-κB blocks cell death
- New therapies prospects

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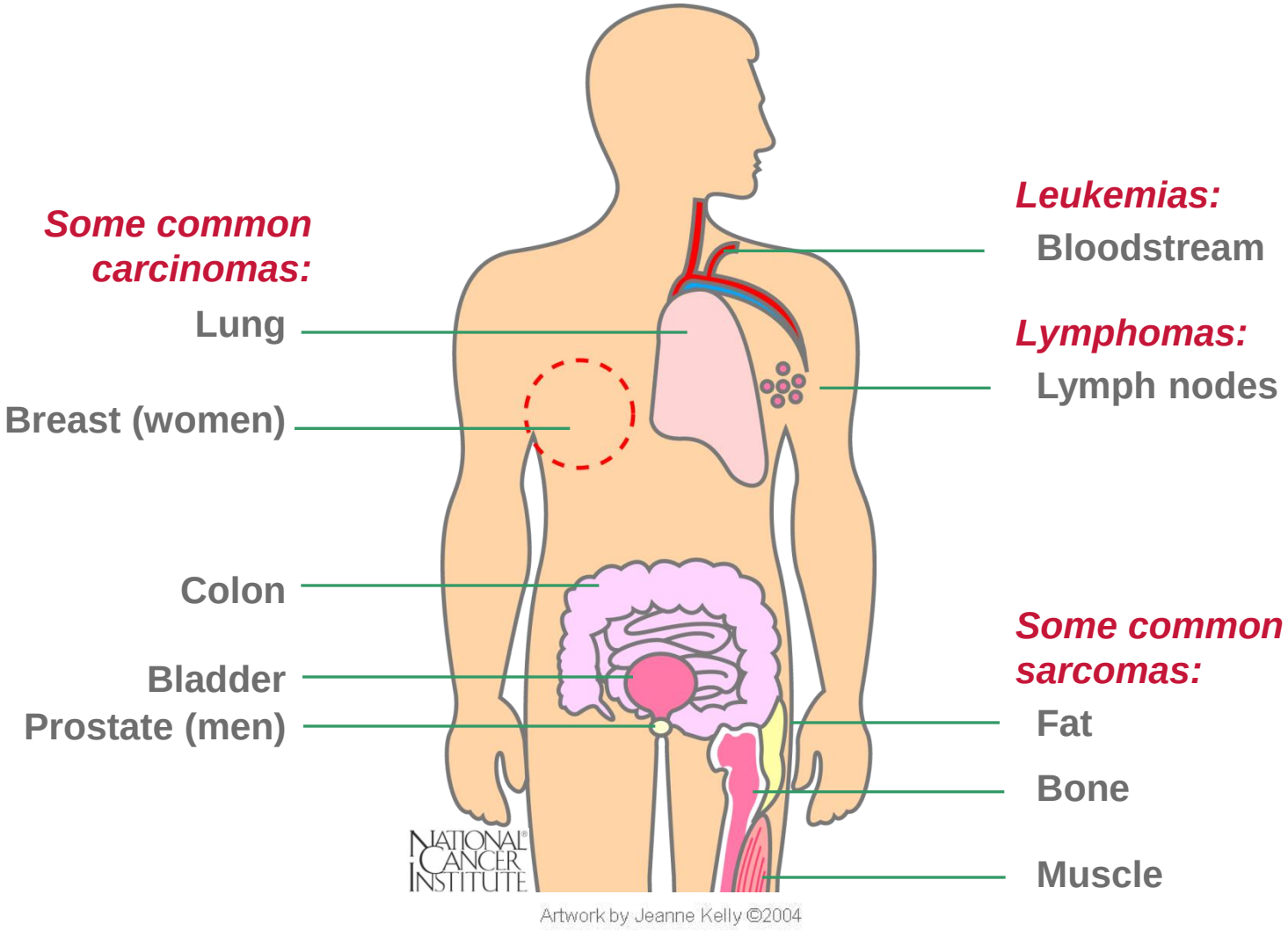
Different kinds of cancer



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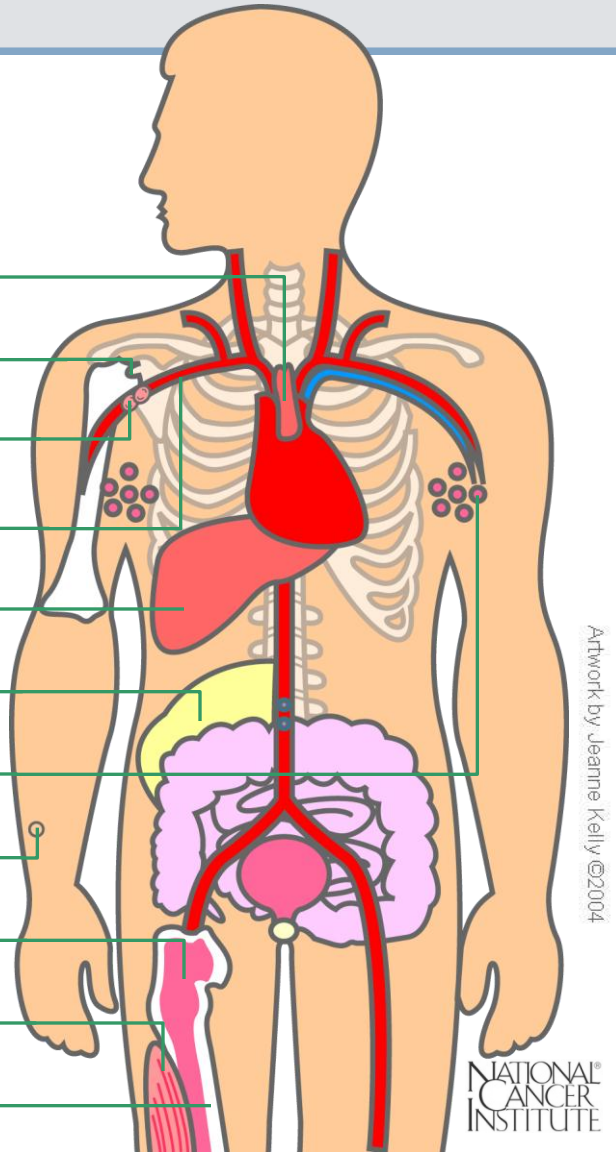
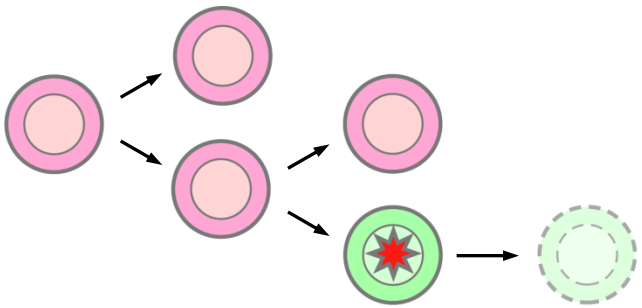
Naming cancer

Prefix	*Meaning*
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



Normal cell growth

Normal
cell division



Cell damage

Cell Suicide or Apoptosis

Regulated by:

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

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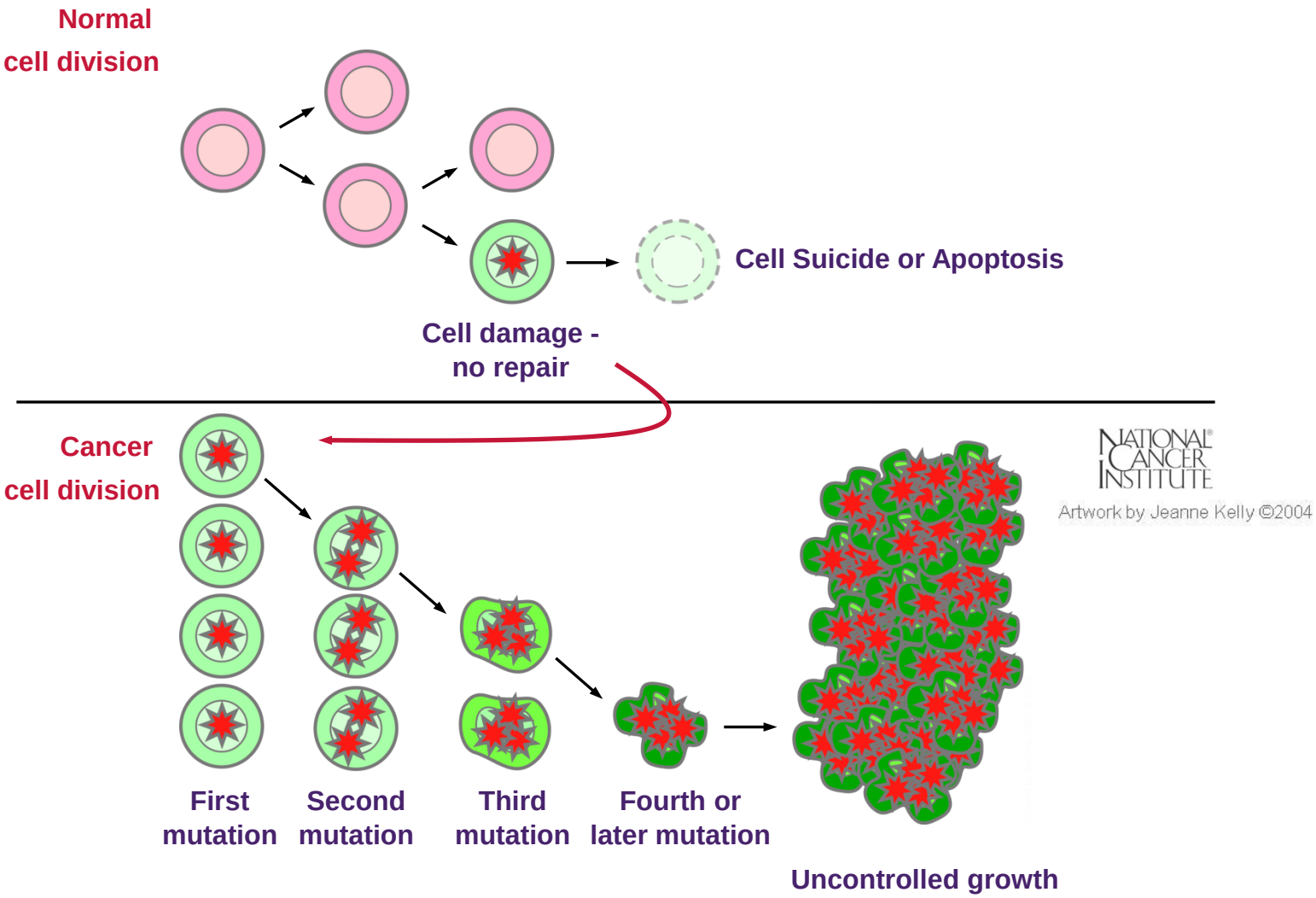
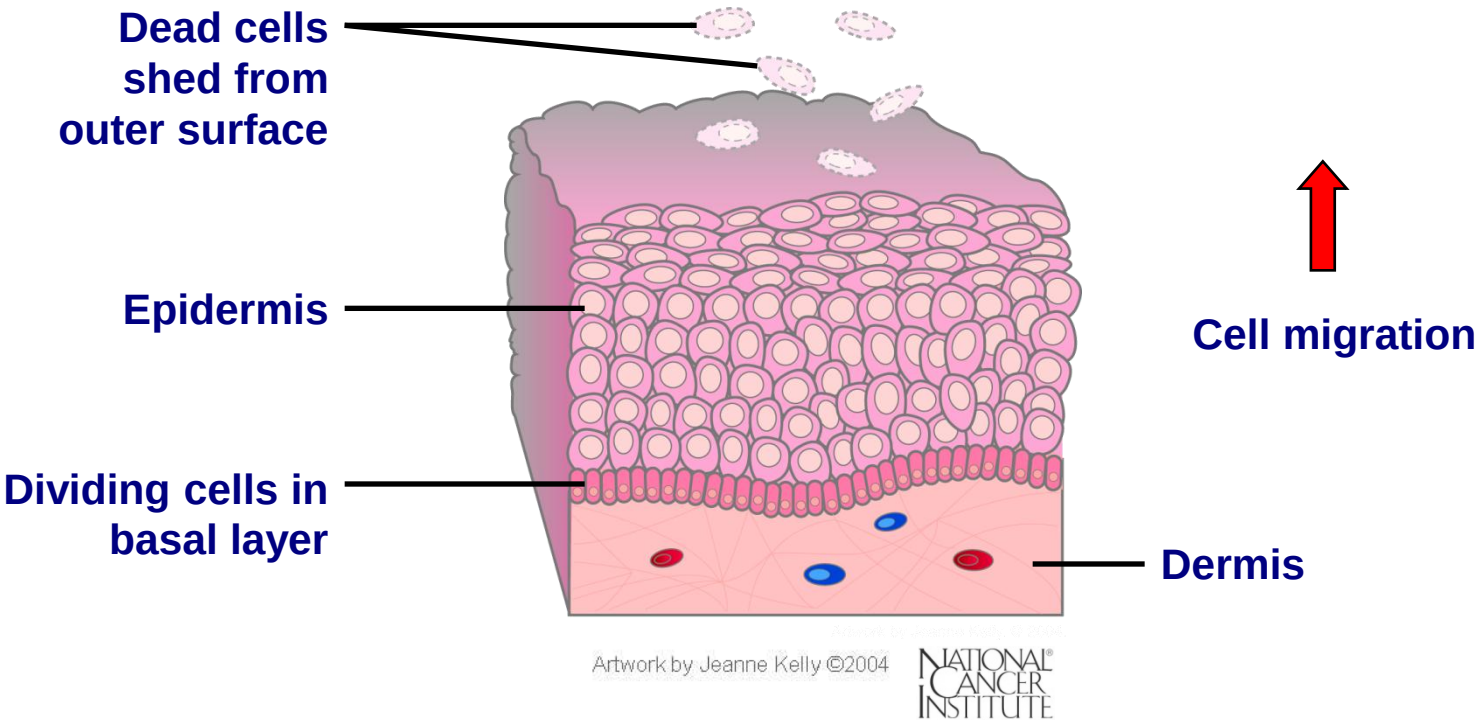
Loss of normal cell growth

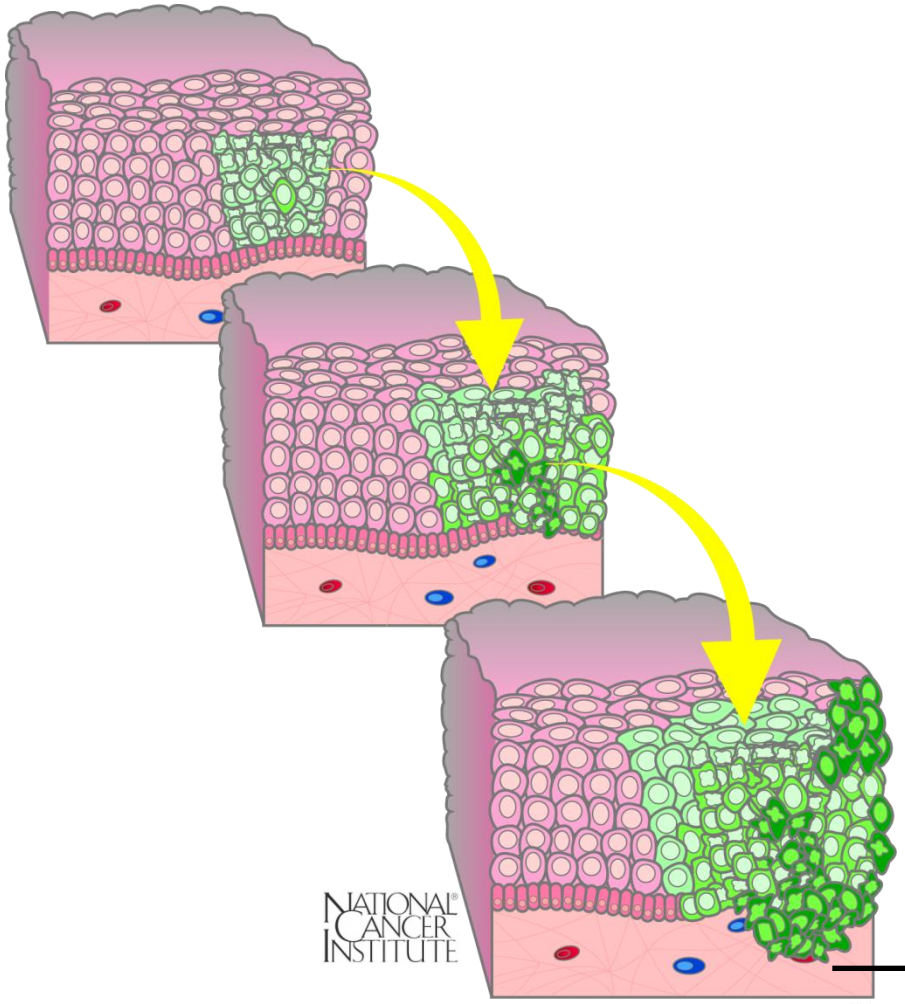


Example of normal growth

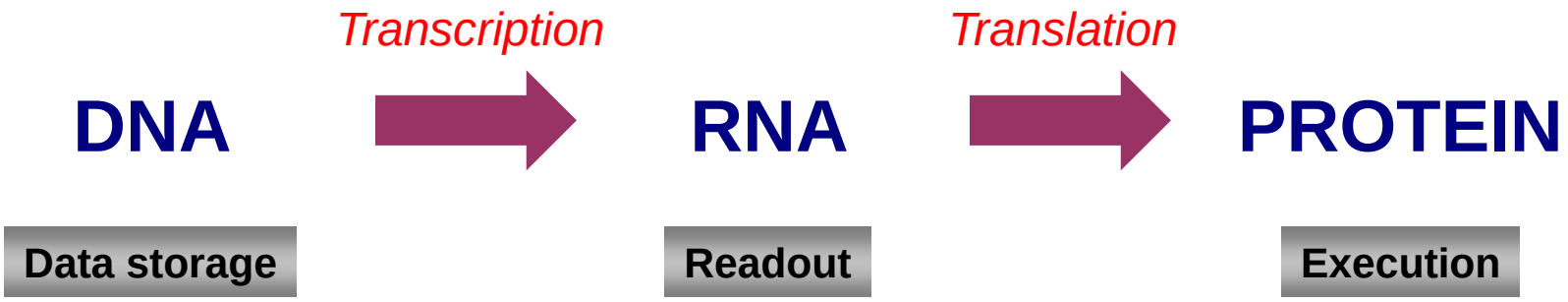


Number of dividing cells in the basal layer = Number of dead cells that are lost from the surface

Tumors


NATIONAL CANCER INSTITUTE
Underlying tissue
Artwork by Jeanne Kelly ©2004

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

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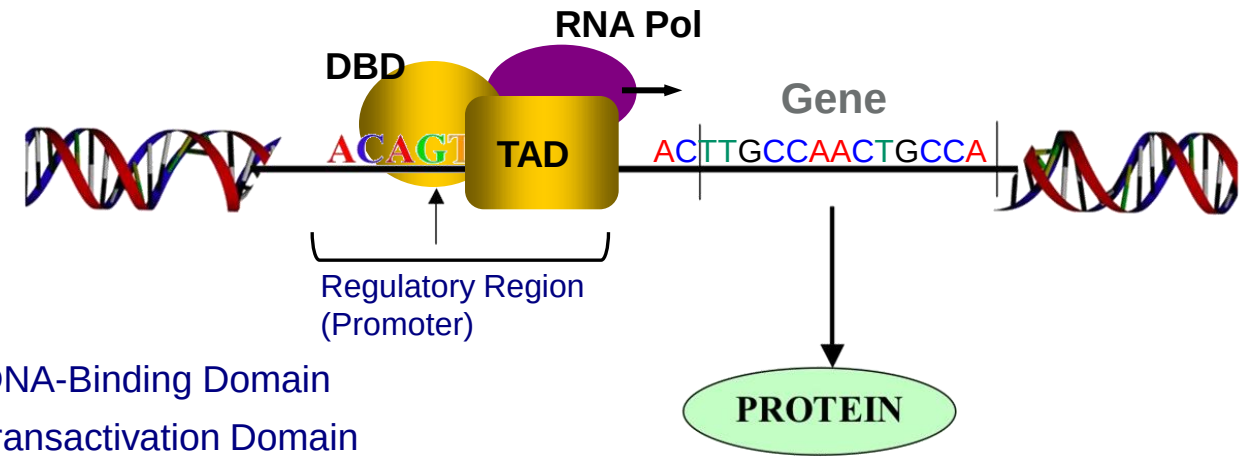
Transcription factor: how it works?



DBD = DNA-Binding Domain

TAD = Transactivation Domain

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1986: discovery of the Nuclear Transcription Factor, NF-κB

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

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

Ranjan Sen and David Baltimore
Whitehead Institute for Biomedical Research
Cambridge, Massachusetts 02142
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

NF-κB 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-κB, 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 ex-

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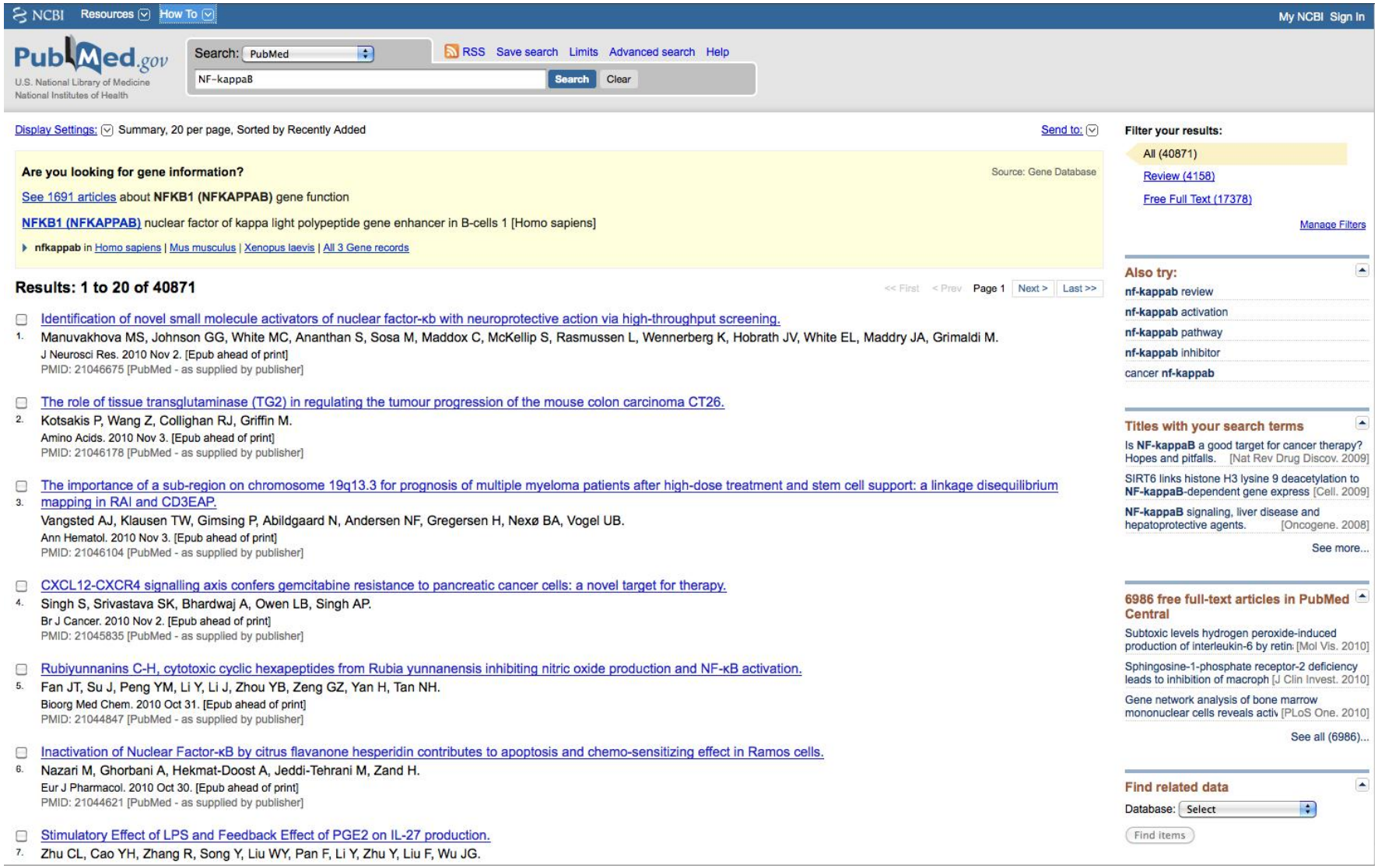
40,871 articles on the subject NF-κB… and counting: Why then?



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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

Results: 1 to 20 of 40871 — << First < Prev Page 1 Next > Last >>

1. Identification of novel small molecule activators of nuclear factor-κb with neuroprotective action via high-throughput screening.
   Manuvakhova MS, Johnson GG, White MC, Ananthan S, Sosa M, Maddox C, McKellip S, Rasmussen L, Wennerberg K, Hobrath JV, White EL, Maddry JA, Grimaldi M.
   J Neurosci Res. 2010 Nov 2. [Epub ahead of print]
   PMID: 21046675 [PubMed - as supplied by publisher]

2. The role of tissue transglutaminase (TG2) in regulating the tumour progression of the mouse colon carcinoma CT26.
   Kotsakis P, Wang Z, Collighan RJ, Griffin M.
   Amino Acids. 2010 Nov 3. [Epub ahead of print]
   PMID: 21046178 [PubMed - as supplied by publisher]

3. The importance of a sub-region on chromosome 19q13.3 for prognosis of multiple myeloma patients after high-dose treatment and stem cell support: a linkage disequilibrium mapping in RAI and CD3EAP.
   Vangsted AJ, Klausen TW, Gimsing P, Abildgaard N, Andersen NF, Gregersen H, Nexø BA, Vogel UB.
   Ann Hematol. 2010 Nov 3. [Epub ahead of print]
   PMID: 21046104 [PubMed - as supplied by publisher]

4. CXCL12-CXCR4 signalling axis confers gemcitabine resistance to pancreatic cancer cells: a novel target for therapy.
   Singh S, Srivastava SK, Bhardwaj A, Owen LB, Singh AP.
   Br J Cancer. 2010 Nov 2. [Epub ahead of print]
   PMID: 21045835 [PubMed - as supplied by publisher]

5. Rubiyunnanins C-H, cytotoxic cyclic hexapeptides from Rubia yunnanensis inhibiting nitric oxide production and NF-κB activation.
   Fan JT, Su J, Peng YM, Li Y, Li J, Zhou YB, Zeng GZ, Yan H, Tan NH.
   Bioorg Med Chem. 2010 Oct 31. [Epub ahead of print]
   PMID: 21044847 [PubMed - as supplied by publisher]

6. Inactivation of Nuclear Factor-κB by citrus flavanone hesperidin contributes to apoptosis and chemo-sensitizing effect in Ramos cells.
   Nazari M, Ghorbani A, Hekmat-Doost A, Jeddi-Tehrani M, Zand H.
   Eur J Pharmacol. 2010 Oct 30. [Epub ahead of print]
   PMID: 21044621 [PubMed - as supplied by publisher]

7. Stimulatory Effect of LPS and Feedback Effect of PGE2 on IL-27 production.
   Zhu CL, Cao YH, Zhang R, Song Y, Liu WY, Pan F, Li Y, Zhu Y, Liu F, Wu JG.

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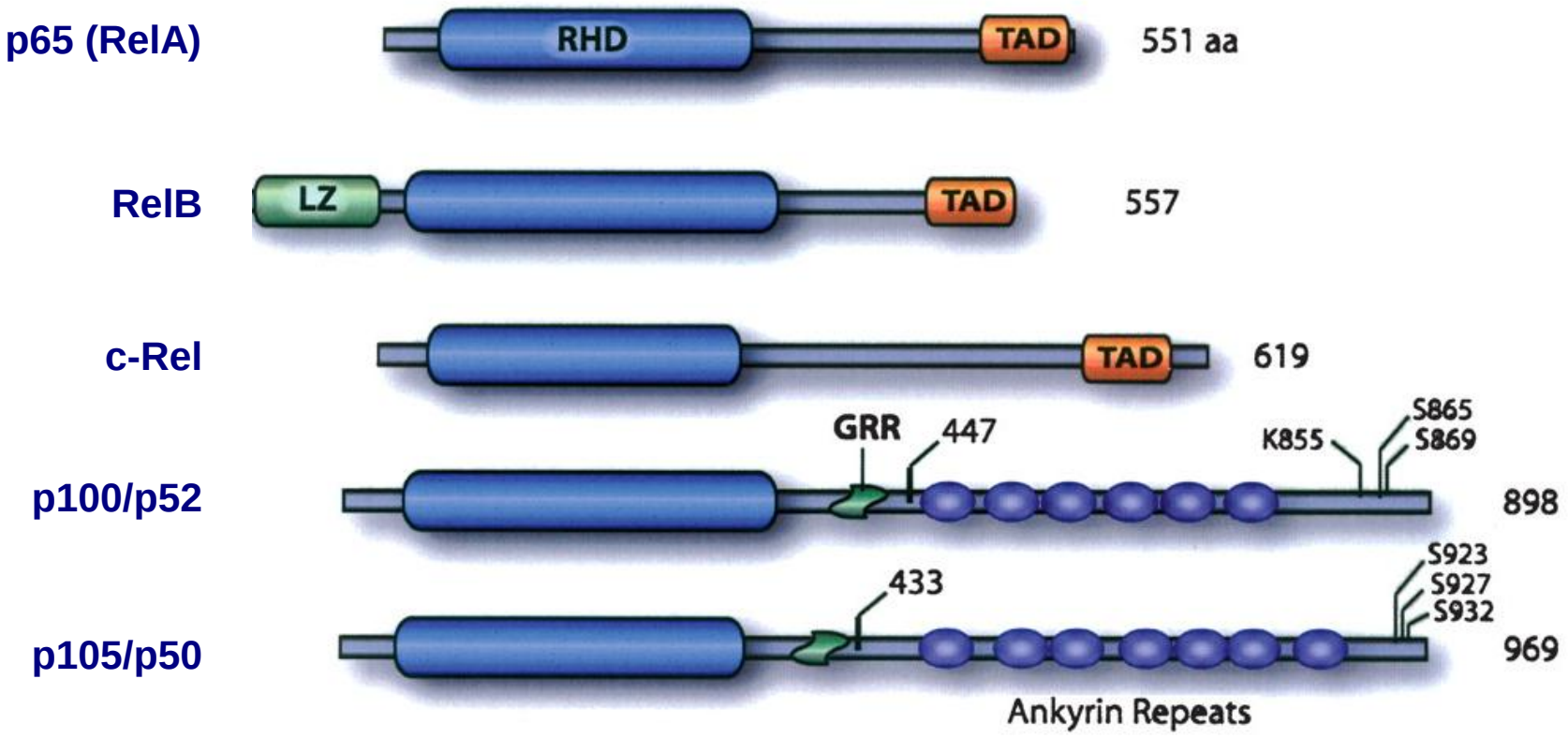
Nuclear Factor-kappa B (NF-κB) protein family



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

NF-κB: assembling details

The term NF-κB refers to a family of inducible dimeric transcription factors that recognize a common sequence motif, the "κB site"

The possible dimeric combination are:

p50:p50

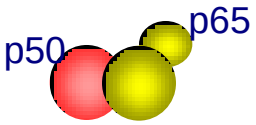
p65:p65

RelB:RelB

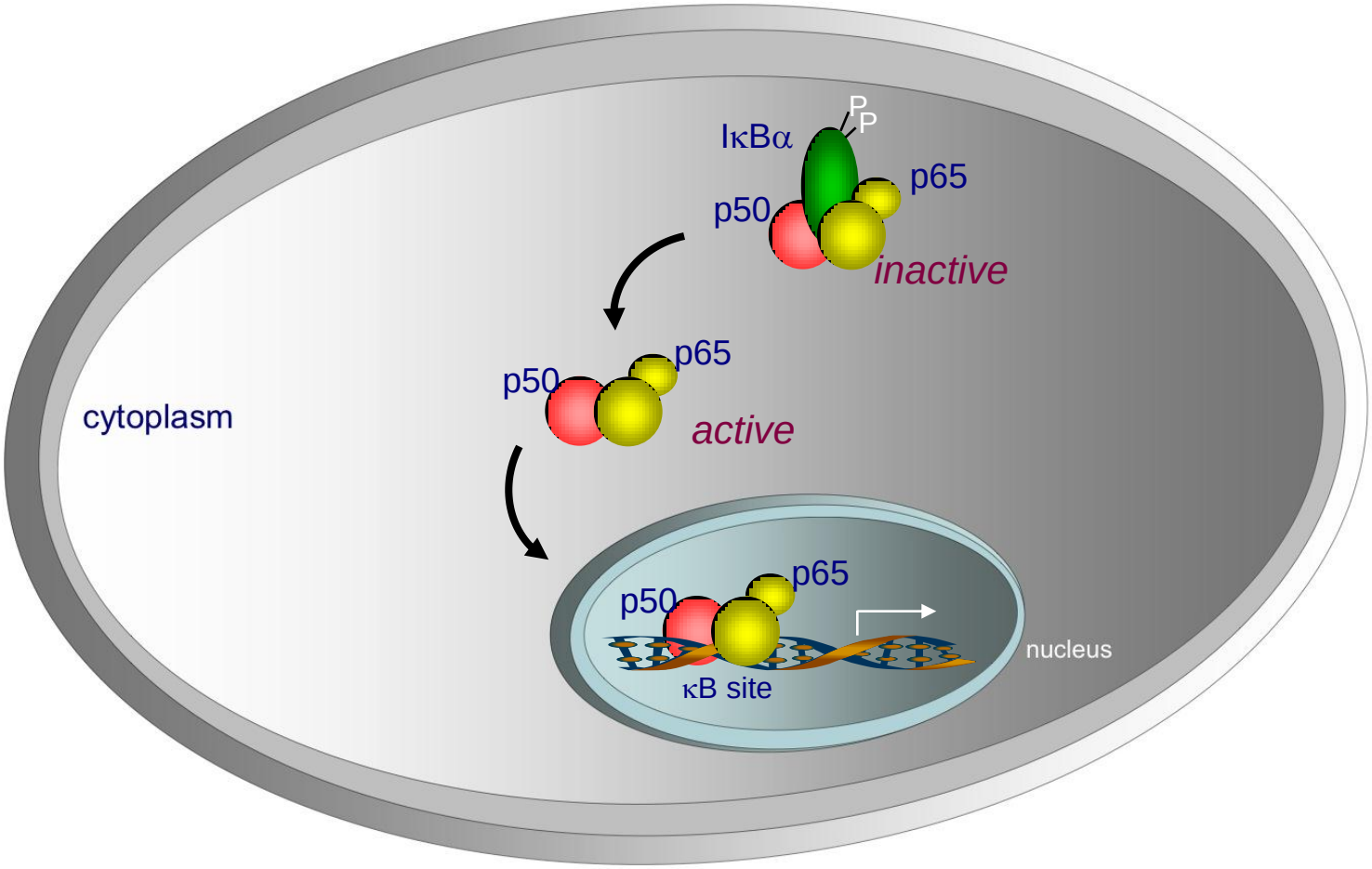
p50:p65

p50:RelB

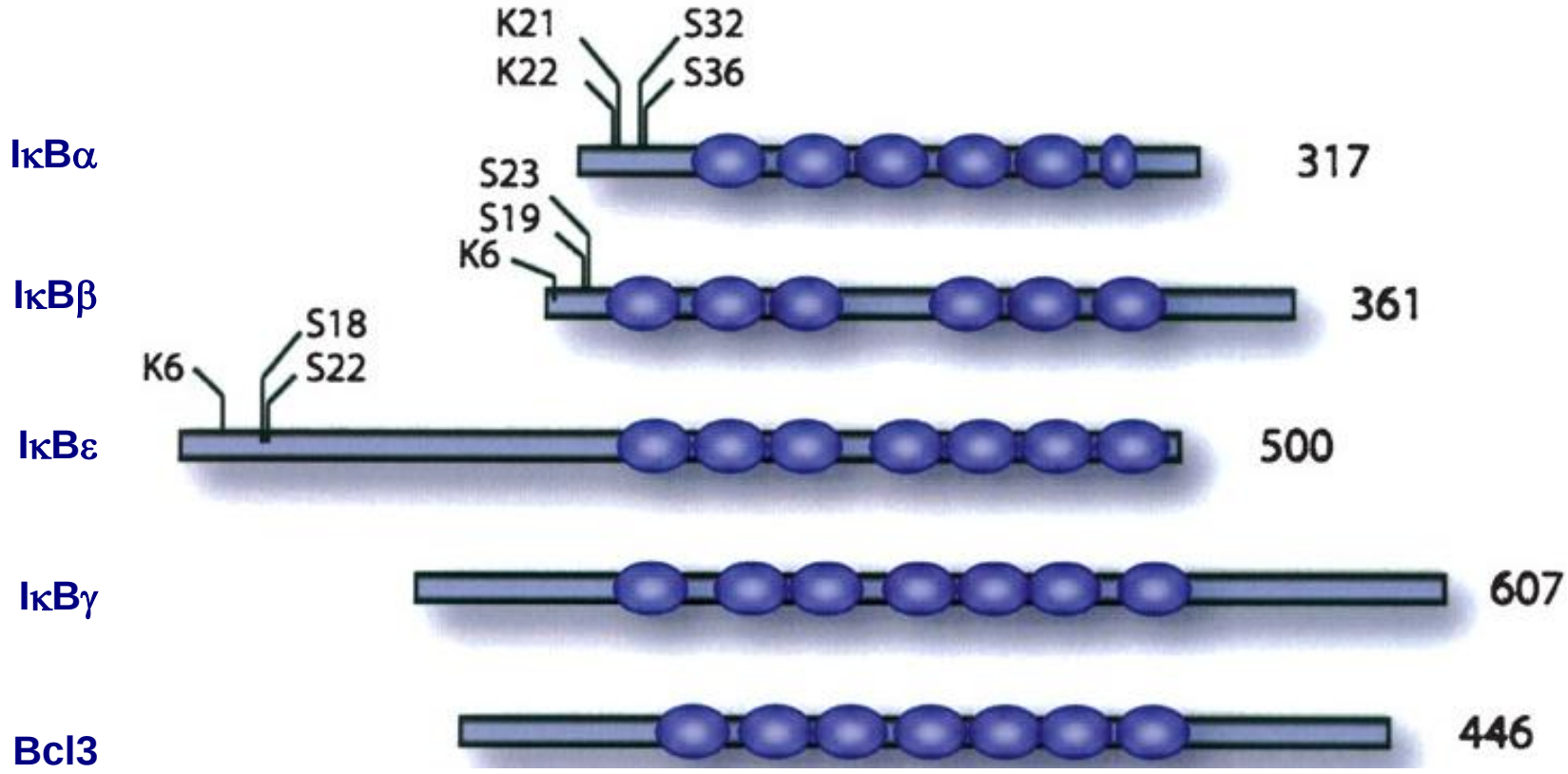
p52:RelB



NF-κB: assembling details

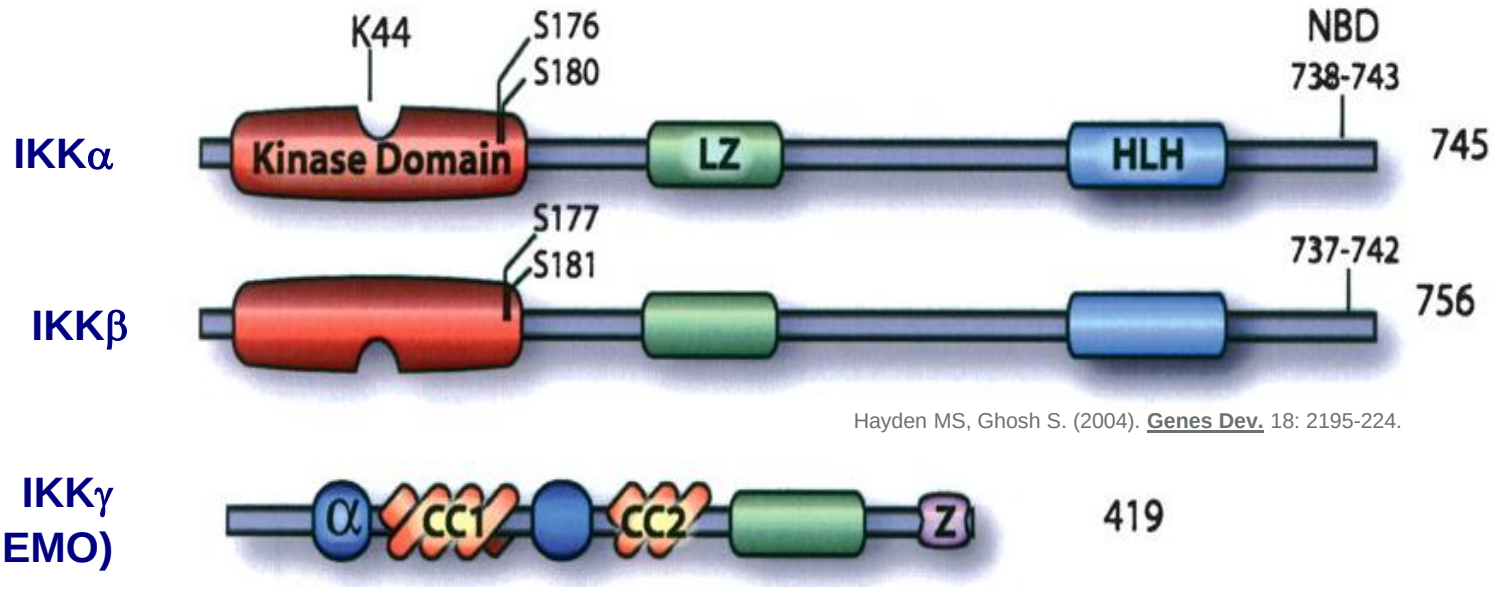


Inhibitor-kappa B (IκB) proteins family



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

Inhibitor-kappaB kinase (IKK) proteins family



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

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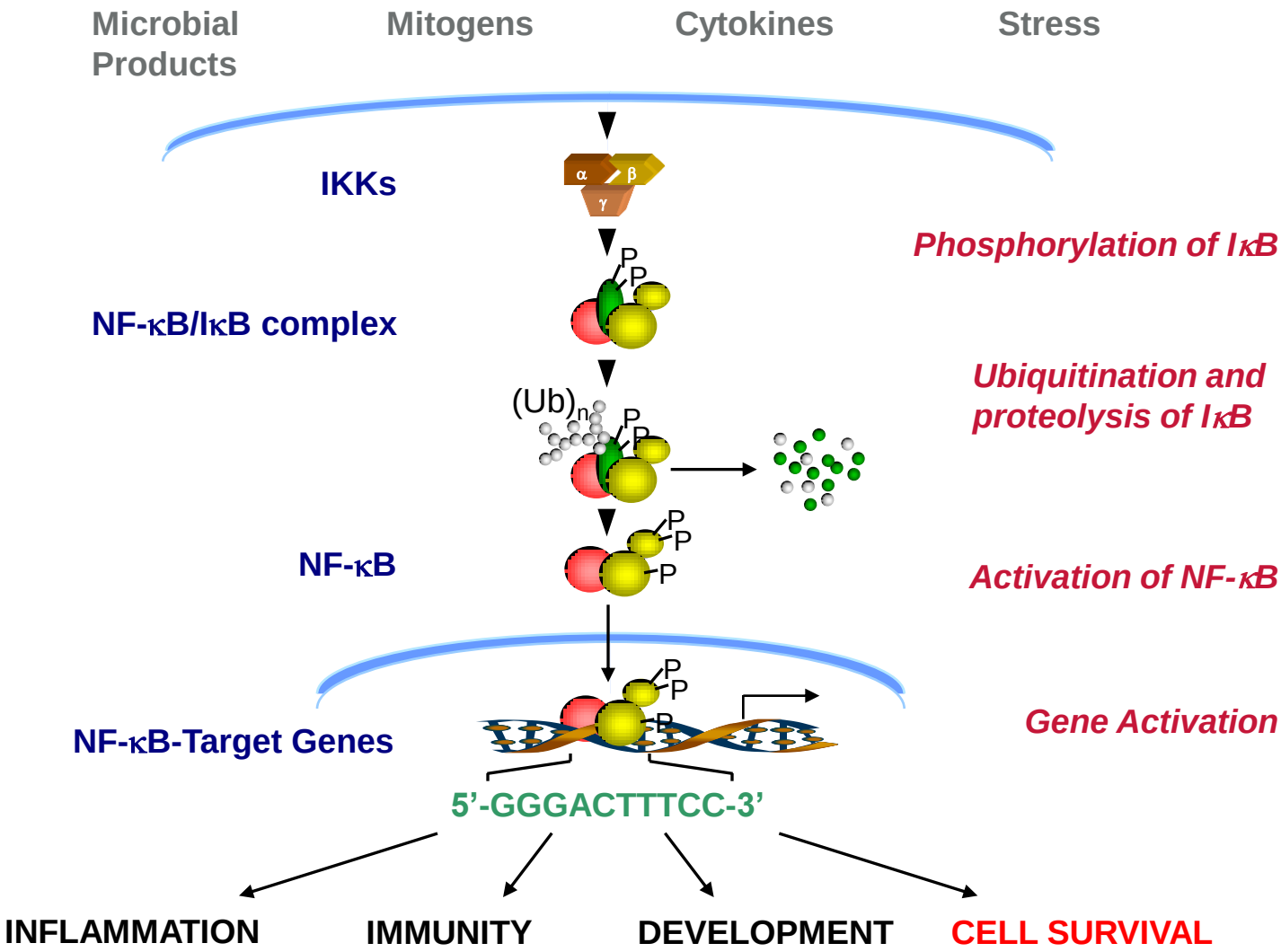
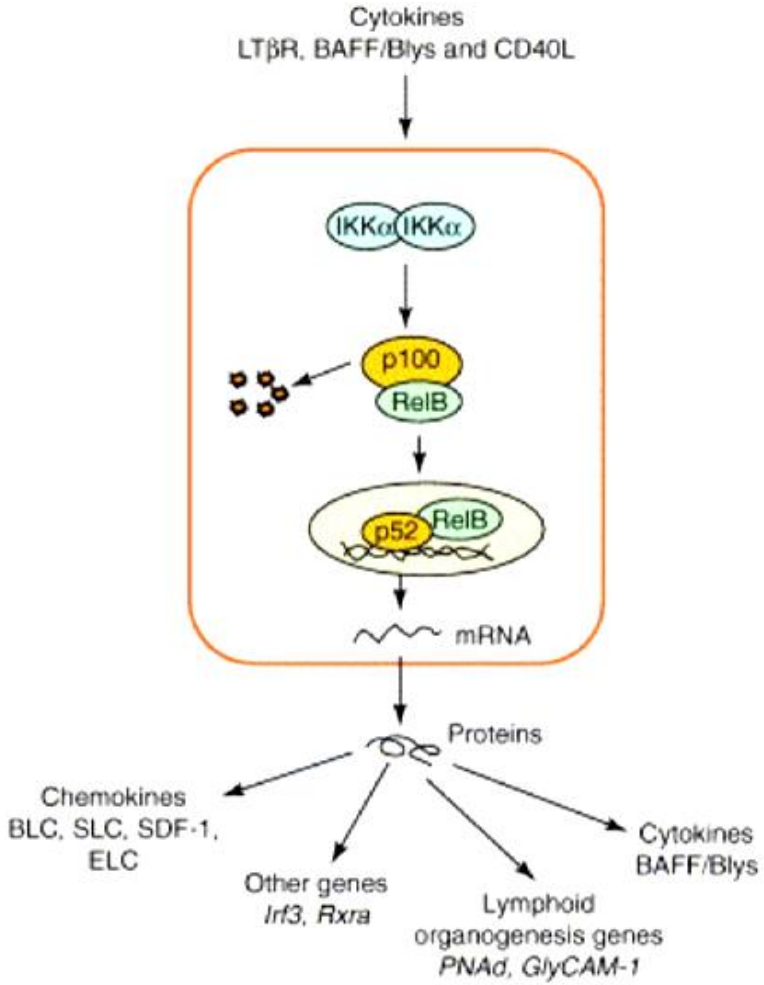
The classical NF-κB pathway

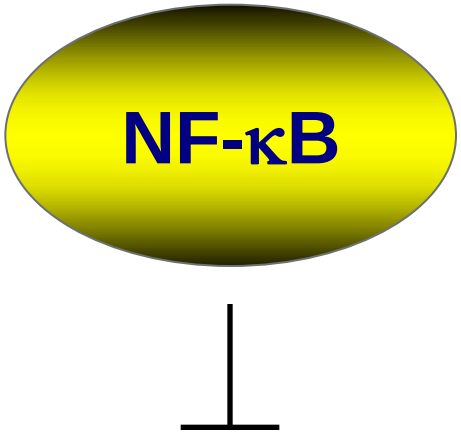


The alternative NF-κB pathway



Bonizzi G and Karin M (2004). Trends Immunol 25: 280-288

The pro-survival activity of NF-κB



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

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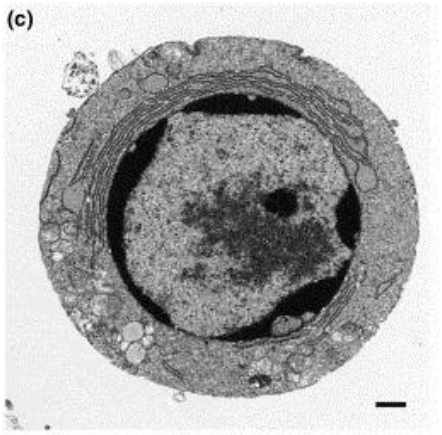
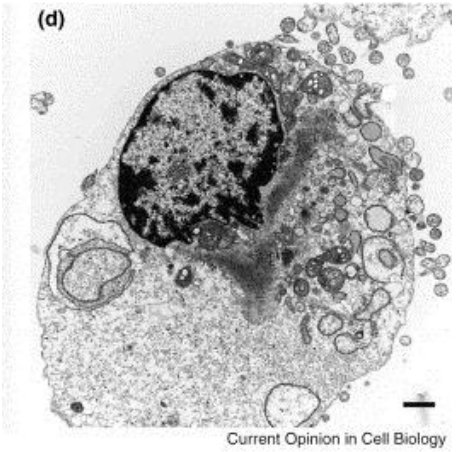
Programmed Cell Death

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-κB

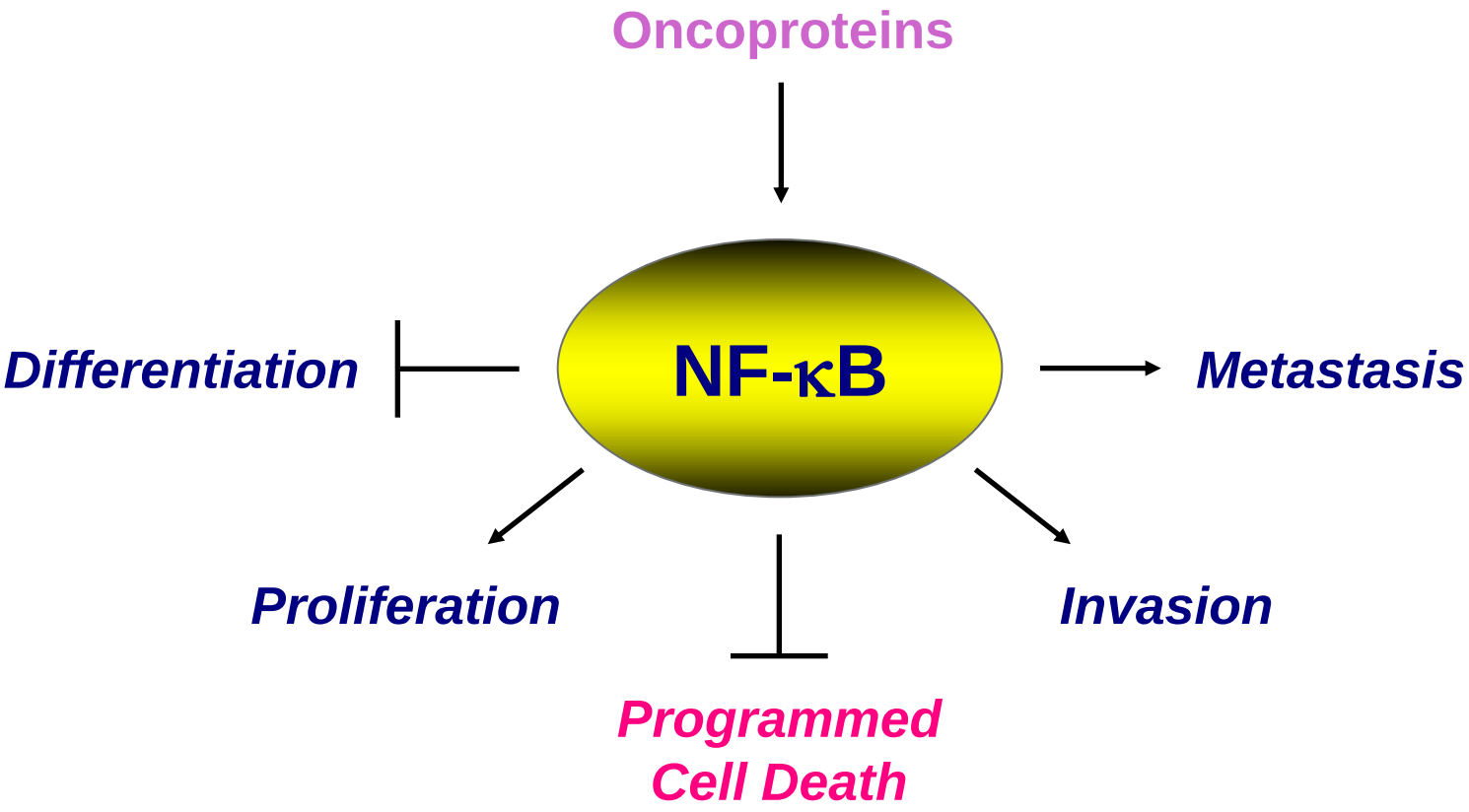
- Cellular Responses to the Triggering of
- TNF-Rs, TRAIL-Rs and Fas
- B Lymphopoiesis
- Bone Morphogenesis
- B- and T-Cell Costimulation (CD40, CD28, etc.)
- Liver Development

The pro-survival activity of NF-κB 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-κB in cancer



Role of the NF-κB pro-survival activity in cancer

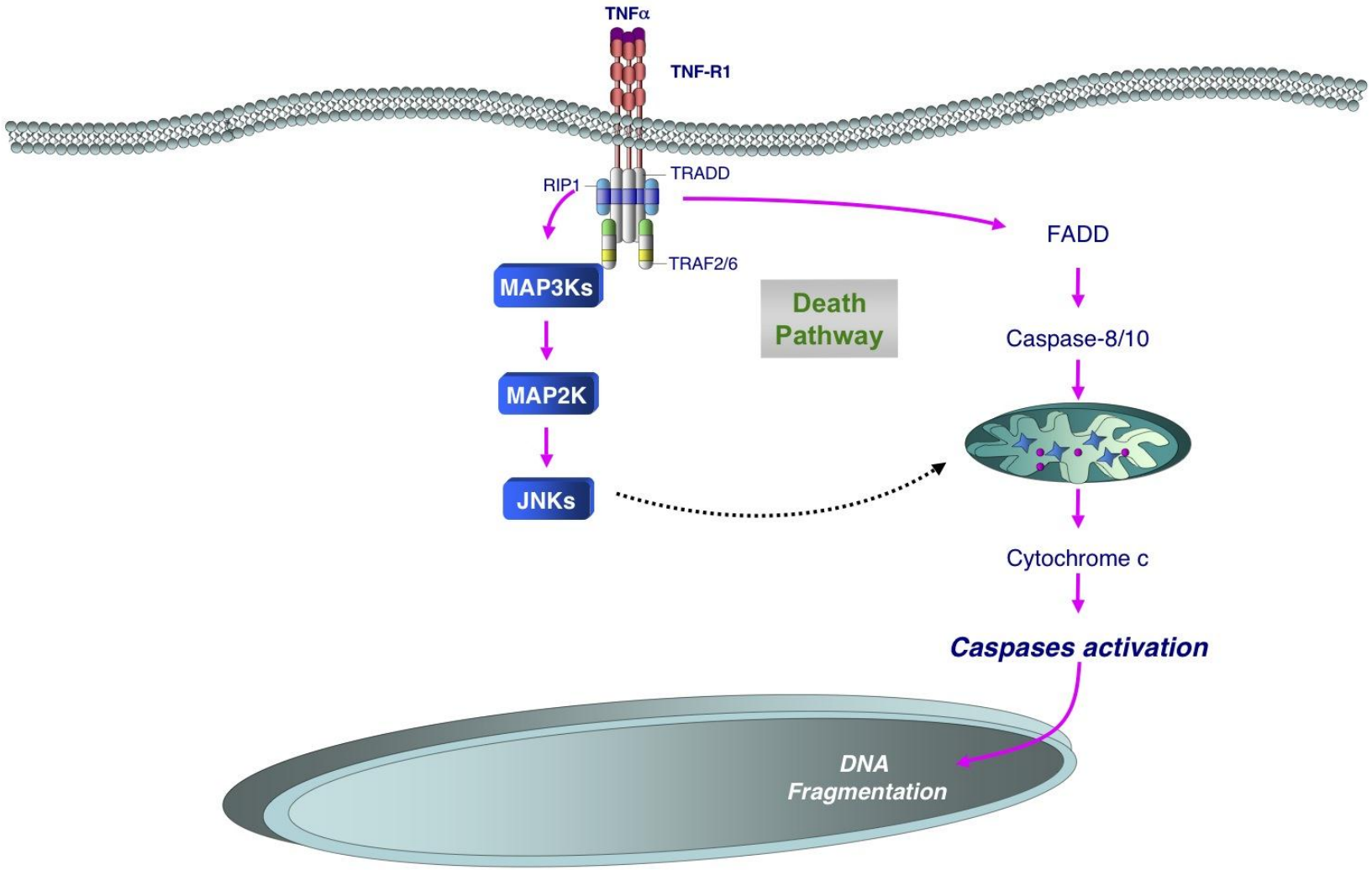
- ***Suppression of Transformation***
  - ***Associated Apoptosis:*** **- oncogenic Ras, Bcr-Abl, etc.**
- ***Survival of Late Stage Tumors:***
  - **- Hodgkin's Lymphoma**
  - **- Breast Cancer**
  - **- Diffuse Large B Cell Lymphoma**
  - **- Multiple Myeloma**
  - **- Colon Cancer**
  - **- Prostate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

Summary: part 1

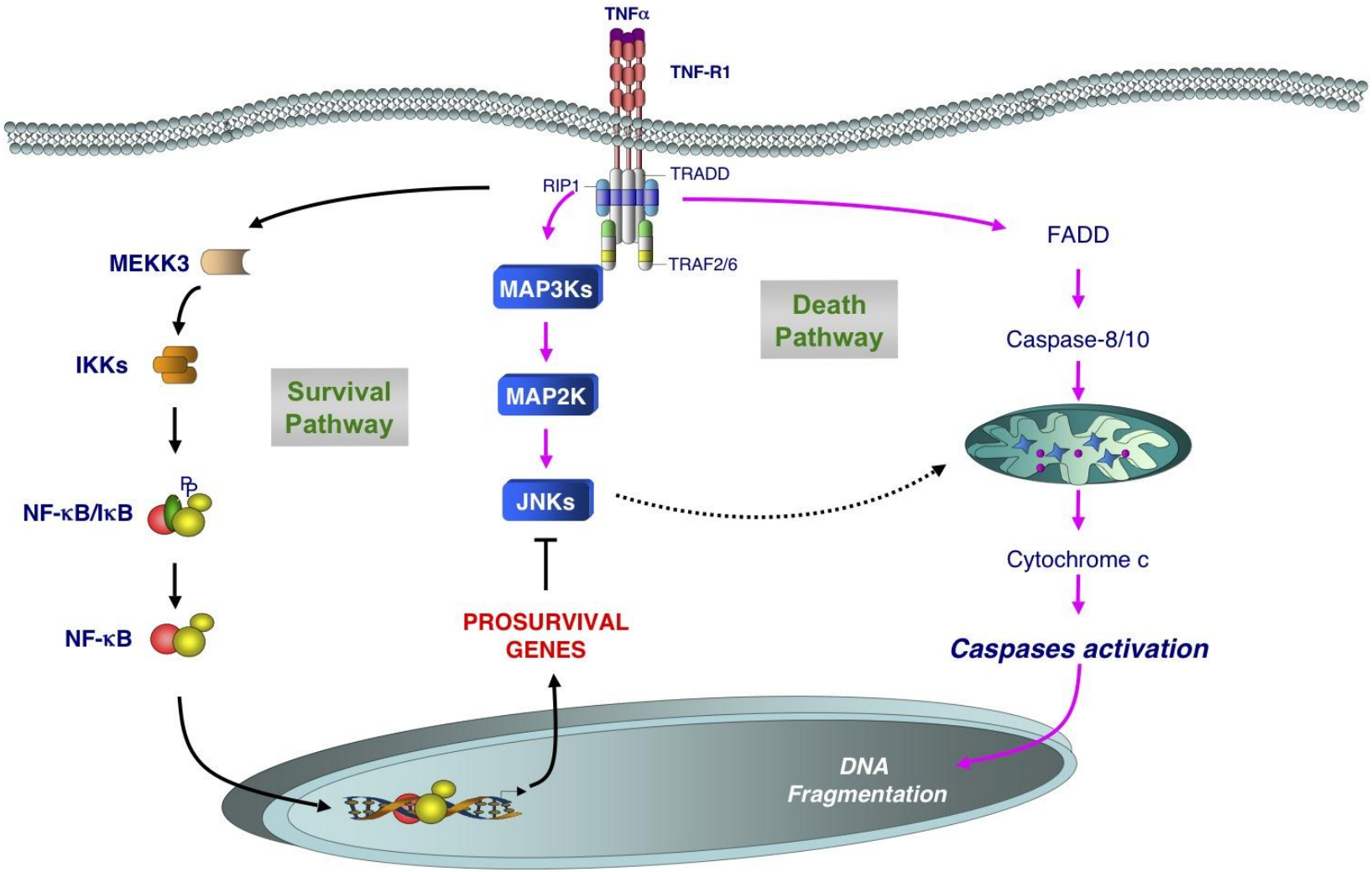
- NF-κB are transcription factors that block Programmed Cell Death (PCD)
- Apoptosis and necrosis are two forms of PCD
- Deregulation of the ability of NF-κB to control PCD leads to oncogenesis

What is the molecular mechanism by which NF-κB control Programmed Cell Death?

TNF-R1 signalling



TNF-R1 signalling



Protein kinase: definition

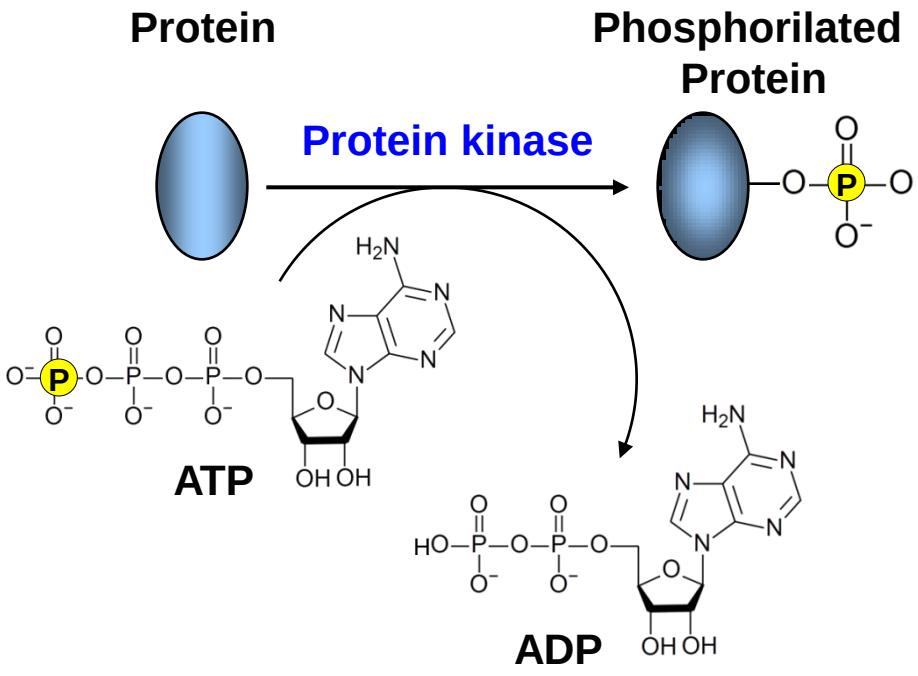
A kinase is an enzyme that modifies other proteins by adding phosphate groups (phosphorylation).

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.



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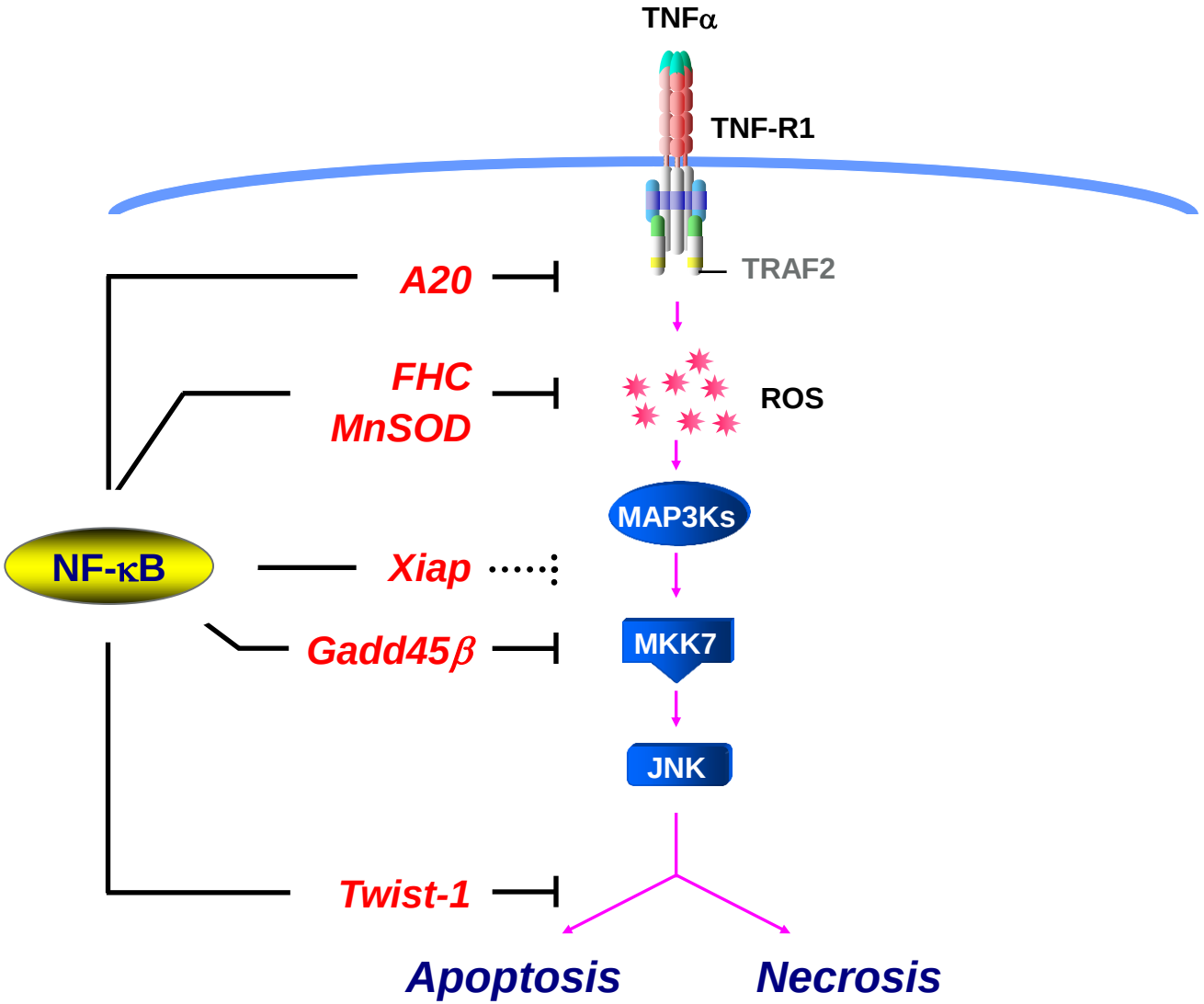
Distinct mechanisma of PCD inhibition



Gadd45β/Myd118

- ~21 kDa
- Predominantly Nuclear
- Member of the Gadd45 Family of Inducible Factors,
- Also Including Gadd45α and Gadd45γ
- Proposed Functions of Gadd45β:
- Specific inhibitor of JNK cascade
- Ectopic expression of Gadd45β inhibits the
- JNK-mediated PCD downstream of TNFR

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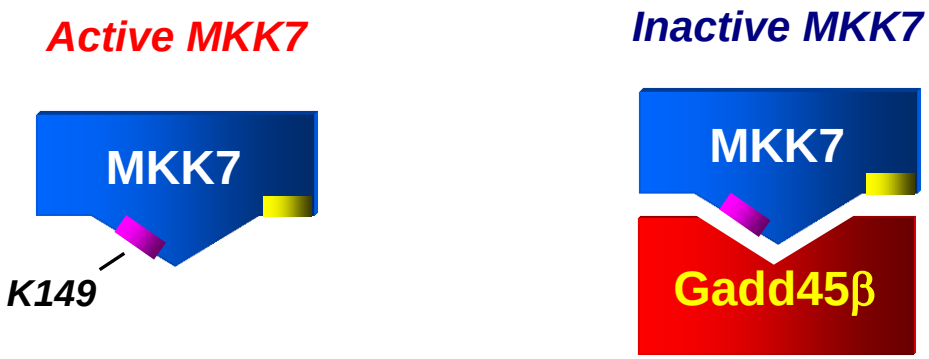
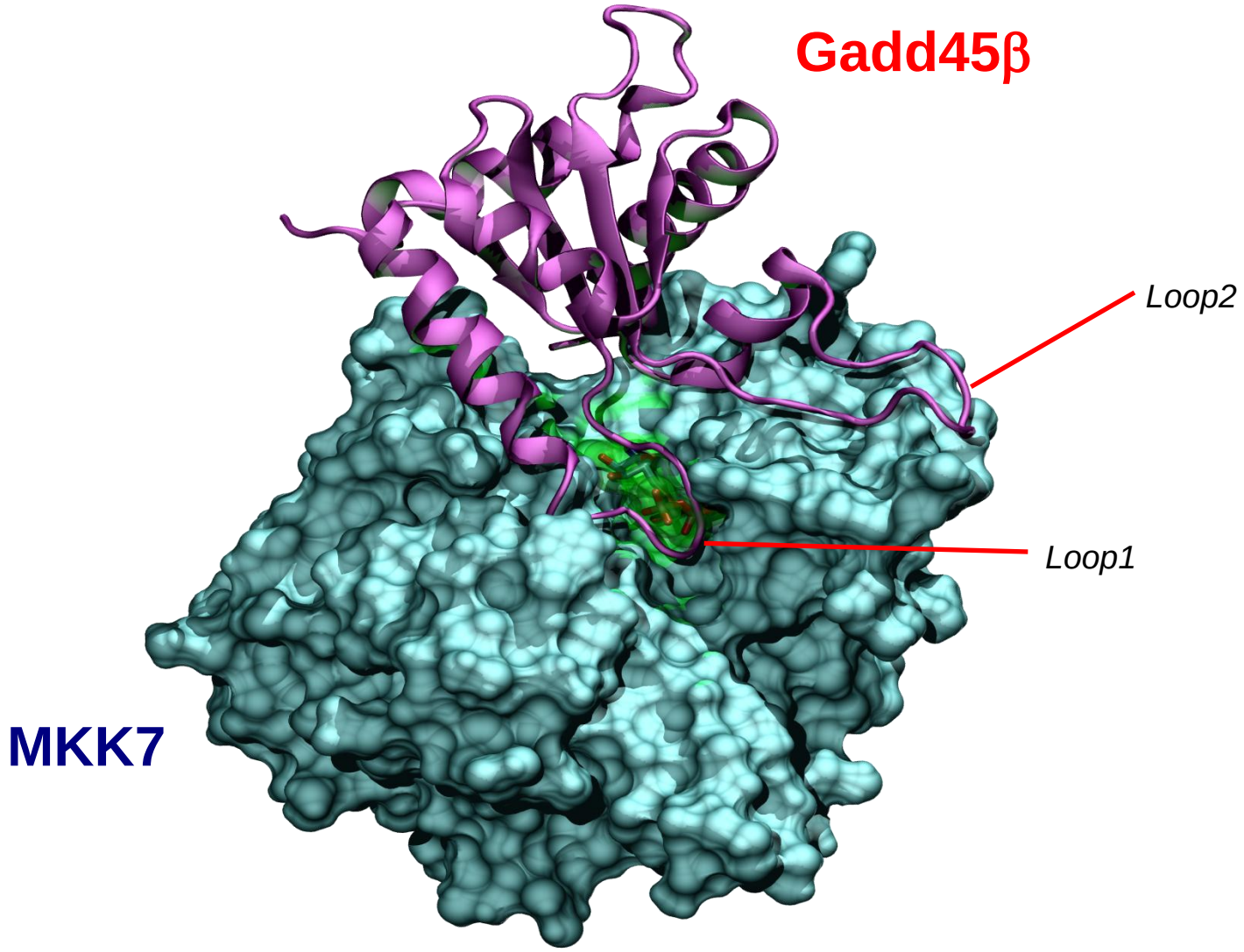
Gadd45β interact with MKK7



Gadd45β bind the ATP-binding site of MKK7



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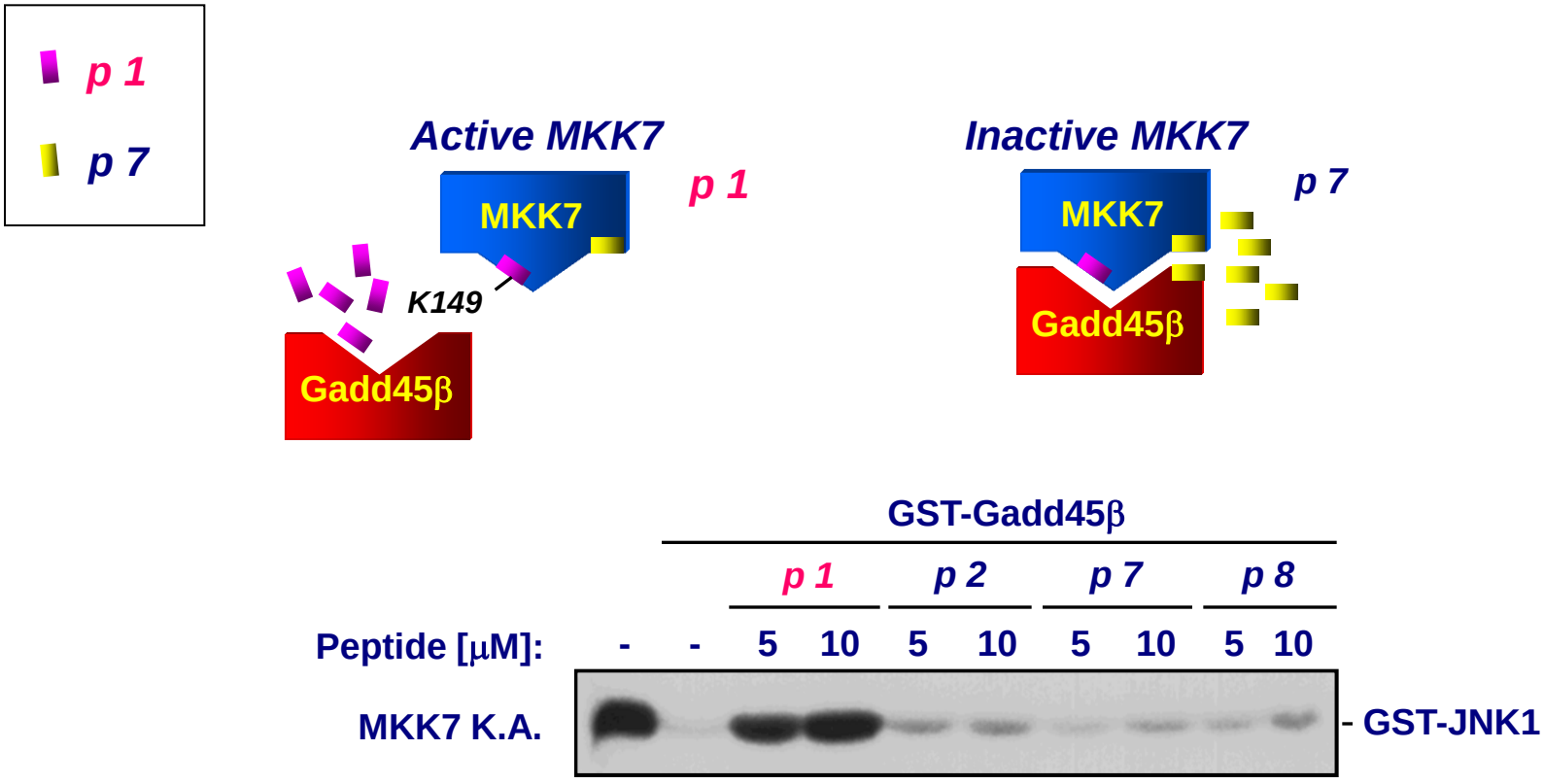
Peptides (P1) disrupting Gadd45β/MKK7 interaction restores MKK7 activity



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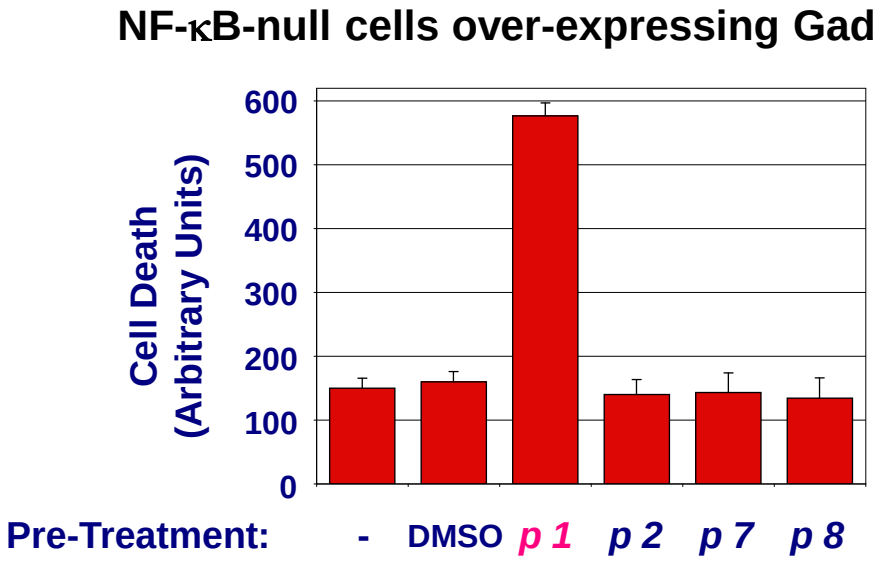
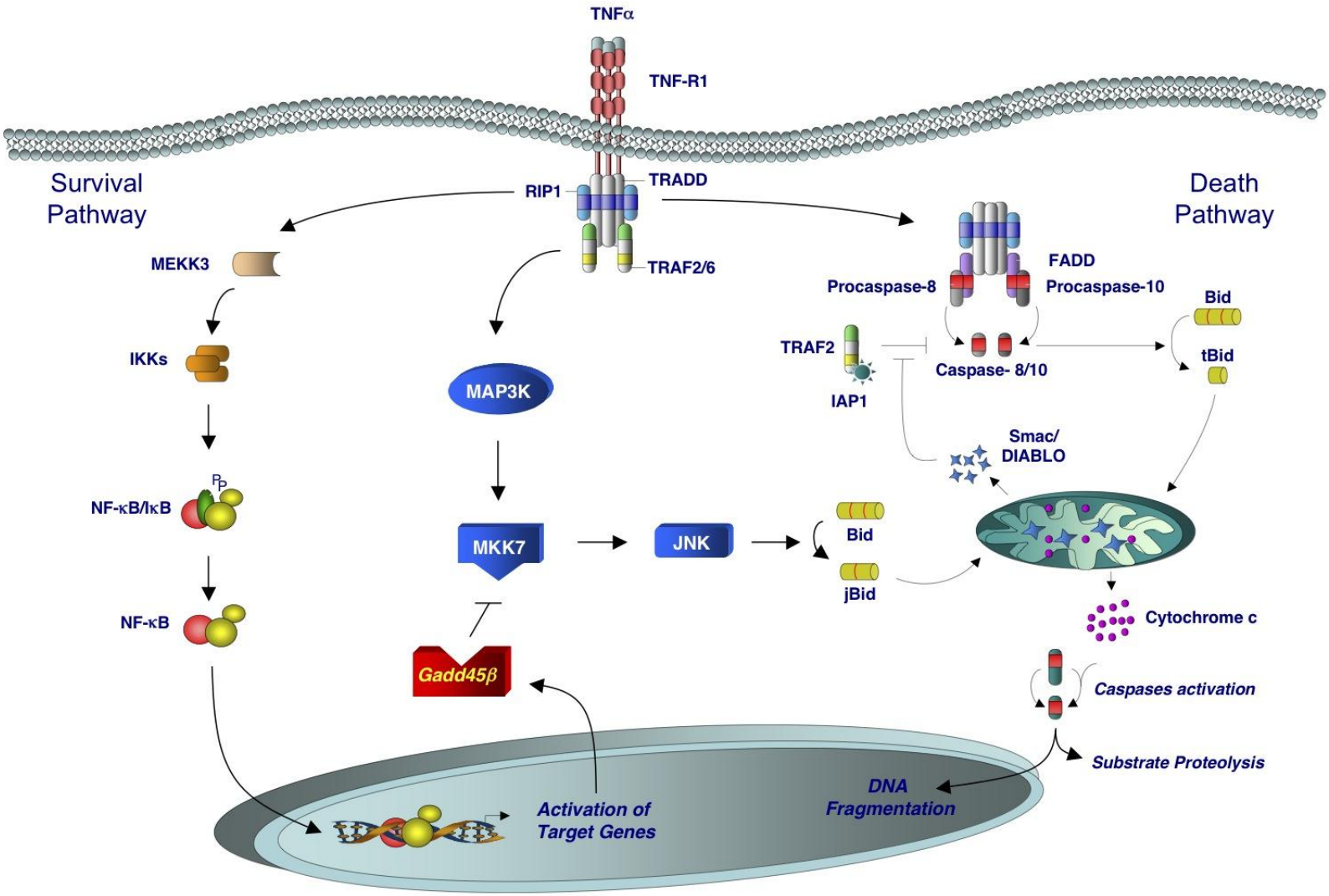
Cell-permeable Peptide 1 (P1) prevents inhibition of TNFα-induced apotosis



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

Summary: part 2

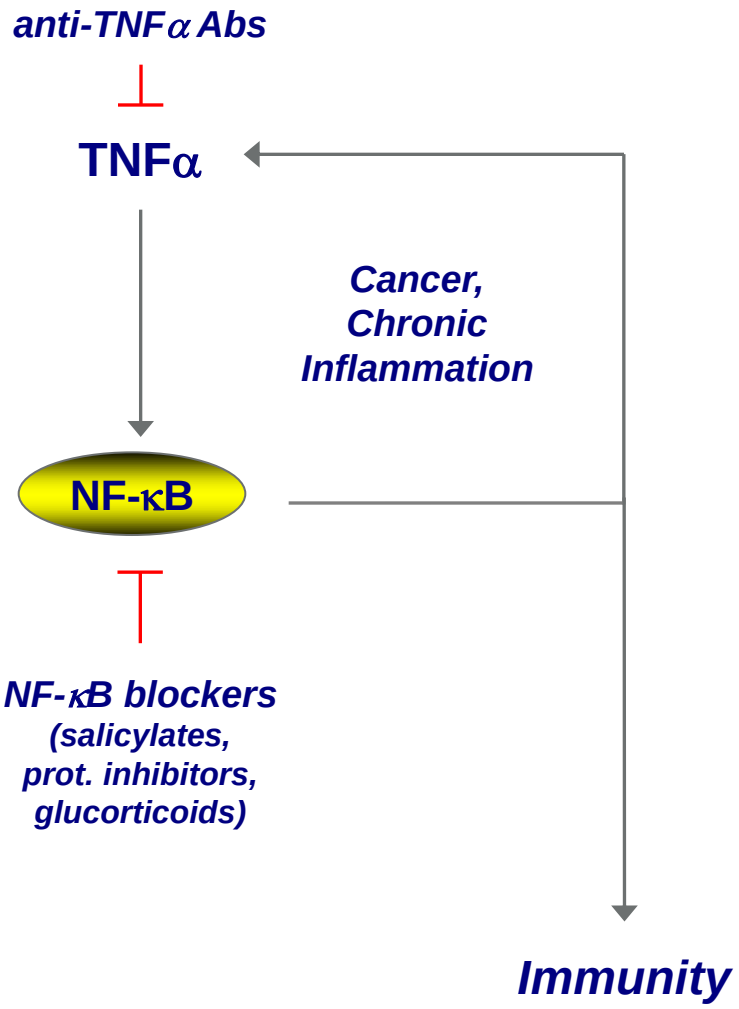


Why study molecular mechanisms by which NF-κB blocks PCD are important ???

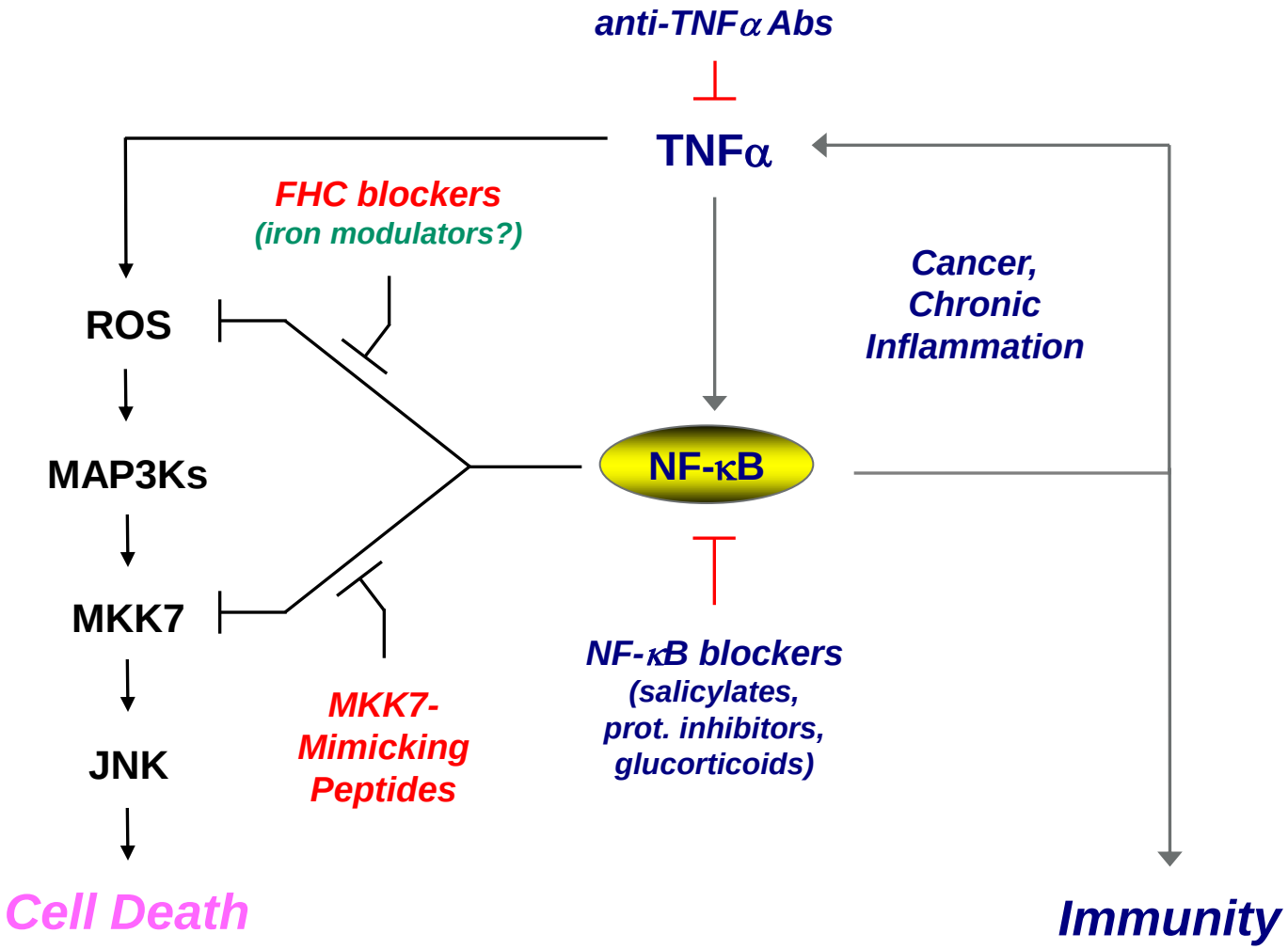
NF-κB blockers as pharmacological compound

Inhibition of NF-κB 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-κB and have considerable side effects, which limit their use in humans.



Future therapies: Gadd45β and FHC may represent new targets



Conclusions

- NF-κB are transcription factors that block Programmed Cell Death (PCD)
- Apoptosis and necrosis are two forms of PCD
- Deregulation of the ability of NF-κB to control PCD leads to oncogenesis
- Downstream TNFR1, Gadd45β is up-regulated by NF-κB and inhibits JNK pathway by blocking MKK7
- New anticancer therapy can be developed based on inhibitors of Gadd45β–MKK7 interaction (?)

Learning outcomes

- **Describe the NF-κB 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-κB in response to TNFa**
- **Describe one of the molecular mechanism by which NF-κB blocks PCD**
  - *Draw the JNK pathway*
- **Relate the survival activity of NF-κB to the therapeutic potential of its inhibition in cancer and the possible complications**

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References

- Baud V and Karin M. (2009). *Is NF-κB a good target for cancer therapy? Hopes and pitfalls.* **Nature**, 8:33-40.
- Ghosh S, and Hayden MS. (2008). *New regulators of NF-κB in inflammation.* **Nat. Rev. Immunology,** 8:837-848.
- Staudt L. (2010). *Oncogenic activation of NF-κB*. **Cold Spring Harb. Perspect. Biol.,** 2:a000109.
- Papa S, Zazzeroni F, Bubici C *et al* Franzoso G. (2004). *Gadd45b mediates the NF-κB suppression of JNK signalling by targeting MKK7/JNKK2.* **Nat. Cell Biol,** 6: 146-153.
- Rock KL & Kono H (2008). *The inflammatory Response to cell Death.* **Annu.Rev.Pathol.Mech.Dis.,** 3: 99-126.
- Papa S, Bubici C, Zazzeroni F, Pham CG, Kuntzen C, Knabb JR, Dean K, Franzoso G. (2006). The NF-κB-mediated control of the JNK cascade in the antagonism of programmed cell death in health and disease. **Cell Death Differ,** 13: 712-729