The Virtual Free Radical School

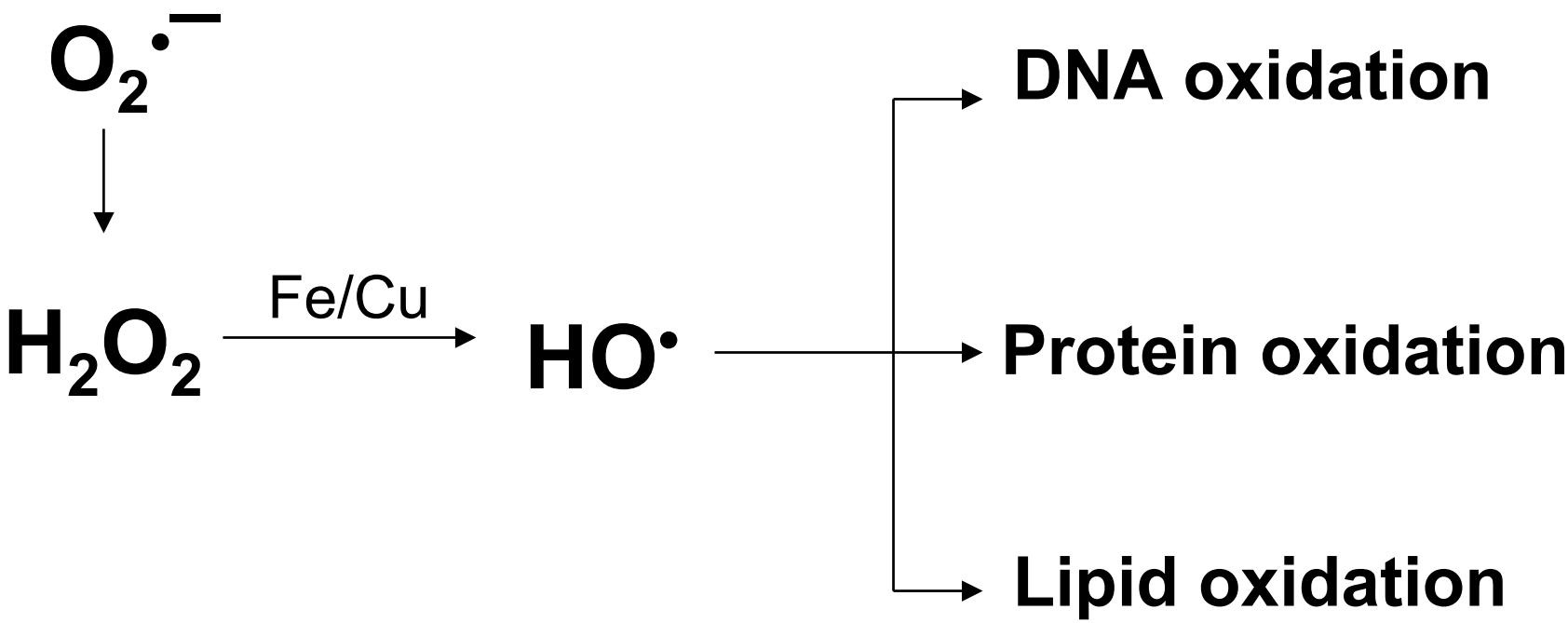
NFκB – What is it and What's the deal with radicals?

Emily Ho, Ph.D

Linus Pauling Institute Scientist
Department of Nutrition and Food Management
Oregon State University
117 Milam Hall
Corvallis, OR 97331

emily.ho@oregonstate.edu (Tel) 541-737-9559; (Fax) 541-737-6914

Cytotoxicity of ROS

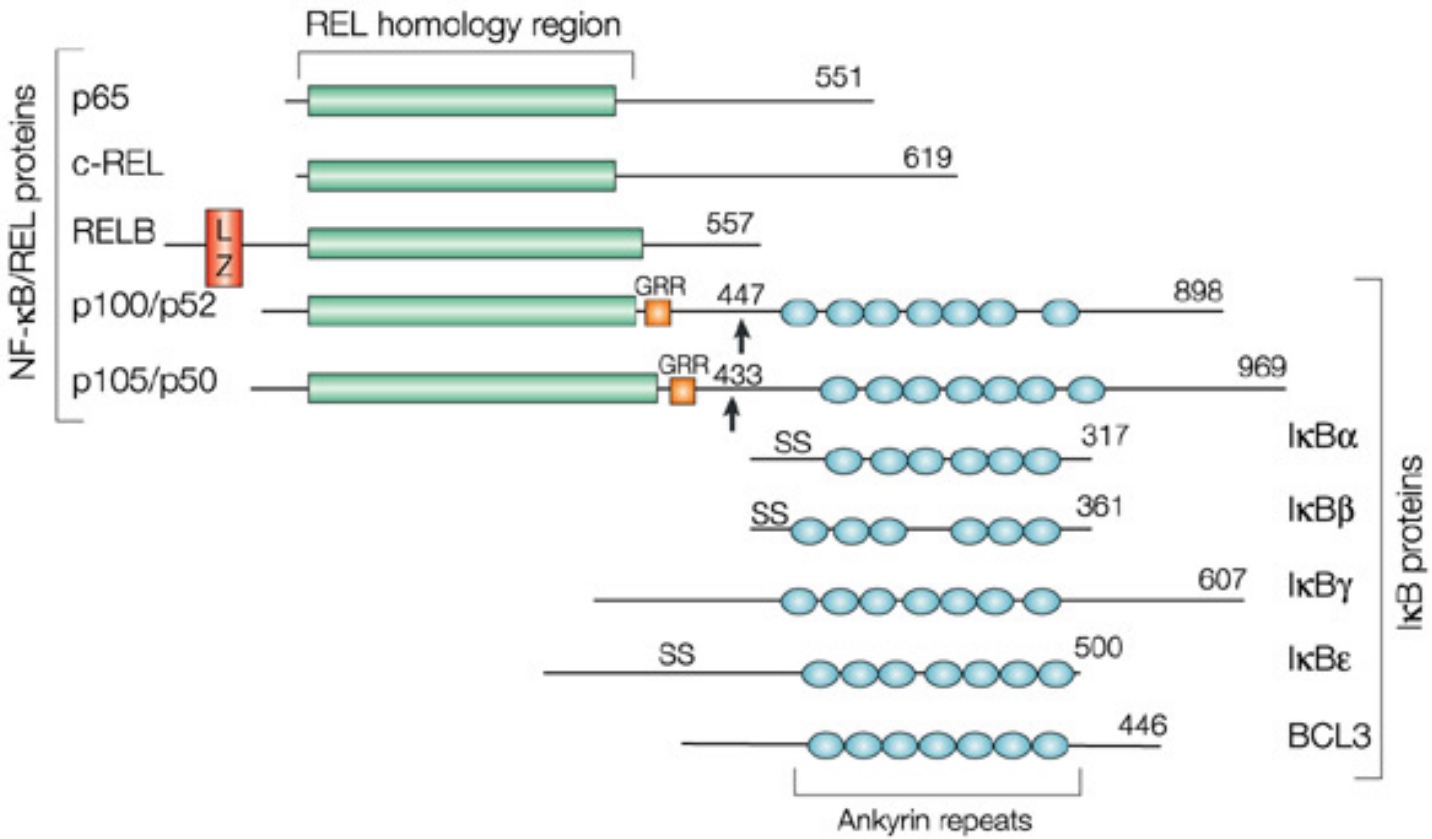


More recently, the role of ROS as a signal molecules has gained increasing attention. The cytotoxicity of ROS may be associated with the ability of ROS to signal distinct pathways, such as the NFkB pathway, to induce pathology.

What is NFκB?

- First discovered by Baltimore & Sen as a B cell specific nuclear protein that binds to a site in the immunoglobulin κ light chain gene enhancer (Cell **47**:921-928, 1986)
- NFκB comprises a family of transcription factors that are involved in regulating a large number of genes related to immune function, inflammation, apoptosis and cell proliferation.
- Mammalian cells have 5 distinct NFkB subunits based on a highly conserved 300 amino acid dimerization domain called the rel homology domain.
- Several different combinations of subunits in the cytoplasm, the most common being a heterodimer of p50/p65 (Rel A) and the inhibitory IκBα inhibitory subunit.

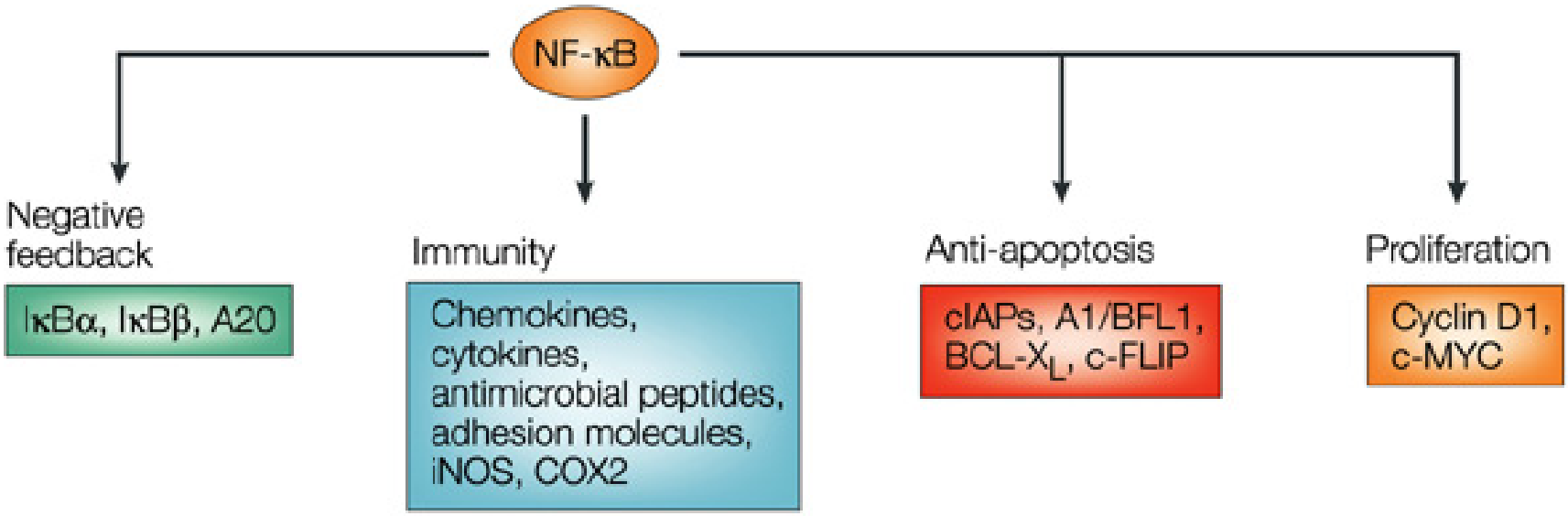
Family of NFκB and Inhibitory κB (IκB) proteins



Nat.Rev.Cancer **2**:301-310, 2002

Nature Reviews | Cancer

Genes Regulated by NFκB



Nature Reviews | Cancer

Nat.Rev.Cancer **2**:301-310, 2002

Function of NFκB

- Key mediator of a variety of cellular responses
  - Immune and inflammatory response
  - Cell proliferation and survival
    - Protecting cells from undergoing apoptosis in response to DNA damage or cytokine treatment

Many chronic disease states have been associated with aberrant activation of NFκB, and several therapeutic strategies targeting NFκB activation have been considered for the treatment of inflammation and cancer.

Disorders associated with aberrant NFκB activation

- Rheumatoid arthritis
- Atherosclerosis
- Vascular dysfunction
- Multiple sclerosis
- Neurodegenerative disorders
- Inflammatory bowel disease
- *H. pylori*-associated gastritis
- Systemic inflammatory response syndrome
- Autoimmune thyroid disease
- Cystic fibrosis
- Diabetes
- Aging
- Macular degeneration
- HIV/AIDS
- Cancer
- Septic shock

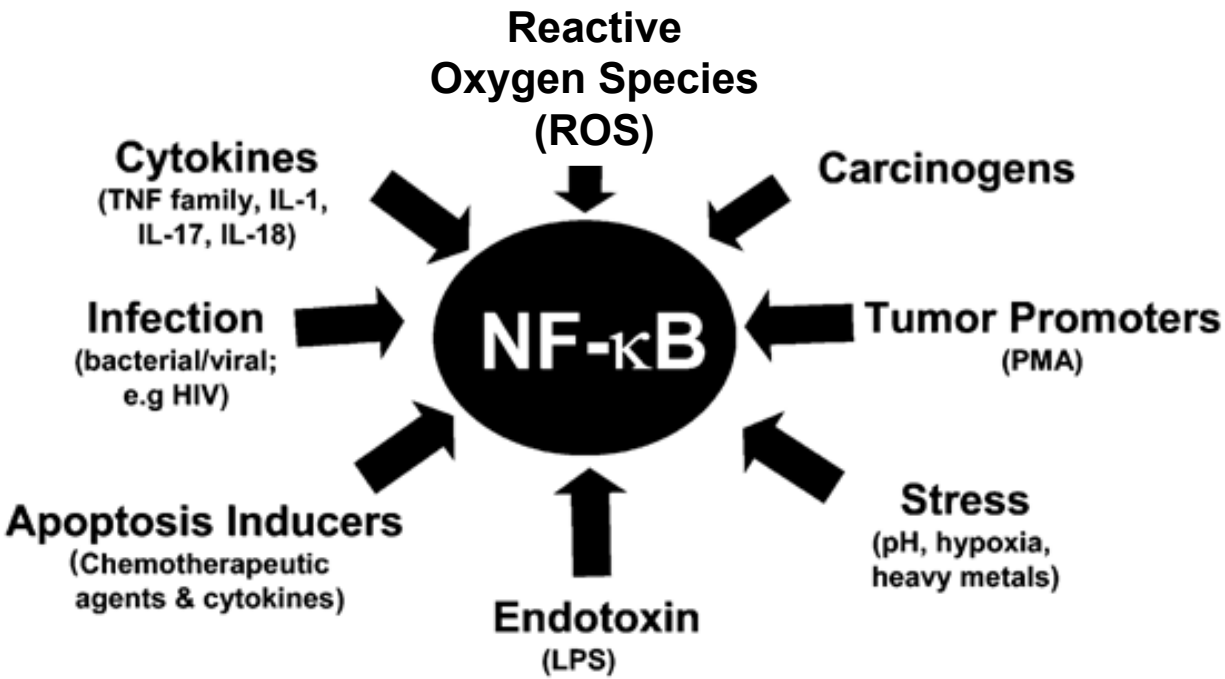
- And the list is growing…

Tumors that express constitutively active NFκB

- B cell lymphoma
- Hodgkin's disease
- T-cell lymphoma
- Acute lymphoblastic leukemia
- Breast
- Liver
- Thyroid
- Prostate
- Melanoma
- Head and neck SCC
- Colon
- Multiple myeloma
- Ovarian
- Bladder
- Lung

Leukemia **16:**1053-1068, 2002

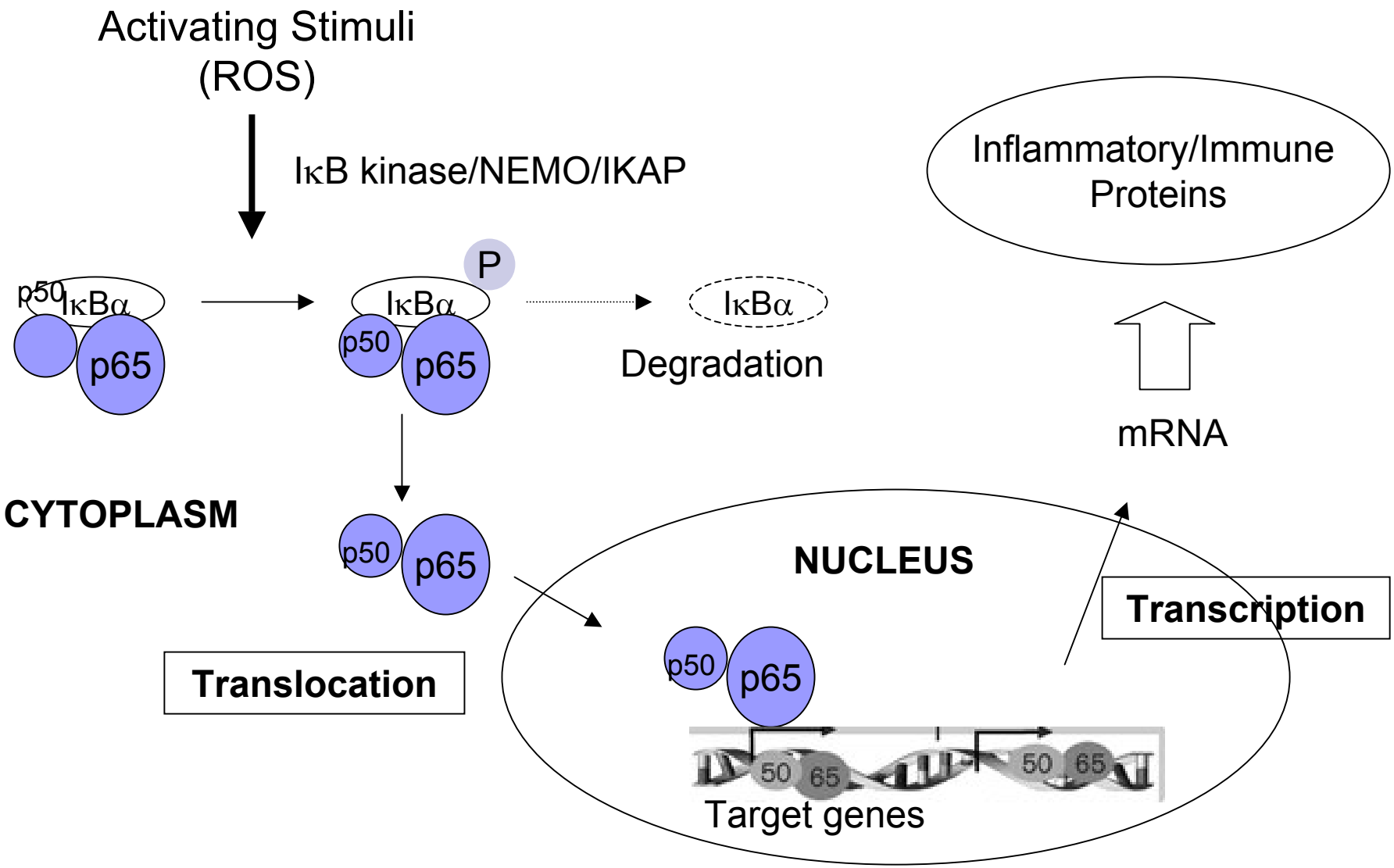
Factors that induce NFκB



Leukemia **16**:1053-1068, 2002

Activation of NFκB

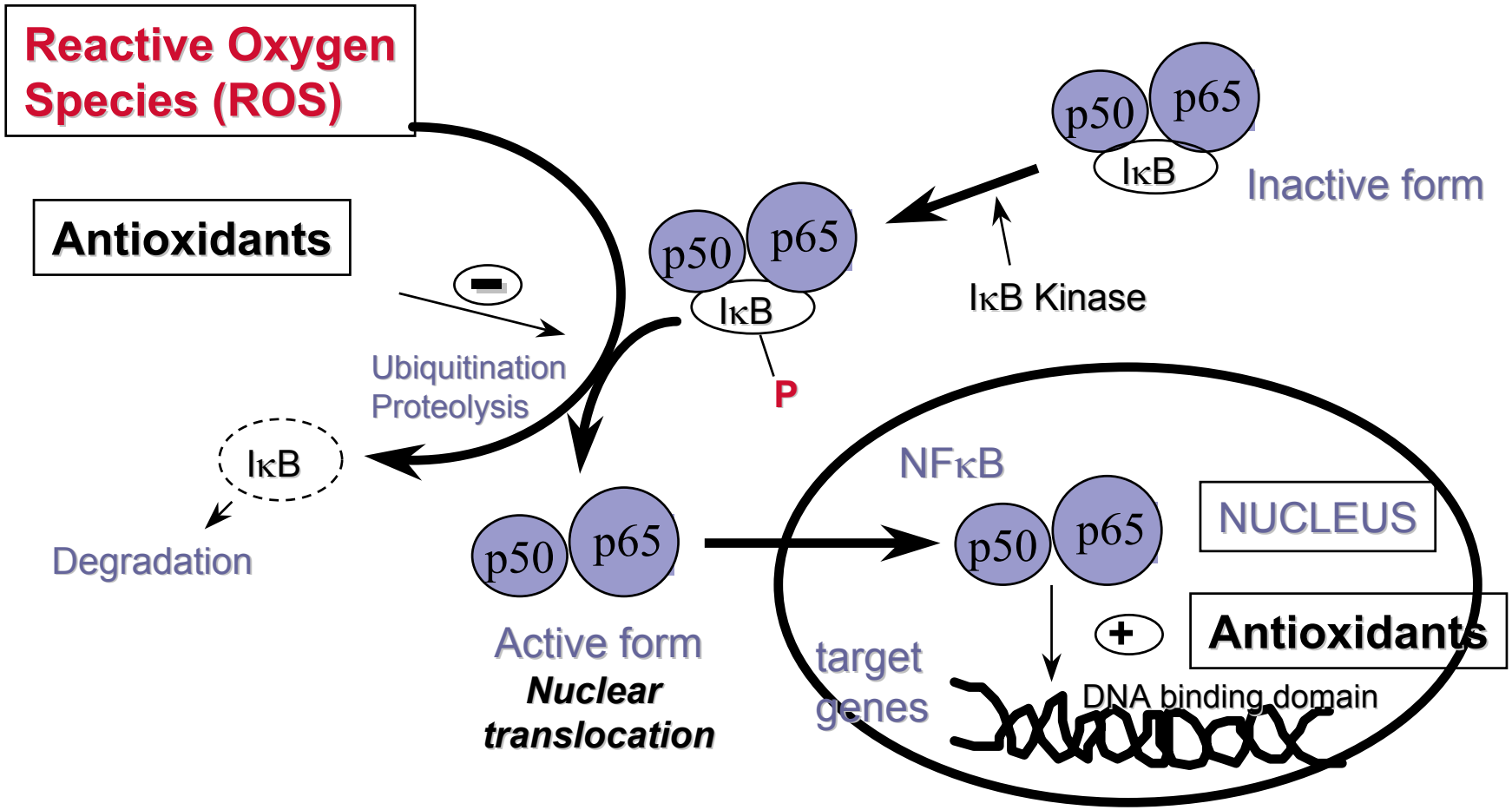
- NFκB is normally found in its inactive form in the cytosol as the heterodimer p50/65 bound to its inhibitory unit IκBα
- In response to extracellular inducers, such as ROS, the IκBα kinase complex is activated and IκBα becomes phosphorylated at serines 32 and 36, and leads to ubiquination at lysines 21 and 22
- This leads to degradation of IκBα subunit by the 26S proteosome
- Degradation of the inhibitory subunit, releases the p50/p65 complex, allowing the complex to translocate from the cytoplasm to the nucleus
- In the nucleus, the transcription factor binds to a consensus sequence (5'-GGGACTTTC-3') and activates gene expression

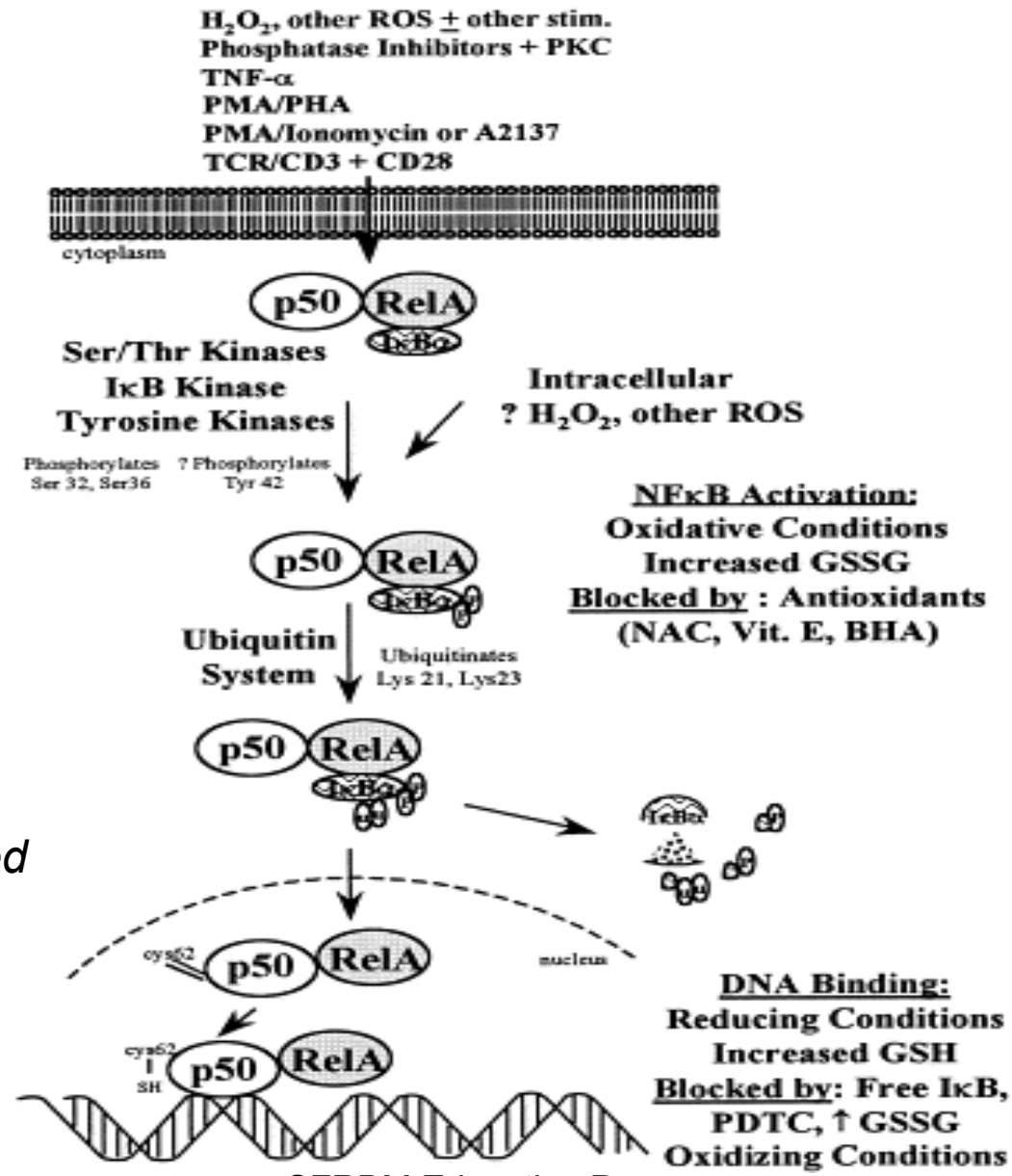

Activating Stimuli
(ROS)
IκB kinase/NEMO/IKAP
Inflammatory/Immune
Proteins
p50
IκBα
p65
P
IκBα
p50
p65
IκBα
Degradation
mRNA
CYTOPLASM
p50
p65
NUCLEUS
Transcription
Translocation
p50
p65
50
65
50
65
Target genes

Role of ROS and Redox Status in NFκB activation

- Many factors that activate NFκB also produce ROS.
- Hypoxia/reoxygenation and oxidants (such as hydrogen peroxide) have been shown to induce NFκB activation in some cell types.
- Inhibition or overexpression of enzymes that affect intracellular ROS can modulate activation of NFκB.
- Antioxidant supplementation can block NFκB activation.
- The DNA binding domain needs to be in reduced form, (especially cysteine 62) *in vitro*, to bind to its responsive elements.
- Redox regulation may be dual-fold: reducing conditions can block IkB degradation but can enhance transcriptional activity by enhancing its ability to bind in the nucleus

Modulation of NFκB Activation by Antioxidants





Free Radic Biol Med
25:346-361

Some Considerations…

- Activation of NFκB by hydrogen peroxide may be cell specific.
- Not all activation pathways require oxidative stress as a component
- Lipid peroxides may be important in activation in some cell types
- Antioxidants may inhibit NFκB activity through mechanisms distinct from redox regulation

- However, in certain cases, oxidative stress is a potent activator of NFκB and has an important role in regulating cell survival and immune response.

Summary

- NFκB plays an important role in regulating immune and inflammatory response, apoptosis and cell survival.
- ROS and redox status plays an important role in NFκB activation in some cases.
- Several steps in the activation cascade may be affected by redox status, including IKK complex phosphorylation and DNA binding.
- Antioxidants may be an effective strategy in modulating excess NFκB activation in chronic inflammatory states and cancer.