Redox Regulation of Gene Expression or... The Alphabet Soup of Transcription Factors

by

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Multiple Levels of Regulation are Possible

- *Signal transduction*
    - The flow or relay of information from a "cause", often an extracellular stimulus, to an "effect", usually a change in the pattern of gene expression
- *Transcription*
    - The synthesis of an RNA molecule from a DNA template
- *Translation*
    - The synthesis of a polypeptide chain from an mRNA template
- *Post-Translational Modification*
    - Modification of protein structure and function by addition of various prosthetic groups

Signal Transduction - The Basics

- *Activation of Kinase Cascades*

  - **Receptor Kinases**
    - Transmembrane receptors with extracellular ligand binding domain and intracellular kinase domain
    - Ligand binding activates receptor dimerization and autophosphorylation, followed by phosphorylation of other target molecules
    - Examples
      - Epidermal growth factor (EGF) receptor
      - Insulin receptor
      - Platelet derived growth factor (PDGF) receptor
        - *Hydrogen peroxide* is involved PDGF receptor signaling

  - **Non-receptor Kinases**
    - Intracellular proteins regulated by transmembrane receptor molecules such as cytokine receptors

    Interaction with intracellular domain of receptor proteins through "src-homology" (SH) domains 2 and 3 (SH2 domains bind phosphotyrosine, SH3 domains bind proline-rich stretches) activates their kinase activity
    - Examples
      - Src, the oncogene product of the Rous ***s**a**rc**oma* virus
        - At least two members of the Src family, $p56^{lck}$ and $p59^{fyn}$, can be activated by hydrogen peroxide and the oxidizing agent diamide
      - JAK, so-called ***Ja**nus **k**inases*

Signal Transduction - The Basics (cont.)

- *Ras Activation*
    - Ras, an onco-protein named for a tumor virus which causes ***ra***t ***s***arcoma
    - Ras is a small (~21 kDa) membrane associated guanine nucleotide binding protein, or G-protein.
    - Ras is activated by receptor mediated activation of guanine nucleotide exchange factors (GEFs) which facilitate the exchange of Ras-bound GDP with GTP.
    - Ras activation initiates a "cascade" of downstream phosphorylation events which proceed through Raf kinase and MEK (***M***AP/***E***RK ***k***inase).
        - MAP kinase: ***M***itogen ***A***ctivated ***P***rotein kinase
        - ERK kinase: ***E***xtracellular signal ***R***egulated ***K***inase
    - Ras auto-inactivates by hydrolyzing GTP to GDP with the help of GAP (***G***TPase ***A***ctivating ***P***rotein)

- ***S****tress-****A****ctivated ****P****rotein ****K****inases (SAPKs)*
    - A stress activated phosphorylation cascade
    - May be initiated by cytokines, UV radiation, oxidants; signaling occurs through the G-protein Rac
    - MKKK (***M***AP ***k***inase ***k***inase ***k***inase)
    - MKK (***M***AP ***k***inase ***k***inase)
    - MAP kinases
        - JNK (***J***un ***N***-terminal ***K***inase) -- Phosphorylates the transactivating domain of Jun thus enhancing its transactivating ability.
        - p38

Examples of Redox Regulated Signal Transduction

- *Ras Activation by Nitric Oxide (˙NO)*

  - Ras can be S-nitrosylated on cysteine 118 (Cys^{118}) by $^{\bullet}NO$
  - S-nitrosylation of Ras on Cys^{118} stimulates Ras guanine nucleotide exchange, GTPase activity, and downstream signaling through ERKs.

- *Activated Ras can lead to Superoxide ($O_2^{\cdot-}$) Generation*

  - Ras interacts with Rac, another G-protein, to stimulate NADPH oxidase production of superoxide in ras-transformed fibroblasts

  Increased superoxide associated with increased DNA synthesis

- *Activation of guanylate cyclase by ˙NO*

  - ˙NO reacts with the metal at the catalytic site of guanylate cyclase to activate the enzyme, thus leading to increased production of cyclic GMP.

- *PDGF Receptor signaling is mediated in part by production of* H_2O_2.

  - H_2O_2 is produced intracellularly after PDGF binds to the PDGF receptor

The ABC's of Transcriptional Control

- *Transcription Factors*

  - Proteins, phosphoproteins
  - Basal transcription factors vs. Specific transcription factors
  - Cis and Trans Actions
    - Cis-elements are specific sequences of nucleic acid through which proteins interact. They might be considered "receptors" in a way, in that a given nucleotide sequence is specific for a particular transcription factor.
    - Trans-factors are the proteins which interact with nucleic acids through specific cis-elements and modulate the transcriptional activity of nearby gene
  - Structure -- Transcription Factors have a modular domain structure
    - DNA binding domain
    - Dimerization domain
    - Transactivation domain
    - Ligand binding domain
  - Dimerization -- A common feature of transcription factors is that they bind DNA as homo- or hetero-dimers
  - Activity -- Transcription factors require two distinct activities to activate specific gene expression.
    - DNA binding activity - The DNA binding domain must be able to interact with its cognate cis-element.
    - Trans-activating activity - The transactivating domain must be able to interact with other participants in the transcription complex.
  - Transcription Factor Inhibitors
    - Regulatory factors which bind to and inhibit the activity of transcription factors
  - Examples
    - IκB (***I***nhibitor of NF-*k**B***) Cytoplasmic inhibitor of NF-κB nuclear localization and transactivation. IκB is regulated by phosphorylation and ubiquitin-mediated degradation to release active transcriptional active NF-κB
    - AP-2β (***A***ctivator ***P***rotein-***2*** *b*) Acts as a dominant inhibitor of AP-2.

Redox Regulation of Transcription Factors

In Vitro -- Oxidants generally decrease transcription factor DNA binding activity
vs.
In Vivo -- Oxidants often increase transcription factor DNA binding activity

Molecular mechanisms to explain this apparent paradox are a subject of intense investigation in this field. One attractive idea is that oxidative stress activates reductases which then act upon transcription factors to enhance their DNA binding activities in the face of increased intracellular oxidants. Perhaps the best candidate identified to date with such a role is Ref-1.

Examples of Redox Regulated Transcription Factors

- *Eukaryotes*
  - AP-1 (***A***ctivator ***P***rotein-***1***), a dimer consisting of any two of various members of the Jun and Fos families.
  - NF-κB (***N***uclear ***F***actor inducing immunoglobulin *k* light chain in ***B***-cells) consists of a heterodimer of two subunits known as p50 and p65.
  - Egr-1 (***E***arly ***g***rowth ***r***esponse-***1***) An early response gene with fos-like kinetics.
  - AP-2 (***A***ctivator ***P***rotein-***2***) A developmentally regulated trans-acting factor, binds DNA as a homo- or heterodimer with other members of the AP-2 gene family.

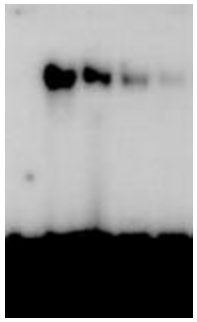
Examples of Redox Regulated Transcription Factors (cont.)

- *Prokaryotes*
  - OxyR
    - A transcriptional regulator of genes induced by H_2O_2 in *E. coli* and *S. typhimurium*. Induced genes include the following:
      - hydroperoxidase I
      - alkyl hydroperoxide reductase
      - glutathione reductase
  - SoxR
    - A sensor and regulator of the adaptive response to superoxide and nitric oxide in *E. coli*.
    - SoxR contains an Fe-S cluster which is redox sensitive, and activates transcription of SoxS, another transcriptional regulator. SoxS, in turn, trans-activates the expression of a variety of other stress response genes.

Redox Modulation of AP-2 DNA Binding Activity

Treatment with diamide, a thiol oxidant, inhibits DNA binding of AP-2

[diamide]



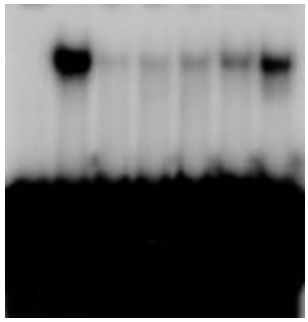
1 2 3 4 5

Oxidation by diamide inhibits AP-2 DNA binding. Purified rhAP-2 was pre-incubated for 20 minutes at room temperature with increasing concentrations of diamide and then tested for DNA binding activity. Lane 1, probe only: lane 2, untreated AP-2 protein; lane 3, 1 mM diamide; lane 4, 5 mM diamide; lane 5, 10 mM diamide.

Treatment with b-ME, a reducing agent, restores AP-2 DNA binding

b-ME - - -

Diamide - - + + + + +



Oxidant induced inhibition of AP-2 DNA binding activity is reversible by the reductant β-mercapto-ethanol (β-ME) in a dose dependent manner. rhAP-2 was treated with 20 mM diamide for 20 min at room temperature followed by incubation for 20 min at room temperature with increasing concentrations of β-ME ranging from 0.02% to 2.5%. Lane 1, probe only; lane 2 probe plus rhAP-2 only.

1 2 3 4 5 6 7

Molecular Mechanisms for Redox Regulation *in vivo*

Little is known about the precise mechanisms underlying ROS (***R***eactive ***O***xygen ***S***pecies) mediated activation of transcription factors.

NFkB

- NFκB is bound in the cytoplasm in an inactive form by IκB.
- Oxidants somehow induce IκB phosphorylation, leading to its ubiquitination and degradation, thus liberating NFκB to translocate to the nucleus to bind DNA and activate transcription of NFκB responsive genes.

AP-1

- Oxidants somehow change the phosphorylation status of Jun, perhaps through activation of MAP kinases such as JNK.
- Ref-1, in the presence of reducing equivalents (NADPH), thioredoxin and thioredoxin reductase reduces conserved cysteines in the DNA binding domains of several transcription factors.

Conserved Cysteines

Conserved cysteine residues reside in the DNA binding domains of many transcription factors. They are often involved with Zn^{2+} coordination in the formation of "zinc fingers" which interact directly with DNA cis-elements. These cysteines must be reduced for the transcription factors to bind DNA *in vitro*. Examples include the following:

- NF-kB; Cys^{62}
  - Reduced cysteine required for DNA binding activity
  - Reduced by Ref-1 (***Re***dox ***f***actor-***1***), which is identical to a DNA repair enzyme with an AP (apurinic) endonuclease activity
  - Reduced by thioredoxin
- AP-1; Cys^{252}
  - Reduced by Ref-1
- Egr-1
  - Cys oxidation inhibits DNA binding
  - DNA binding restored by Ref-1
- Myb; Cys^{130}

Iron-Sulfur Centers

Protein activity can be changed by disruption of Fe-S cluster. This can be accomplished through abundance of Fe or by

- SoxR
- OxyR
- IRE, IRP
  - IRP=c-aconitase

Redox Regulation of Translation

- IRE, IRP (IRE-BP)
- Antioxidant Enzyme mRNA binding proteins
  - MnSOD mRNA binding protein
  - Catalase mRNA binding protein

Iron Regulatory Element (IRE)

- A stem-loop secondary structure in mRNA
  - Ferritin mRNA, single IRE on 5' end of transcript
- Transferrin receptor mRNA, five IREs on 3' end of transcript

IRP, Iron regulatory protein (a.k.a. IRE-BP)

- An iron-sulfur center regulates protein conformation
  - Iron abundant, [4Fe-4S], does <u>not</u> bind IRE
    - [4Fe-4S] IRP equivalent to cytosolic aconitase
    - Allows efficient translation of ferritin
    - Does not inhibit transferrin receptor mRNA degradation
  - Iron deficient, [3Fe-4S], <u>does</u> bind IRE
    - Inhibits ferritin translation
    - Stabilizes transferrin receptor mRNA

OxyS, an RNA Stem Loop

- *OxyS*
  - small stable RNA induced in E. coli rapidly in response to oxidative stress (H_2O_2)
  - stem-loop secondary structure
  - anti-mutator
  - mechanism--unknown

Conclusions

- Redox regulation of transcription can be direct or indirect
- ROS induce changes in cellular signal transduction
- Redox activated signal transduction pathways impinge on transcription factors
- ROS lead to changes in gene expression

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