GENE NETWORK OF REDOX REGULATION AND THE PROBLEM OF INTEGRATING LOCAL GENE NETWORKS

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Summary

Motivation: Systematization and analysis of the miscellaneous experimental data on molecular genetic mechanisms regulating gene expression under redox changes in the cell.

Availability: r*edox regulation* (http://wwwmgs.bionet.nsc.ru/systems/mgl/genenet).

Introduction

Reactive oxygen species (ROS)—including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (–OH)—are formed in various metabolic processes. The level of ROS is an important characteristic of cell function. An excess formation of reactive oxygen species is named the oxidative stress. A variety of pathological states result from or are accompanied by an increased ROS level. Therefore, the insight into molecular mechanisms underlying the cell response to oxidative stress and maintenance of normal ROS level is of great interest.

Discovery of ROS direct involvement in intracellular signal transduction and expression regulation of the genes other than those of the antioxidant systems changed basically the conception of ROS biological role. Studies of the redox (reduction–oxidation) regulation of gene expression are becoming an actively developed direction in the field of molecular biological investigations into regulation of prokaryotic and eukaryotic genetic processes. Resulting is the concept of *redox-sensitive genes,* whose expression is efficiently regulated by the intracellular redox status.

A rapid progress in gene redox regulation studies resulted in accumulating a considerable volume of the relevant data, although yet miscellaneous, mostly in bibliographical databases. Recent studies have demonstrated the roles of redox regulation in various processes, including cell proliferation, apoptosis, and stress response. Thioredoxin and glutathione systems are involved in intracellular transducing redox signals from external factors through NADPH-dependent reduction of glutathione, thioredoxin, glutaredoxin, and redox factor to certain transcription factors, thereby casing their post-translational modification. Redox regulation may affect correct protein packing, their assembly in multimeric complexes, and binding to DNA.

The system of redox-sensitive genes is a complex ensemble of interacting genes, regulated by gene networks. The redox regulation may be considered as a natural integrator of all local gene networks, producing ROS while functioning. Therefore, the goal of this work was to describe the molecular mechanisms underlying the gene network function of eukaryotic redox regulation.

Methods

The redox regulation of gene expression is described using the computer technology GeneNet (Kolpakov *et al.,* 1998; Kolpakov and Ananko, 1999) available at http://wwwmgs.bionet.nsc.ru/systems/mgl/genenet/. The objects of gene networks and their interrelations have references to published experimental data as well as EMBL, SWISS-PROT, and MEDLINE databases. The information on transcription regulation of 60 redox-sensitive genes, their regulatory regions, and transcription factor binding sites is stored with the TRRD database (http://www.bionet.nsc.ru/trrd/), referred to in the gene descriptions.

Results

Scheme illustrating basic principles of redox regulation of gene expression is shown in Fig. 1. The scheme is visualized with the GeneNet viewer basing on the formalized information contained in the GeneNet database. This database compiles the information on signal molecules, transcription factors modified with redox changes, and redox-sensitive genes controlling main cellular processes. The TRRD database (Kolchanov *et al.,* 2000) contains formalized descriptions of these genes.

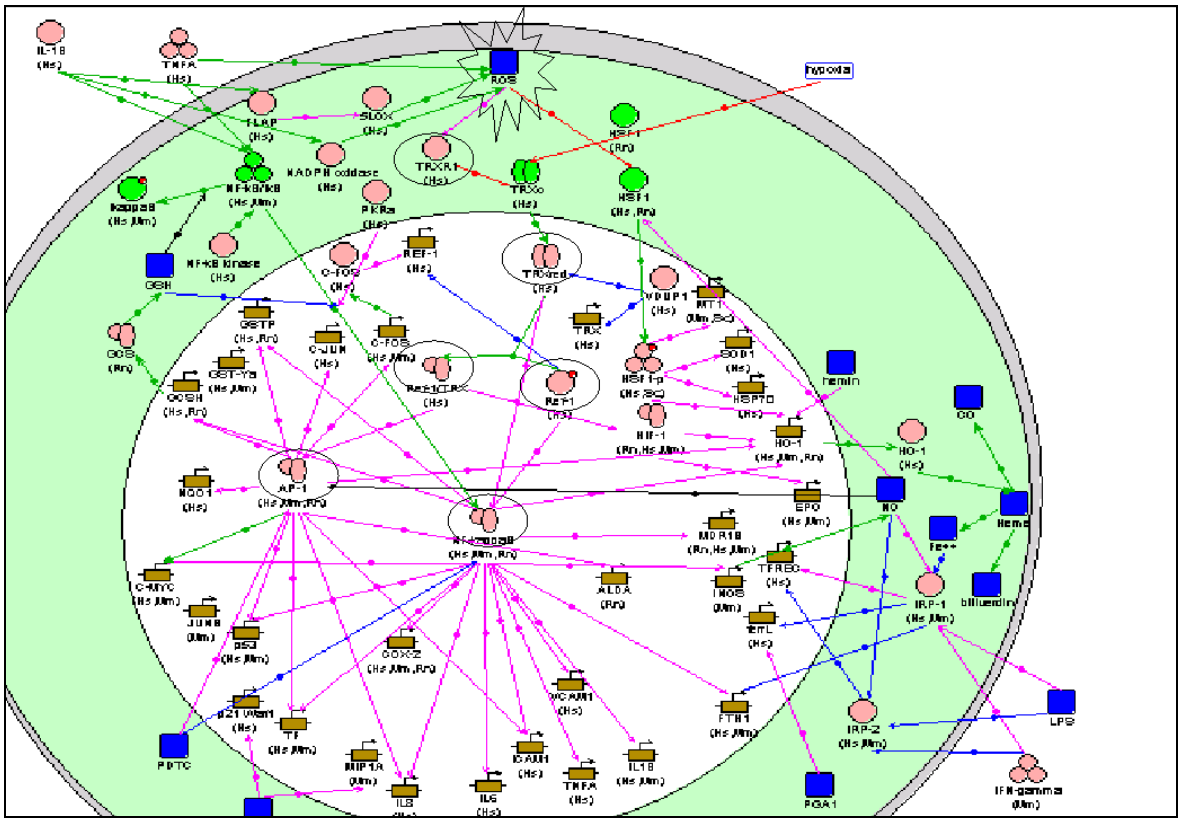

Figure 1. Scheme of redox regulation comprising the antioxidant genes induced by high ROS concentrations and the genes regulated by low ROS concentrations according to redox signaling pathway, namely, the genes involved in anti-inflammatory response.

Major activators of redox regulation gene network. Thioredoxin, one of the major factors with the thiol-mediating system, occurs in the cell in either reduced or oxidized forms and is involved in redox regulation through a reversible cysteine oxidation in its active center. In turn, the activity of thioredoxin depends on the redox status of thioredoxin reductase and its selenocysteine residues. ROS production results in selenocysteine oxidation and consequent increase in expression of this enzyme. Thus, selenocysteine is a redox sensor in the cell. Vitamin D_3–regulated protein (VDUP1), one of the known thioredoxin negative regulators, binds redox-active cysteines of the reduced thioredoxin and inhibits its biological activity as well as thioredoxin gene expression.

Another factor, the so-called redox factor Ref-1, which is a DNA repairing endonuclease (APE), is involved, together with thioredoxin, in redox regulation of DNA binding activities of several transcription factors, such as AP-1 and NF-kB. Ref-1 gene is activated by oxidative stress through induction of c-fos, the Ref-1 factor acting as a repressor of its own synthesis.

Modification of transcription factor AP-1 redox-sensitive residues regulates its DNA-binding activity and induction of a number of genes. The conservative cysteine residues in DNA-binding domains of Fos and Jun proteins mediate the redox regulation. Two proteins—thioredoxin and Ref-1, forming a heterodimer under oxidizing conditions—are necessary for AP-1 redox regulation. Thioredoxin and Ref-1 restore the NF-kB DNA-binding and transcriptional activities through interaction with cysteine at position 62 of its p50 subunit. The family of redox-regulated

transcription factors includes also HSF1, a heat shock factor; p53, the product of tumor suppressor gene and key factor regulating cell cycle–related genes; and HIF-1, a hypoxia-inducible factor.

Basal response of gene network to increase in cell ROS level. ROS are formed in all cells through oxygen metabolism. Once ROS level exceeds certain threshold value, the redox regulation gene network is triggered, causing its decrease. This is provided for through activating numerous genes of the antioxidant system (genes of superoxide dismutase, catalase, glutathione reductase, peroxidases, etc.). The redox regulation is a dynamic process maintaining the balance between productions of ROS, oxidants, and antioxidants and providing for cell homeostasis. As is shown in figure, antioxidants activate the DNA-binding and transcription activities of AP-1 complex, which thereon binds to conservative ARE elements of genes.

Gene network response to increased ROS level in catabolism processes involving iron. Catabolism processes involving iron may be considered as a model of the oxidative stress. HO-1 gene expression under the effects of various stress factors is controlled by redox-regulated transcription factors AP-1, NF-kB, HIF-1, and HSF1. Heme oxygenase is the key enzyme in heme degradation to biliverdin, carbon dioxide, and iron; in this process, biliverdin and its product bilirubin are antioxidants, whereas iron increases the oxidative stress. Ferritin binds the released iron. Regulatory proteins IRP-1 and IRP-2 control syntheses of ferritin and transferrin receptor through binding to IRE, localized to 5'- and 3'-untranslated regions of their mRNAs, according to the redox pathway via nitric oxide and cell iron content. Nitric oxide activates IRP-1 and inhibits IRP-2, an RNA-binding activity; increases ferritin expression; and decreases the level of transferrin receptor gene mRNA.

Gene network response to an ROS level increase during inflammation. NF-kB is involved in expression regulation of many genes determining the anti-inflammatory response, cell growth, and its differentiation, including those of cytokines, growth factors, and adhesins (IL-1b, IL-2, IL-6, IL-8, TNF, ICAM, iNOS, and GM-CSF). Stimulation of a cell with bacterial lipopolysaccharides, IL-1b, and TNFa results in ROS production, dissociation of inhibitor from NF-kB/IkB complex, and translocation of the active NF-kB factor into the nucleus. Depending on cell types, ROS are produced differently—through activation of either NADPH oxygenase or 5-lipoxygenase. Activation of NF-kB involves both the kinase cascade (NF-kB and IkB kinases) and redox signaling pathway, since oxidized NF-kB fails to bind to the sites of the genes it regulates. Various antioxidants, including natural antioxidant—reduced glutathione (GSH), are NF-kB inhibitors. In redox signal transduction from ROS to genes, glutathione acts as a buffer and suppresses phosphorylation of IkB inhibitor and TNFa-induced NF-kB expression. The tissue specific gene expression in response to ROS formation might be connected with different activation and binding of redox-regulated factors AP-1 and NF-kB. In case of inflammation, NF-kB activation results in induction of inducible nitric oxide synthase (iNOS) to produce nitric oxide (NO), inhibiting AP-1 DNA-binding activity.

Gene network response to an increase in ROS level under hypoxia. A low oxygen concentration (hypoxia) alters expressions of a number of genes, such as erythropoietin, heme oxygenase, and enzymes of glycolysis. The signal is transduced through thioredoxin and redox factor 1 to HIF-1 transcription factor, regulating expression of these genes. In this case, the thiol groups of cysteines are necessary for HIF-1 interaction with its coactivator CBP/p300.

Conclusion

Many key events in regulation of cellular processes, such as phosphorylation of protein transcription factors and transcription factor binding to DNA regulatory sites, are controlled by physiological redox homeostasis, in particular, thiol–disulfide balance, affected by ROS. Thus, such ROS as superoxide radical and hydrogen peroxide trigger the redox regulatory mechanism, while glutathione and thioredoxin redox systems are key expression regulators of many redox-sensitive genes, acting through changing the thiol–disulfide balance in molecules of the corresponding transcription factors. This work systematizes the available information on redox regulation of activities of p53, AP-1, NF–kappa B, HIF-1, and HSF1 transcription factors, realized through cysteine residues of DNA-binding domains with these proteins.

The redox regulation is an evolutionary conservative system controlling a vital parameter—the level of reactive oxygen species in both prokaryotic and eukaryotic cells. Any specialized gene network that appeared in the course of multicellular organism evolution had to meet the limitations imposed by the redox regulatory system. How the integration of novel gene networks into the redox regulatory system and implementation of its control function could be provided? The analysis performed demonstrates that involvement of key transcription factors controlling the function of

gene networks integrated is a possible way. This integration mechanism corresponds to the principle of limiting stage in gene networks. If a gene network while functioning produces an increased ROS level, it is integrated with the redox regulatory system through its key regulatory elements—transcription factors. In this process, the following versions are possible: (1) ROS level decreasing with involvement of redox systems of major cellular biothiols—glutathione and thioredoxin; (2) the function correction (activity regulation of the system under control); and (3) cell death in case the ROS level exceeds the norm considerably.

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