SIGNAL TRANSDUCTION

A Bridge to Control

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Aerobic metabolism conferred great energy benefits to the organisms in which it evolved. But it came with a price: the potential for oxidative damage from respiratory by-products. Organisms combat this threat with various devices, but some damage does occur and is often cited as a contributor to degenerative diseases and aging (*1*) or apoptotic cell death (*2*). Two recent reports, one in this issue (*3*, *4*), show how the tables can be turned on oxidative damage: A reaction that usually inactivates cytoplasmic proteins—the oxidation of cysteines to form intramolecular disulfides—has been exploited as an on-off swtich in signal transduction pathways that control gene expression. Such a mechanism provides for a sensitive and dynamic link from cellular energy economy and thiol metabolism to gene expression.

Antioxidant defenses such as superoxide dismutase, catalase, and the reduced form of glutathione (GSH) are almost universally present in aerobic organisms and help prevent the oxidative decay of cellular structures and DNA information (*5*). The extents of these activities can be adjusted in response to environmental cues in many organisms, and the coordinate expression of multiple defense functions constitutes an oxidative stress response. The two best characterized oxidative stress responses are controlled by the OxyR and SoxR transcriptional activators of *Escherichia coli* (*6*). The OxyR system is activated by treating cells with low concentrations of H_2O_2, whereas the SoxR system is activated by treatment with superoxide-generating compounds or nitric oxide. The activation of these responses provides greatly increased cellular resistance to oxidative agents (*6*).

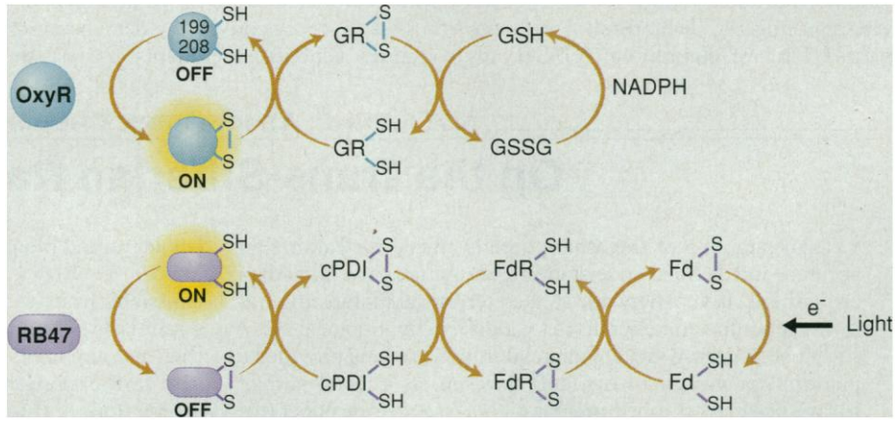
Both OxyR and SoxR are present in "unstressed" cells, where they await activation. The nature of the biochemical signaling events that unleash the latent transcriptional activity of these proteins has been uncertain for some time. Last year, activation of SoxR protein was shown to result from reversible one-electron oxidation of its iron-sulfur centers (*7*, *8*). Now, on page 1718 of this issue, Zheng *et al.* provide evidence that OxyR is reversibly activated by the formation of an intramolecular disulfide. This activation is balanced by reduction through the thiol donors glutaredoxin and GSH.

The analysis of redox signaling events like these has been beset by some chronic problems. Most vexing is the tendency of all cellular molecules, proteins included, to undergo spontaneous oxidation as cells are lysed. Many proteins lose activity upon oxidation, but unexpectedly, OxyR could activate transcription in vitro even when the protein was isolated from untreated bacteria (*9*). This result was evidently attributable to adventitious oxidation during protein purification; treating OxyR with thiol-reducing agents such as dithiothreitol switched the activity off (*9*). An obvious mechanism would have been the formation of protein disulfide bonds, but biochemical and protein engineering experiments seemed to eliminate roles for disulfides between or within OxyR molecules. It was proposed instead that OxyR activation is caused by partial oxidation of a cysteine thiol to form the sulfenate or sulfinate (*9*).

Cysteine-199 of OxyR was initially thought to be the only cysteine critical for activation in vivo (*9*), but more complete studies (*10*) recently revealed a contribution, albeit smaller, by cysteine-208. Protein engineering and biochemical approaches (*4*) have now resolved the genetic uncertainty. The new work depends on an OxyR derivative that exhibits normal regulation in vivo but retains only Cys^{199} and Cys^{208} (the others are replaced by alanines). Proteolytic digestion of active OxyR and analysis by mass spectrometry revealed a specific dipeptide linked by a disulfide between Cys^{199} and Cys^{208}; this disulfide disappears from OxyR upon chemical reduction in vitro.

When Cys^{208} of OxyR is replaced by serine, Cys^{199} residues from separate OxyR molecules may become crosslinked (*4*). Such a form could account for the activity retained in OxyR derivatives that retain only Cys^{199}. It is not known, however, whether OxyR with such an *inter*molecular disulfide mimics the structure of the protein with the *intra*molecular crosslink.

OxyR activation is reversed by cellular disulfide-reducing machinery, with particular dependence on glutaredoxin, a small protein with cysteines active in thiol-disulfide exchange. Glutaredoxin links OxyR to GSH metabolism: Like glutaredoxin-deficient *E. coli*, both GSH-deficient and GSH reductase–deficient strains switch off OxyR activity more slowly than do wild-type cells. Another thiol-disulfide exchange protein, thioredoxin, has no obvious role in OxyR down-regulation, but it may contribute to maintaining the reduced state in the absence of oxidative stress.



Regulation by oxidation. OxyR and RB47 can be switched on and off by alterations in the redox state of the cell. GR, glutaredoxin; FdR, ferredoxin-thioredoxin; and Fd, ferredoxin.

The new observations resolve some confusion and yield a model in which the activation of a redox-regulated transcription activator is linked to the cellular thiol economy. This model (see the figure) places OxyR between opposing pathways: activation by H_2O_2-mediated oxidation to form an intramolecular disulfide, and inactivation by glutaredoxin- and GSH-dependent reduction. Having an oxidative stress sensor poised between contrary pathways of oxidation and reduction parallels the situation for SoxR (*7*). The opposing pathways produce a dynamic response, and both OxyR (*4*) and SoxR (*11*) are inactivated rapidly after withdrawal of oxidative stress.

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Redox signaling that exploits the oxidative formation of protein disulfide bonds may occur widely. Another recent report links a chloroplast disulfide isomerase in *Chlamydomonas reinhardtii* with light-regulated translational control by an RNA binding protein (*3*). Binding of two proteins is required to activate translation of the *psbA* transcript (encoding a photosystem component), and Mayfield's group has isolated these proteins by affinity binding and cloned the structural genes. The sequence of one of these, a 60-kilodalton polypeptide, revealed a surprise: clear homology to both plant and mammalian protein disulfide isomerases (PDIs). These proteins contain pairs of thiols in the sequence Cys-Gly-His-Cys that are active in thiol-disulfide exchange, and two of these motifs appear in the 60-kilodalton chloroplast PDI (cPDI).

The cPDI seems to transmit metabolic signals to a 47-kilodalton protein (RB47) that binds the 5′-untranslated region of *psbA* mRNA (*3*). When RB47 is active, the presence of cPDI and dithiothreitol has no further effect, which suggests that the RB47 cysteines are already fully reduced. However, replacing the dithiothreitol with the oxidized form of glutathione (GSSG) allowed cPDI to inactivate mRNA binding by RB47; GSSG on its own had only a small inactivating effect. The cPDI can thus transmit disulfides from GSSG to RB47 and inactivate it. If the cysteines of RB47 are chemically oxidized to disulfides, which inactivates mRNA binding, cPDI can transmit reduced thiols from dithiothreitol to RB47 and reactivate it. The overall model (*3*) hypothesizes that reducing equivalents arise from the chloroplast photosystem during light exposure and are relayed through ferredoxin to ferredoxin-thioredoxin reductase, and thence to cPDI and RB47 (see the figure). In darkness, phosphorylation of cPDI might reverse the process, allowing oxidized RB47 to accumulate (*3*).

The concept of gene regulation by the formation and reversal of protein disulfides has been mooted before. Numerous mammalian transcription factors, such as nuclear factor κB and AP-1, have been proposed to be redox-regulated through key cysteine residues (*12*). However, the corresponding experimental data are not always consistent and rely most often on in vitro experiments, which are subject to the limitations discussed above. A convincing demonstration requires genetic experiments to test the roles of proposed redox-sensitive protein residues. Nonetheless, it would be surprising if such a facile regulatory mechanism were not used repeatedly in biology. We can look forward to new examples of thiol-disulfide exchange as a molecular on-off switch in gene control.

References

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NOTA BENE: ATMOSPHERIC CHEMISTRY

On the Trans-Siberian Railroad

Global sampling of the atmosphere is crucial for gaining an accurate and detailed understanding of its chemistry. Vital data are gathered from a network of fixed terrestrial monitoring stations, but unfortunately, this network is patchy in some parts of the globe. Ocean-going commercial ships have long provided a platform for measurements, and they are an integral part of today's ocean monitoring programs (*1*). To cover the upper troposphere, recent campaigns have used not only research planes but also commercial aircraft, most notably in the Measurement of Ozone by Airbus-in-Service Aircraft (MOZAIC) program. With five aircraft, ozone and water vapor have been measured on thousands of flights (*2*). Another program, CARIBIC, uses a fully automated equipment payload to obtain a more extensive set of measurements on various flight routes (*3*).

Such mobile commercial platforms have distinct advantages for atmospheric measurements, such as regular service, rapid coverage of large areas, and modest cost. Over land, measurements from trains could be used to complement fixed monitoring stations; however, this idea has only recently been realized. Crutzen *et al.* (*4*) and Bergamaschi *et al.* (*5*) now report measurements within the project TROICA (Trans-Siberian Investigation of the Chemistry of the Atmosphere). A laboratory wagon located behind the electric pulling locomotive of a passenger express train traveled along the Trans-Siberian railroad during the summer of 1996 and measured a set of chemicals (O_3, NO, NO_2, CO, CH_4, SF_6, and black carbon aerosol). The route from Moscow to Vladivostok covers 9000 km across regions with extremely limited data coverage before this measurement campaign. Isotope analysis has identified biomass burning as the source of extended enhanced levels of CO; elevated CH_4 levels are attributed to emissions from west Siberian wetlands (*6*), rather than to natural gas escaping during exploitation and distribution. Further measurements during different seasons are under way to gain further insights into the source regions, transport, and interactions of these trace gases.

These results show that trains as atmospheric monitoring platforms, especially for remote areas, can provide a wealth of information that would otherwise require a prohibitively expensive fixed monitoring network.

References and Notes

1. Such a program is the international effort for studying the El Niño–Southern Oscillation in the Pacific Ocean; see, for example, "Learning to Predict Climate Variations Associated with El Niño and the Southern Oscillation: Accomplishments and Legacies of the TOGA Program", National Research Council (National Academy Press, Washington, DC, 1996).
2. Results from flights over the tropical Atlantic Ocean within this program have shown that there are pockets of unexpectedly high ozone, the origin of which is not yet well understood [K. Suhre *et al.*, *Nature* **388**, 661 (1997)].
3. The CARIBIC (Civil Aircraft for Remote Sensing and In-Situ Measurement of the Troposphere and Lower Stratosphere Based on the Instrument Container Concept) measuring campaign is performed on board Lufttransport-Unternehmen GmbH (LTU) aircraft. First results will be published soon (C. A. M. Brenninkmeijer *et al.*, *J. Atmos. Sci.*, in press).
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