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Commentary

# Redox control of gene expression involving iron–sulfur proteins. Change of oxidation-state or assembly/disassembly of Fe-S clusters?

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**Abstract Attention is drawn to a mechanism of redox control of gene expression involving Fe–S proteins which depends on the disassembly and reassembly of Fe–S clusters rather than a change in oxidation state. Iron Regulatory Protein (IRP)/ aconitase and FNR are discussed as examples for such a mechanism.**

Two years ago, in this journal, J.F. Allen [1] pointed out features common to several regulators of gene expression that have been implicated in 'Redox control' of transcription. Similar views have been presented elsewhere [2,3]. In Allen's paper, six regulatory proteins were postulated to be directly controlled by oxidation-reduction potential. One was FNR of *Escherichia coli*, which we now know is an iron-sulfur (Fe–S) protein [4,5] and five others were suggested to involve Fe–S proteins.

In this commentary, we would like to expand upon the interpretation of 'redox control', to consider the situation when iron sulfur clusters are involved in the process. In this case, it may not be as important whether the cluster is oxidized or reduced as, whether it is actually there or not. Probably, to date the best explored example is that of the iron regulatory protein (IRP) found in eucaryotes. IRP functions as aconitase (cytoplasmic), when it contains a [4Fe–4S] cluster and as IRP when it is in the apo-form [6]. Only after loss of the Fe–S cluster is the apo-aconitase able to bind the iron-responsive elements (IRE), the hairpin loops in the untranslated regions of the mRNAs for transferrin receptor or ferritin (large subunit) that control translation [6–8]. Initially, redox control of this activity via a 'sulfhydryl switch' had been postulated [9]. We know now that, what was thought to be the sulfhydryl switch was due to oxidation-reduction reactions of thiol groups liberated after iron removal from the cluster under conditions when iron is limiting in the cell.

The FNR protein of *Escherichia coli* has been mentioned repeatedly among prominent examples for redox-control of gene expression in prokaryotes [1–3]. In vivo experiments have shown that FNR requires iron for its activity in triggering the transcription of genes for anaerobic respiration [10]. FNR contains five Cys residues, which could furnish ligands to the bound iron. Four of these Cys residues were shown by mutational studies to be essential for activity [11,12]. Initially, only about one Fe per monomer of FNR had been found, apparently excluding the presence of Fe–S clusters [13]. Evidence that the redox state of this iron determined whether FNR was active or not (the reduced form being the active one) had also been reported [14]. Furthermore, in vivo experiments demonstrated that upon raising the redox potential of anaerobic cells by addition of ferricyanide, FNR lost its activity [15]. In addition, we [4,5] have reported an in vitro experiment in which DNA binding by FNR was greatly diminished, if the binding assay was carried out aerobically instead of anaerobically in the presence of dithionite. However, more recent experiments [5] have shown that FNR in fact contains an Fe–S cluster (one [4Fe–4S] cluster per monomer) and that not only may the Fe–S cluster of FNR be oxidized when ferricyanide or atmospheric oxygen are added, but it is actually destroyed [5]. We found that the Fe–S cluster in WT FNR is lost within seconds after exposure to atmospheric oxygen, as monitored by the typical visible absorption in the 360–450 nm region and by assaying DNA binding and labile sulfide [5]. The cluster stability can be enhanced in some mutant proteins, thus facilitating experimentation [4]. Thus, the picture emerges that presumably FNR is present in cells in both forms, containing or lacking the Fe–S cluster, and that in the presence of oxygen, this equilibrium is changed towards all apo-FNR, the form that is less competent for dimerization and site specific DNA binding.

We conclude from the examples of IRP/aconitase and of FNR, that disassembly and reassembly of Fe–S clusters in response to certain signals, such as $O_2$ or iron availability, is a common mechanism of regulation of gene expression. FNR is known to control the expression of at least 75 proteins, and there is a sizable number of bacterial transcription factors that share sequence homology to FNR including the N-terminal region which contains three of the essential cysteines [2]. Thus, FNR and these related proteins may already represent a large group of proteins controlled by an Fe–S cluster assembly/disassembly mechanism.

Other examples for a regulatory function of Fe–S clusters have recently come to light: ferrochelatase [16], or anacrobic ribonucleotide reductase of *E. coli* [17], both of which are inactive without a [2Fe–2S] cluster; the SoxR protein of *E. coli* where [2Fe–2S] clusters are required for the defense response against $O_2^-$ [18]. Apparently, the oxidation state of the SoxR cluster, i.e. whether $[2Fe–2S]^{2+}$ or $^{1+}$ is of minor importance. In this case, the apo-protein appears to be the inactive form in cells [18].

Assembly of Fe–S clusters in vitro occurs spontaneously, if the ingredients, Fe, sulfide and RSH are supplied and anaerobic conditions are maintained. One may, therefore, assume that this can also occur in vivo when the same ingredients are available. In this case, in addition to the proper constellation

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of Cys residues, carriers for Fe and $S^{2-}$ may also be necessary. Fe-citrate or Fe-ADP can serve the role of an Fe-carrier. Alternatively, special proteins may be present in the cell for this purpose. Sulfide derived from Cys may be provided by the apparently widely distributed enzymes of the NifS type [19]. These proteins can act as carriers of $S^0$ (sulfan sulfur), which under reducing conditions will furnish $S^{2-}$. Other sulfur transferases may also be used.

The degradation of Fe–S clusters under aerobic conditions also proceeds spontaneously, unless the cluster is shielded by protective ligands or altogether isolated by a hydrophobic environment from access via the aqueous phase. The so-called 'high-potential' Fe–S proteins have relatively well shielded, stable [4Fe–4S] clusters. A protein such as aconitase, on the other hand, provides an example of an unstable [4Fe–4S] cluster. When functioning as aconitase, the protein offers water soluble substrates access to one specific iron of the cluster. This iron has no Cys but rather an $OH^-$ ligand. Thus, this iron, if not protected by a bound substrate or inhibitor, is readily lost on exposure to air. The attack by oxygen is thought to be mainly directed to the thiolate bonds. In vivo, other factors could, of course, also come into play, such as NO and its derivatives or labile reaction products with reduced forms of oxygen [20]. Both of these fields, biosynthesis or biodegradation of Fe–S clusters are still in their early development and the next few years are likely to provide a clearer picture than what we have at this time.

In many cases, changes in redox-potential have been suggested to be responsible for changes in gene expression in response to oxygen availability [1–3]. However, it must also be considered that with changing the redox potential, e.g. by addition of ferricyanide, dithionite, or dyes, reactions, other than reversible oxidation-reduction may take place. There are few, if any, examples that involve a redox component such as heme, in which case the state of oxidation or ligation of the presumed sensor could be unambiguously assessed. The two component regulatory system, FixL (a heme protein) and FixJ, of *Rhizobium meliloti* or *Bradyrhizobium japonicum* attracted considerable attention in this respect [21]. FixL is an oxygen sensor where binding of oxygen to a heme prosthetic group regulates the protein kinase activity of FixL. At low levels of oxygen, autophosphorylation of FixL is stimulated, triggering a phosphorylation cascade which leads to activation of FixJ and transcription stimulation of a number of genes. The state of oxidation and coordination of the heme have been examined by spectroscopy which have shown that it is a change in spin rather than oxidation state of the heme that exerts the control [22,23]. Apparently the change in spin state is coupled to a conformational change in FixL. Reversible oxidation-reduction of sulfhydryls (2 R–S–H) to disulfide (R–S–S–R) may also be used as a mechanism of redox regulation. However, as was discussed above, involvement of this redox system may actually point to the presence of a vacant Fe–S binding site.

In summary, we have drawn attention to a novel and potentially simple regulatory mechanism for gene expression, which is based on the great sensitivity of Fe–S clusters to oxygen and the ease with which Fe–S clusters can apparently be disassembled and reassembled. Similar agents, such as reduction products of oxygen or NO might also play a similar role as $O_2$, but quantitative aspects of the reversibility have yet to be explored. In addition, the availability of iron is likely to play a role in this process. Since the protein environment of Fe–S clusters is presumed responsible for determining how stable a given cluster is to $O_2$ or other agents, one can easily see how protein bound Fe–S clusters could provide a mechanism for detecting a wide range of concentrations of $O_2$ and related species.

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