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Current Opinion in Chemical Biology

Iron–sulfur cluster sensor-regulators

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Regulatory proteins that contain an iron–sulfur cluster cofactor constitute a group that is growing both in number and importance, with a range of functions that include sensing of molecular oxygen, stress response, and iron regulation. In all cases, the cluster plays a central role, as a sensory module, in controlling the activity of the regulator. In some cases, the cluster is required for the protein to attain its regulatory form, while in others the active form requires loss or modification of the cluster. In this way, nature has exploited the inherent reactivity of iron–sulfur clusters. Here, we focus on recent advances that provide new insight into the remarkable chemistries exhibited by these regulators, and how they achieve the required levels of sensitivity and specificity.

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Current Opinion in Chemical Biology 2012, **16**:35–44

This review comes from a themed issue on
Bioinorganic Chemistry
Edited by Fraser Armstrong and Lawrence Que

Available online 2nd March 2012

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2012.02.009

Introduction

Iron–sulfur clusters are extremely widespread in nature, occurring as protein-cofactors that perform a remarkable variety of roles [1,2]. A major function of these cofactors that has recently emerged is in signal transduction within DNA-binding and RNA-binding regulatory proteins, in which an analyte (resulting, for example, from oxidative or nitrosative stress) is sensed, resulting in differential nucleic acid binding and subsequent transcriptional or translational re-programming [3].

Iron–sulfur regulatory proteins contain either [2Fe–2S] or [4Fe–4S] clusters, see Table 1. The [4Fe–4S] cluster consists of two interpenetrating tetrahedra of iron (as Fe^{3+} or Fe^{2+}) and sulfide (S^{2-}) ions, forming a cube that is linked to the protein framework by four amino acid residues at the vertices of a tetrahedron. Loss of one of the iron ions can lead to the formation of a [3Fe–4S] cluster, and although these are found in electron-transfer proteins, they have not yet been identified as a stable form in any iron–sulfur cluster regulator. The [2Fe–2S] cluster consists of a [Fe_2-(μ_2-$S)_2$] rhomb, coordinated by protein amino acid residues that lie in a plane perpendicular to the plane of the rhomb. Cysteine thiolates (RS^-) are most commonly found as coordinating ligands to [2Fe–2S] and [4Fe–4S] cluster irons, but other residues, such as histidine (–N=), serine ($R–O^-$) and aspartate (RCO_2^-), sometimes occur.

Iron–sulfur clusters are usually redox active, with reduction potentials highly dependent on which redox couple is involved and, importantly, the protein environment of the cluster [1]. Cluster stability tends to decrease as the overall oxidation level increases. This redox activity, which underpins the functional importance of many clusters, also means that iron–sulfur clusters are susceptible to damage from reactions with reactive oxygen and nitrogen species, often leading to cluster conversion or even complete loss [4]. They are also prone to damage in the presence of strongly coordinating species, which may target iron or thiolates/sulfides. For example, copper and cobalt toxicity results in part from the high affinities of these metals for the sulfur ligands of iron–sulfur clusters [5•,6]. Nitric oxide (NO) also reacts with iron–sulfur clusters forming various iron-nitrosyl species [7].

The inherent reactivity of iron–sulfur clusters makes them ideally suited to roles in sensing stress caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). Although such regulators are still relatively few (see Table 1), they exhibit complex and fascinating chemistries, in which the sensing mechanism is invariably centered on the cluster, and involves significant structural rearrangements leading to protein conformational changes, which are sufficient either to promote or to abolish nucleic acid binding. Here we review some of the major recent advances in our understanding of iron–sulfur cluster-based sensory mechanisms and consider the outstanding issues to be addressed.

Tuning reactivity and specificity

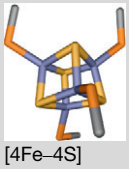
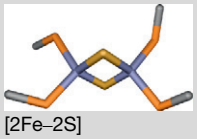
In order that iron–sulfur clusters can function effectively as sensory components of regulatory proteins, they must exhibit appropriate sensitivity and specificity. Recent studies of the O_2-sensing global regulator fumarate and nitrate reduction (FNR) [8] have begun to address questions about the evolution of optimum reactivity. FNR, a member of the CRP-FNR superfamily of regulators, contains an N-terminal sensory domain and a C-terminal DNA-binding domain [9]. *Escherichia coli* FNR becomes activated under anaerobic conditions through

Glossary
CRP: catabolite repressor protein. Founding member of a family of regulators structurally related to FNR.
DNIC: dinitrosyl iron complex.
EPR: electron paramagnetic resonance. A spectroscopic technique useful for studying systems with unpaired electrons (paramagnets), including many transition metal complexes.
NRVS: nuclear resonance vibrational spectroscopy. A technique in which inelastic scattering of high energy radiation allows the measurement of vibrations between 500 and 700 cm^{-1} which, in the case of iron-nitrosyls, are diagnostic of the type of complex.
RRE: Roussin's red ester.

the binding, by four conserved Cys residues, of an O_2-labile [4Fe–4S] cluster into the N-terminal sensory domain, which causes the ~30 kDa protein to dimerize and facilitates specific binding (of the C-terminal domain) to specific sequences in FNR-controlled promoters [8,10], see Figure 1a. Upon exposure to O_2, the FNR $[4Fe–4S]^{2+}$ cluster undergoes a one-electron oxidation reaction that very likely involves the initial binding of O_2 directly at the cluster. The superoxidized cluster is unstable, yielding first a $[3Fe–4S]^{1+}$ form and then a $[2Fe–2S]^{2+}$ form, with the overall generation of two S^{2-} ions and two irons (one Fe^{2+} and one Fe^{3+}). The reaction also results in the generation of superoxide,

Table 1

Iron–sulfur cluster regulatory proteins.

Regulator	Cluster type	Function/comments	Reference
FNR	 [4Fe–4S]	Global regulator O_2 sensor in a wide range of bacteria. Secondary function in NO sensing. Activator and repressor of genes involved in anaerobic and aerobic respiration, respectively. Undergoes cluster conversion via a [3Fe–4S] intermediate to a [2Fe–2S] form that no longer binds DNA. Variation in cluster depending on organism; can be N-terminal or C-terminal.	[8,10]
NreB	[4Fe–4S]	O_2 sensor in staphylococci Functions as a [4Fe–4S]-dependent cytoplasmic histidine kinase that phosphorylates NreC, thereby activating it to bind to specific sequences upstream of anaerobic respiratory *nar* and *nir* operons. [4Fe–4S] NreB is O_2 sensitive, decaying to [2Fe–2S]/apo forms.	[49]
ArnR	Unknown	O_2 sensor in corynebacteria Functions as a repressor of the *narKGHJI* operon under aerobic conditions.	[50]
SoxR	 [2Fe–2S]	Redox/O_2^- stress sensor Long believed to be a sensor of superoxide, but recently proposed to sense small natural redox-active molecules. The only iron–sulfur sensor known to activate transcription through oxidation state change at the cluster (+1 to +2). Part of a two component system, with SoxS in enteric bacteria, and as a one component regulator in nonenteric bacteria.	[20••,33••]
NsrR	[2Fe–2S]/[4Fe–4S]	Global regulator of NO stress response Rrf2-family regulator. Uncertainty about the nature of the cluster, which may be variable. Cluster-bound form binds upstream of NsrR-regulated genes. Nitrosylation abolishes DNA-binding. Some evidence for additional, cluster-independent DNA-binding.	[46,48•]
Wbl	[4Fe–4S]	Key roles in cell developmental processes in actinomycetes Found only in actinomycetes, which usually contain multiple Wbl proteins. These regulatory proteins are involved in major metabolic reorganization (sporulation in *Streptomyces*, transition to dormancy in *Mycobacterium*). Evidence accumulating that at least some Wbl proteins may act as NO-sensors.	[22•,25••]
IscR	[2Fe–2S]	Sensor of cellular iron–sulfur cluster levels Rrf2-family regulator. Regulator of both the Isc and Suf iron–sulfur cluster biogenesis pathways of *E. coli*. [2Fe–2S] form acts as a repressor of Isc, while apo-form activates the Suf system.	[41,45•]
SufR	[4Fe–4S]	Sensor of cellular iron–sulfur cluster levels in cyanobacteria [4Fe–4S] form acts as a repressor of the Suf system, which is the primary iron–sulfur biogenesis system in cyanobacteria.	[51]
IRP1	[4Fe–4S]	Iron regulation; integration of NO/iron regulation [4Fe–4S] form is cytoplasmic aconitase with no regulatory activity. Under low iron or oxidative/nitrosative stress, the apo-form accumulates which binds iron regulatory elements (IREs) at the ends of transferrin receptor and ferritin mRNAs, stabilizing them or enhancing their degradation.	[27•,52]