Iron-Sulfur Proteins and Redox Regulation

Limei Zhang, Ph.D.
BIOC983
Lincoln, NE, June 11, 2016
Outline

• Iron-Sulfur (Fe-S) proteins – a general introduction
• Fe-S transcriptional regulators
  – FNR: a [4Fe-4S] O₂ Sensor
  – SoxR: a global [2Fe-2S] oxidative stress sensor
  – IscR: [2Fe-2S] regulator sensing oxidative stress, Fe and Fe-S cluters
  – [4Fe-4S] NO sensors
• Studies on Fe-S regulators: significance and challenges
• A tour to my lab
Fe-S Proteins

• Fe-S proteins: a group of proteins containing one or more metalloclusters (Fe-S clusters) with multiple iron and inorganic sulfide

• Fe-S clusters:
  – Among the most ancient cofactors
  – Ubiquitous and essential for all living organisms

• Three types of the most common Fe-S clusters:

![Diagram of Fe-S clusters]

Beinert, 1997, *Science*
Fe-S Proteins

- Three types of the most common Fe-S clusters:

- Fe-S cluster ligands:
  - Cys-S, the most common protein-based ligand
  - His-N/Arg-N
  - Asp-O
  - Glutathione-S

Fe-S Proteins

- Fe-S clusters possess rich redox properties:

2Fe-2S

- \([2\text{Fe}^{3+}:2\text{S}]^{2+}\)
- \([\text{Fe}^{3+}:\text{Fe}^{2+}:2\text{S}]^{+}\)
- \([2\text{Fe}^{2+}:2\text{S}]^{0}\)

3Fe-4S

- \([3\text{Fe}^{3+}:4\text{S}]^{+}\)
- \([2\text{Fe}^{3+}:\text{Fe}^{2+}:4\text{S}]^{0}\)
- \([\text{Fe}^{3+}:2\text{Fe}^{2+}:4\text{S}]^{-}\)
- \([3\text{Fe}^{2+}:4\text{S}]^{2-}\)

4Fe-4S

- \([4\text{Fe}^{3+}:4\text{S}]^{4+}\)
- \([3\text{Fe}^{3+}:\text{Fe}^{2+}:4\text{S}]^{3+}\)
- \([2\text{Fe}^{3+}:2\text{Fe}^{2+}:4\text{S}]^{2+}\)
- \([3\text{Fe}^{3+}:\text{Fe}^{2+}:4\text{S}]^{+}\)
- \([4\text{Fe}^{2+}:4\text{S}]^{0}\)
Fe-S Proteins

- Fe-S clusters possess rich redox properties:

Span over 1 V

Fe-S Proteins

- The redox properties account for the versatile functions of Fe-S proteins from electron transfer to catalysis to regulation of gene expression

<table>
<thead>
<tr>
<th>Function</th>
<th>Examples</th>
<th>Cluster type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron transfer</td>
<td>Ferredoxins; redox enzymes</td>
<td>[2Fe-2S]; [3Fe-4S]; [4Fe-4S]</td>
</tr>
<tr>
<td>Coupled electron/proton transfer</td>
<td>Rieske protein</td>
<td>[2Fe-2S]</td>
</tr>
<tr>
<td></td>
<td>Nitrogenase</td>
<td>[8Fe-7S]</td>
</tr>
<tr>
<td>Substrate binding and activation</td>
<td>(de)Hydratases</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Radical SAM enzymes</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Acetyl-CoA synthase</td>
<td>Ni-Ni-[4Fe-4S], [Ni-4Fe-5S]</td>
</tr>
<tr>
<td></td>
<td>Sulfite reductase</td>
<td>[4Fe-4S]-siroheme</td>
</tr>
<tr>
<td>Fe or cluster storage</td>
<td>Ferredoxins</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Polyferredoxins</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td>Structural</td>
<td>Endonuclease III</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>MutY</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td>Regulation of gene expression</td>
<td>SoxR</td>
<td>[2Fe-2S]</td>
</tr>
<tr>
<td></td>
<td>FNR</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>IRP</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>IscR</td>
<td>[2Fe-2S]</td>
</tr>
<tr>
<td>Regulation of enzyme activity</td>
<td>Glutamine PRPP amidotransferase</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Ferrochelatase</td>
<td>[2Fe-2S]</td>
</tr>
<tr>
<td>Disulfide reduction</td>
<td>Ferredoxin thioredoxin reductase</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Heterodisulfide reductase</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td>Sulfur donor</td>
<td>Biotin synthase</td>
<td>[2Fe-2S]</td>
</tr>
</tbody>
</table>

http://genomics.unl.edu/RBC_EDU/fe.html
Biosynthesis of Fe-S Clusters

- Biosynthesis of the Fe-S clusters requires a specific set of proteins and is tightly regulated

Biosynthesis of Fe-S Clusters

- Biosynthesis of the Fe-S clusters requires a specific set of proteins and is tightly regulated.

# Fe-S Proteins

- The redox properties of Fe-S proteins may account for their versatile functions: from electron transfer to catalysis to regulation of gene expression.

<table>
<thead>
<tr>
<th>Function</th>
<th>Examples</th>
<th>Cluster type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron transfer</td>
<td>Ferredoxins; redox enzymes</td>
<td>[2Fe-2S]; [3Fe-4S]; [4Fe-4S]</td>
</tr>
<tr>
<td>Coupled electron/proton transfer</td>
<td>Rieske protein</td>
<td>[2Fe-2S]</td>
</tr>
<tr>
<td></td>
<td>Nitrogenase</td>
<td>[8Fe-7S]</td>
</tr>
<tr>
<td>Substrate binding and activation</td>
<td>(de)Hydratases</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Radical SAM enzymes</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Acetyl-CoA synthase</td>
<td>Ni-Ni-[4Fe-4S], [Ni-4Fe-5S]</td>
</tr>
<tr>
<td></td>
<td>Sulfite reductase</td>
<td>[4Fe-4S]-siroheme</td>
</tr>
<tr>
<td>Fe or cluster storage</td>
<td>Ferredoxins</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Polyferredoxins</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td>Structural</td>
<td>Endonuclease III</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>MutY</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td>Regulation of gene expression</td>
<td>SoxR</td>
<td>[2Fe-2S]</td>
</tr>
<tr>
<td></td>
<td>FNR</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>IRP</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>IscR</td>
<td>[2Fe-2S]</td>
</tr>
<tr>
<td>Regulation of enzyme activity</td>
<td>Glutamine PRPP amidotransferase</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Ferrochelatase</td>
<td>[2Fe-2S]</td>
</tr>
<tr>
<td>Disulfide reduction</td>
<td>Ferredoxin thioredoxin reductase</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td></td>
<td>Heterodisulfide reductase</td>
<td>[4Fe-4S]</td>
</tr>
<tr>
<td>Sulfur donor</td>
<td>Biotin synthase</td>
<td>[2Fe-2S]</td>
</tr>
</tbody>
</table>
Fe-S Proteins as Redox Sensors/Regulators

- Fe-S proteins may react rapidly with oxygen, reactive oxygen species (ROS), and reactive nitrogen species (RNS)
Fe-S Proteins as Redox Sensors/Regulators

- Fe-S proteins may react rapidly with oxygen, reactive oxygen species (ROS), and reactive nitrogen species (RNS).

- Fe-S proteins are also be involved in Fe and Fe-S cluster homeostasis.
**FNR: [4Fe-4S] Sensor of $O_2$ and ROS**

- Fumarate and Nitrate Reduction Regulator (FNR):
  - Contains a [4Fe-4S] cluster in its active form
  - Reacts rapidly with $O_2$ through its oxygen-sensitive [4Fe-4S] cluster
  - Globally controls the transition between anaerobic and aerobic respiration in facultative anaerobes

![Diagram depicting the interaction between FNR and RNAP, showing aerobic respiration and activation of the FNR regulon.](Crack, 2014, Acc. Chem. Res.)
FNR: A [4Fe-4S] Sensor of $O_2$ and ROS

- Fe-S clusters may react rapidly with oxygen and reactive oxygen species (ROS) → Conformational change of the Fe-S protein

$$[4\text{Fe}-4\text{S}](\text{CysS})_4 + nO_2 \rightarrow [2\text{Fe}-2\text{S}](\text{CysS})_2(\text{CysSS})_2 + \text{Fe}^{2+} + \text{Fe}^{3+} + \text{reduced } O_2 \text{ species}$$

**FNR: A [4Fe-4S] Sensor of O_2 and ROS**

- **Fumarate and Nitrate Reduction Regulator (FNR):**
  - Site-directed mutagenesis and structural studies on FNR support a [4Fe-4S] cluster-dependent dimerization through the intramolecular hydrophobic interactions and the salt bridges between the monomers.

*Crystallographic structure of holo-FNR from Aliivibrio fischeri at 2.6 Å*

**FNR: A [4Fe-4S] Sensor of O₂ and ROS**

- Fumarate and Nitrate Reduction Regulator (FNR):
  - Substitution of Ser24 by a Phe may stabilize the intramolecular hydrophobic interactions and thus reduce O₂ sensitivity of FNR.

*Crystallographic structure of holo-FNR from Aliivibrio fischeri at 2.6 Å*


- SoxR, a member of the MerR family, is widely distributed in both pathogenic and nonpathogenic bacteria
- SoxR is activated by redox-active molecules and nitric oxide
- The genes regulated by SoxR are species-specific

Proposed model of activation of SoxR by redox-cycling drugs in E. coli


- SoxR dimer binds to the soxS promoter region via the N-terminal helix bundle

- The dimerization helix forms an antiparallel coiled coil to stabilize the dimer

- The Fe-S cluster is in the unstructured loop in the C-terminal of SoxR

*Crystallographic structure of Ec SoxR-soxS promoter complex at 2.8 Å*


- The Fe-S cluster is completely exposed to solvent, and in an asymmetric charge environment.
- It is proposed that the asymmetric charge environment induces the redox-dependent conformational change of SoxR.

*Structure of the Fe-S cluster in Ec SoxR-soxS promoter complex*

- Iron-Sulfur Cluster biosynthesis Regulator (IscR):
  - Regulate Fe-S cluster biosynthesis in response to oxidative stress and Fe limitation


- **Iron-Sulfur Cluster biosynthesis Regulator (IscR):**
  - Regulate Fe-S cluster biosynthesis in response to oxidative stress and Fe limitation
  - Enables the crosstalk between the ISC and (SUF) biosynthetic pathways to coordinate the utilization of iron and cysteine for Fe/S cluster assembly

---


- Iron-Sulfur Cluster biosynthesis Regulator (IscR):
  - IscR binds to one of two distinct DNA motifs in an Fe-S cluster-dependent manner

<table>
<thead>
<tr>
<th>Type 2</th>
<th>Promoter</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>hyaA</td>
<td>5’-ATAAATCCCACACGATTTTGTTTGT-3’</td>
<td></td>
</tr>
<tr>
<td>hybO</td>
<td>5’-CGATAACCAATAAAATGCTGTTAAAAC-3’</td>
<td></td>
</tr>
<tr>
<td>sufA</td>
<td>5’-GTAAAGCCCTTGCCTTTGCTGGTTGAC-3’</td>
<td></td>
</tr>
<tr>
<td>ydiU</td>
<td>5’-CGATAACCCTCTGTCTTGGCTGGTTAA-3’</td>
<td></td>
</tr>
</tbody>
</table>

**apo-IscR**

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Promoter</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>iscRA</td>
<td>5’-ATTGACTTTGACTGATTTAGTGGGTATTT-3’</td>
<td></td>
</tr>
<tr>
<td>iscRB</td>
<td>5’-AAATAGTTGACCAATTATACCTGGGAATGT-3’</td>
<td></td>
</tr>
<tr>
<td>erpA</td>
<td>5’-GAATATGTTGACGAAATACCAGGTATTA-3’</td>
<td></td>
</tr>
<tr>
<td>nfuA</td>
<td>5’-TAATAGTTGACTATTGTTAGTGTATTAA-3’</td>
<td></td>
</tr>
</tbody>
</table>

**holo-IscR**

| motif    | 5’-xxWWWWWCCxxYAxxxxxxxxxxTRxCGWWWxx-3’ |
| motif    | 5’-xxWWWWWxTTGACxxxxxxxxxGTCGGxWWWxx-3’ |

*W represents A or T, and x is undefined


- Iron-Sulfur Cluster biosynthesis Regulator (IscR):
  - IscR binds to one of two distinct sets of promoters in an Fe-S cluster dependent manner
  - Crystallographic studies indicate Glu43 of apo-IscR may form H-bond with C7C8
  - apo-IscR with the Glu43A mutation binds both Type 1 and Type 2 promoters

*Crystallographic structure of Ec apo-IscR–P_{hya} complex at 2.2 Å*


- Iron-Sulfur Cluster biosynthesis Regulator (IscR):
  - IscR binds to one of two distinct DNA motifs in an Fe-S cluster dependent manner
  - Crystallographic studies indicate Glu43 of apo-IscR may form H-bond with C7C8
  - apo-IscR with the Glu43A mutation binds both Type 1 and Type 2 promoters

*Proposed model for IscR discrimination between type 1 and type 2 DNA binding motifs*
Fe-S Sensors of NO and RNS

- NsrR and WhiB1/WhiD have been characterized as NO-specific [4Fe-4S] sensors
- Nitrosylation process of Fe-S Cluster is under the investigation

Overall reaction:

$$[\text{Fe}^{II}_2\text{Fe}^{III}_2\text{S}_4(\text{Cys})_4]^2^- + 8\text{NO} \rightarrow 2[\text{Fe}^I_2(\text{NO})_4(\text{Cys})_2]^0 + \text{S}^2^- + 3\text{S}^0$$
### Studies on Fe-S Regulators: Significance

- **List of characterized/proposed Fe-S sensors**

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Organism</th>
<th>Cluster type</th>
<th>Primary signal</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>FNR</td>
<td>Proteobacteria and Bacilli</td>
<td>[4Fe–4S]</td>
<td>O₂</td>
<td>Controls genes in the adaptive response to anaerobiosis</td>
</tr>
<tr>
<td>NreB</td>
<td>Staphylococci</td>
<td>[4Fe–4S]</td>
<td>O₂</td>
<td>Phosphorylates the response regulator NreC to control genes in nitrate/nitrite respiration</td>
</tr>
<tr>
<td>AirS</td>
<td><em>Staphylococcus aureus</em> strain Newman</td>
<td>[2Fe–2S]</td>
<td>O₂</td>
<td>Alters anaerobic expression of genes involved in quorum sensing, virulence, and oxidative stress</td>
</tr>
<tr>
<td>SoxR</td>
<td>Proteobacteria and Actinobacteria</td>
<td>[2Fe–2S]</td>
<td>Redox-cycling compounds</td>
<td>Regulates the oxidative stress response directly or through the transcription factor SoxS</td>
</tr>
<tr>
<td>IscR</td>
<td>Proteobacteria</td>
<td>[2Fe–2S]</td>
<td>Fe–S cluster levels</td>
<td>Controls genes in Fe–S cluster biogenesis</td>
</tr>
<tr>
<td>SuR</td>
<td>Cyanobacteria</td>
<td>[4Fe–4S]</td>
<td>Fe–S cluster levels</td>
<td>Controls the sufBCDS Fe–S cluster biogenesis pathway</td>
</tr>
<tr>
<td>RirA</td>
<td>Rhizobia</td>
<td>Not yet determined</td>
<td>Fe levels</td>
<td>Regulates genes in Fe uptake</td>
</tr>
<tr>
<td>Fra2–Grx3/4</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>[2Fe–2S]</td>
<td>Fe levels</td>
<td>Controls activity of Fe-uptake regulators Aft1/2</td>
</tr>
<tr>
<td>Aft1/2</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>[2Fe–2S]</td>
<td>Fe levels</td>
<td>Regulates genes in Fe uptake</td>
</tr>
<tr>
<td>IRP1</td>
<td>Mammals</td>
<td>[4Fe–4S]</td>
<td>Fe levels</td>
<td>Alters stability or translation of transcripts involved in Fe uptake</td>
</tr>
<tr>
<td>Bacterial aconitases</td>
<td>Bacteria</td>
<td>[4Fe–4S]</td>
<td>Fe levels and/or ROS/NO</td>
<td>Alters stability or translation of transcripts, including those involved in Fe homeostasis, the oxidative stress response, motility, or sporulation</td>
</tr>
</tbody>
</table>

| NsrR     | Proteobacteria, Bacilli, Streptomyces | [2Fe–2S] or [4Fe–4S] | NO | Regulates genes in the NO stress response |
| Wbl proteins | Actinobacteria | [4Fe–4S] | NO | Regulate genes involved in diverse cellular processes, including development, virulence, or antibiotic resistance |
| ArnR     | *Corynebacterium glutamicum* | Not yet determined | NO | Controls genes in nitrate metabolism |
| RsmA     | *Streptomyces coelicolor* | [2Fe–2S] | Not known | Inhibits activity of αM |
| ThnY     | *Sphingomonas macroglottabida* | [2Fe–2S] | Not known | Promotes activity of the tetralin utilization regulator ThnR |
| VnfA     | *Azotobacter vinelandii* | [3Fe–4S] | Not known | Transcriptional activator of nitrogenase-2 |

- **Fe-S redox sensors are required for the pathogenesis and virulence of bacterial pathogens in the host**

Mettert, 2015, BBA
Studies on Fe-S Regulators: Significance

- WhiB proteins represent a unique family of transcriptional regulators that are found only in Actinobacteria.
- Seven members (WhiB1-7) are found in the mycobacterial pathogen Mycobacterium tuberculosis (Mtb).
- Two members (WhiB5 and 6) of the WhiB family are unique to mycobacterial pathogens.
Proposed functions of WhiBs in the stress response:
• Oxidative stress (WhiB1-7)
• Nitric oxide sensing and dormancy (WhiB1)
• Cell wall division (WhiB2)
• Nutrition starvation, fatty acid metabolism and dormancy (WhiB3)
• Virulence and reactivation (WhiB3, WhiB5 and WhiB6)
• Antibiotic resistance (WhiB2 and WhiB7)
Studies on Fe-S Regulators: Significance

- Mtb WhiB members share 30 -50% sequence identity with significant difference in the redox-properties of the Fe-S cluster

Studies on Fe-S Regulators: Significance

- Mtb WhiBs represent a unique family of transcriptional regulators that have not yet been structurally characterized at atomic levels.

- Functional roles and molecular mechanisms of the WhiB proteins in the stress response and pathogenesis remain to be elucidated.

- Structure-mechanism relationship of the substrate-selectivity by [4Fe-4S] sensors in NO/O$_2$ sensing is to be elaborated.
Studies on Fe-S Regulators: Challenges

- Fe-S proteins are extremely oxygen-sensitive
- Strict anaerobic environment is required for handling Fe-S regulators
Studies on Fe-S Regulators: Challenges

• Investigate the molecular mechanisms of redox-dependent regulation of gene expression by Fe-S proteins:
  – Structural information at atomic levels is highly desired for understanding the mechanism of action of Fe-S regulators
  – Oxygen sensitivity and intrinsic disorder of Fe-S regulators challenges structural studies on these regulators
  – Multiple biophysical approaches have been used to study Fe-S regulators, complemented by molecular dynamics simulation
Studies on Fe-S Regulators: Challenges

• Detect reaction intermediates:
  – Transient, fast reactions with redox molecules make it challenging to detect reaction intermediates
  – Mass spectrometry has been used successfully probe the O₂-induced Fe-S cluster degradation process
  – Other local structure-sensitive techniques such as X-ray absorption spectroscopy may also be considered
Studies on Fe-S Regulators: Challenges

• Identify redox-sensing signal and the members in the regulon of Fe-S regulators
  – Traditional techniques: Chip-Seq, mRNA-seq
  – Omics-based techniques
  – Use fluorescence proteins as reporters

M. smegmatis
**Take Home Message**

- Fe-S sensors/regulators are important for all the living organisms.

- The core structure of the cluster, ligands, and protein folder dictate the sensitivity and functionality of the Fe-S regulators.

- Knowledge on molecular mechanisms of Fe-S sensors is critical for health and disease, while it is limited due to the challenges in oxygen-sensitivity and stability, and their transient, fast reactions with redox molecules.