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Running title: Fe-S proteins acting as redox switch

**Abstract:** Iron-sulfur (Fe-S) clusters are inorganic prosthetic groups composed of only iron and inorganic sulfur atoms with variable nuclearities. Found in all kingdoms of life, they perform numerous critical functions in fundamental processes (e.g. respiration, photosynthesis, nitrogen fixation). Organisms develop different pathways to sense their local environment such as nutrient availability, level of oxidative stress or of an element such as iron, and to respond and adapt to changes. The chemistry of Fe-S clusters makes them ideal for sensing various redox environmental signals and subsequently for mediating appropriate cellular responses. Fe-S cluster-containing sensors can lose their cluster, accommodate another type of cluster (e.g. interconversion between [4Fe-4S] and [2Fe-2S] clusters) or receive/give electrons (change in the redox state of the cluster). The present review focuses on the latter sensing mechanism, which controls the activity of Fe-S proteins in response to redox signals by change in the redox state of its cluster. Proteins using this mechanism can be found in bacteria, yeasts as well as mammals and are involved in enzyme protection (FeSII), Fe-S cluster transfer/repair (mitoNEET), DNA repair (Base Excision Repair (BER) glycosylases and helicases), and regulation of gene expression (ThnY, AirS, SoxR). In all these proteins, when the Fe-S cluster is reduced, proteins are in a "dormant state". When their cluster perceives a signal that induces its oxidation, they switch to an "active state". This sensing mechanism efficiently helps cells to turn on survival pathways quickly and recover from stressful conditions.

**Keywords:** Fe-S cluster, sensor, redox state, cellular adaptive response

## INTRODUCTION

Iron-sulfur (Fe-S) cluster-containing proteins are thought to have been present in the very early forms of anaerobic life. This inorganic prosthetic group is composed of iron and inorganic sulfur atoms with variable nuclearities and geometries and is extremely widespread in nature. Since their discovery in the 1960s [1], they have been found in proteins of every kingdom of life and have been shown to perform numerous critical functions in cellular processes as fundamental for life as respiration and photosynthesis [2]. Organisms often use iron-containing proteins as sensors of the environment. The iron can be directly bound to the protein (Ferric uptake regulator (Fur), PerR…), involved in heme (Dissimilative Nitrate respiration Regulator (DNR), Dos…) but also in an Fe-S cluster [3]. Indeed, the chemistry of Fe-S clusters confers on these cofactors a remarkable plasticity and makes them ideal for sensing redox environmental signals such as gases, e.g. $O_2$ and NO, the level of Fe and Fe-S clusters, reactive oxygen species (ROS), and redox cycling compounds, and subsequently for mediating adaptive cellular responses. In response to a signal, Fe-S cluster-containing sensors can lose their cluster (e.g. cytosolic aconitase, IscR, NreB...), interconvert between Fe-S cluster types (e.g. [4Fe-4S] cluster converted into [2Fe-2S] cluster, Fumarate and Nitrate reduction Regulator (FNR), WhiB3, RirA...) or be chemically modified (Nitric oxide Sensing Rrf2-type regulator Repressor NsrR…) [4-6]. Finally, Fe-S clusters can also receive and give electrons (modulation of the iron oxidation state). Such a cluster redox modulation also stands for a potential sensing mechanism, controlling Fe-S-associated protein activity in response to redox signals. Several examples will be examined in this review, which focuses on how Fe-S cluster-containing sensor proteins act as redox switches and control a cell's rapid and efficient response to a change in its environment.

## 1. Fe-S proteins

Fe-S proteins were first detected as electron paramagnetic resonance signatures in mitochondrial membranes [1]. In 1962, the first Fe-S protein, named ferredoxin, was isolated from *Clostridium pasteurianum* and found to contain non-heme iron and to be involved in electron transport in different low-potential reactions [7]. Since this first discovery, it has been demonstrated that Fe-S proteins assuming a large range of functions are present in all the kingdoms of life –in the prokaryotic archaea and bacteria as well as in the eukaryotic fungi, plants and animals– and in all cellular compartments. They are among the most ubiquitous and versatile metal-containing prosthetic centers in nature and are essential for life by sustaining fundamental processes such as photosynthesis, nitrogen fixation, respiration, DNA synthesis and repair, ribosome biogenesis, and cellular iron homeostasis [8, 9].

### 1.1. Properties of Fe-S clusters

Fe-S clusters are composed of one or more iron ions partially or completely coordinated by sulfur atoms. In many cases, they are associated with other types of

cofactors such as organic groups (e.g. flavin) or other metal atoms including nickel, molybdenum, vanadium, and selenium. Fe-S clusters incorporated into proteins have different atom compositions, cluster structures and core oxidation states. The most common configurations are the [2Fe-2S], [3Fe-4S] and [4Fe-4S] core units. Interestingly, the [2Fe-2S] unit can be considered as the basic building block for the two other types of clusters; the [4Fe-4S] cluster being assembled from two [2Fe-2S] units and the [3Fe-4S] cluster from the loss of one iron of the [4Fe-4S] cluster. The tetrahedral coordination of each iron site is performed by cluster inorganic sulfides ($S^{2-}$) and protein-based ligands, generally, thiolate groups provided by cysteine residues. However, aspartate, histidine, serine, glutamine, arginine, or small molecules (i.e. glutathione, substrate molecule, water …) are occasionally ligand at one unique iron site [8-10].

Fe-S clusters are typically redox active, in other words they can be found in different oxidation states. Thus, each iron ion of the cluster can be either in the oxidized state, Fe(III)(ferric ion), or in the reduced state, Fe(II)(ferrous ion). [2Fe-2S] clusters are generally found only in two redox states: the oxidized form, $[2Fe\text{-}2S]^{2+}$, with two ferric ions and the reduced form, $[2Fe\text{-}2S]^{+}$, with one ferric ion and one ferrous ion. The all-ferrous form, $[2Fe\text{-}2S]^{0}$, has been observed only in the case of the Rieske center [11]. The [4Fe-4S] cluster can be typically found in three redox states: 3+ with three ferric ions and one ferrous ion, 2+ with two ferric ions and two ferrous ions and 1+ with one ferric ion and three ferrous ions. The all-ferrous $[4Fe\text{-}4S]^{0}$ state was obtained *in vitro* using strong reducing reagents with very few proteins including nitrogenase [12]. Typically, each [4Fe-4S] protein can be found only in two redox states. The +1 and +2 levels are found in the low potential [4Fe-4S] proteins whereas the +2 and +3 levels are found in the high potential [4Fe-4S] proteins HiPIP. Finally, the [3Fe-4S] cluster can be found in the oxidized form with a global +1 charge (three ferric ions) and in the reduced form with a global charge of 0 (two ferric ions and one ferrous ion) [13].

### 1.2. Functions of Fe-S proteins

Fe-S proteins are capable of mediating biological electron transport (coupled or not to proton transfer), either through small soluble electron carriers such as ferredoxins or in membrane-bound redox enzymatic systems such as photosynthetic and respiratory electron transport chains. However, it was soon demonstrated that Fe-S clusters could also constitute the site for substrate binding and activation in a wide range of enzymes. Among these, the most characterized example is [4Fe-4S]-containing aconitase [14]. Three of the four iron atoms of the cluster are coordinated by three cysteines of the polypeptide chain and the fourth iron binds one molecule of substrate (citrate) and acts as a Lewis acid for the activation of the substrate. A similar approach is used by the members of the fast-growing and diverse radical-SAM superfamily including the biotin synthase BioB, the hydrogenase biosynthetic enzyme HydE, and the thiamine pyrimidine biosynthetic enzyme ThiC [15].

Fe-S proteins are also involved in iron or Fe-S cluster storage as in polyferredoxins from anaerobic bacteria and archaea [16], in disulfide reduction as in thioredoxin reductase in chloroplasts for example [17], in sulfur donation as in biotin synthase [18], in ribosome synthesis (ABCE1) and in Fe-S cluster biogenesis (IscU, IscA…) or repair (mitoNEET) [19, 20].

Until recently, zinc, a redox inactive metal, was considered as the metal of choice for nucleic acid-binding proteins (Zn-finger proteins). Actually, few DNA-binding proteins (the glycosylases MutY and endonuclease III) were known to contain an Fe-S cluster and it was considered that, in these proteins, the Fe-S cluster played only a structural role similar to that of Zn in Zn-finger proteins. Moreover, strong *in vitro* evidence suggested that they may also use their clusters to search cooperatively for damaged DNA bases within the genome [21, 22](this review). Nevertheless, during the last decade, a growing number of DNA-binding proteins including DNA helicase XPD family members, replicative DNA polymerases, the helicase-nuclease Dna2 and RNA-binding proteins including some tRNA modifying enzymes were characterized as Fe-S proteins [23, 24]. Thus, Fe-S proteins play a major role in copying the genome and maintaining its integrity. The exact function of Fe-S clusters in the majority of these proteins has yet to be uncovered, which will be a major issue in the coming years.

Finally, Fe-S proteins are also involved in transcriptional or translational regulation of gene expression [5]. To sense different types of environmental stimuli, distinct sensing mechanisms are used, involving cluster assembly, conversion, or redox chemistry (redox switch, this review). This activity was first described in the case of the mammalian cytosolic aconitase (holoform of the protein), the apo-protein of which, iron-regulatory protein 1 (IRP-1), binds to mRNA iron-responsive elements and regulates the expression of several proteins involved in cellular iron metabolism [25]. In the case of the bacterial FNR, an oxygen-sensing transcriptional regulator, the conversion of the DNA-binding dimeric $[4Fe\text{-}4S]^{2+}$ form to a monomeric $[2Fe\text{-}2S]^{2+}$ form is used to control the expression of genes involved in the respiratory pathways [26]. Finally, as discussed below, intracellular oxidative stress converts the reduced inactive SoxR into a transcriptional activator of *soxS* gene, which is responsible for activating the transcription of numerous enzymes in the oxidative stress response [27].

## 2. Fe-S cluster proteins acting as redox switches

### 2.1. Enzyme protection: case of the FeSII protein (Shethna protein II)

Nitrogen is an essential component of all living organisms because it is present in nucleic acids, proteins and many other biological molecules. Although molecular nitrogen ($N_2$) gas is abundant in air, it cannot be readily used by most organisms. Typically, they use ammonia as substrate for insertion of nitrogen in molecules. Thus, the reduction of $N_2$ to ammonia (nitrogen fixation) is a key process for the synthesis of many organic compounds and is one of the most common ways to make nitrogen available for plants.

Biological nitrogen fixation is catalyzed by the nitrogenase present in some microorganisms known as diazotrophs, mainly bacteria such as *Azotobacter* species [28]. Nitrogenase is composed of two proteins, the Fe protein and the MoFe protein. Both proteins contain complex Fe-S clusters and are irreversibly inactivated by oxygen within minutes by irreversible oxidation of their clusters [29].

*Azotobacter* species are obligatory aerobic nitrogen-fixing organisms and they develop two main mechanisms to protect nitrogenase from *in vivo* inactivation by oxygen. When this organism fixes $N_2$ during energy-supplemented growth, its respiratory system increases to reduce the concentration of intracellular dioxygen (respiratory protection). But, in the case of oxygen stress or under substrate-limiting conditions, this protection ceases to be effective, and nitrogenase is rapidly and reversibly inhibited (temporary conformational protection) [30]. Thus, in crude *A. vinelandii* extracts, nitrogenase is more resistant to $O_2$ than the purified enzyme. Analysis of this $O_2$-insensitive inactive nitrogenase form shows that the two components of nitrogenase are complexed to a small [2Fe-2S] protein known as FeSII or Shethna protein II [30-32]. FeSII protein is not essential for anaerobic nitrogen fixation of *A. vinelandii* [33] but strains lacking FeSII lose viability upon carbon substrate deprivation in the presence of oxygen [34]. *In vitro* studies show that only the oxidized holo-FeSII can form a complex with the two components of nitrogenase (oxidation state-dependent interaction) [35]. This complex is more resistant to oxygen than nitrogenase alone, but is inactive ("switch off state")[36, 37]. In favorable redox conditions, the cluster of FeSII is reduced and the protein dissociates from nitrogenase and its activity recovers [35]. A similar protective mechanism was described in a few other diazotrophs such as *Gluconacetobacter diazotrophicus*, which plays a major role in the supply of nitrogen to sugar cane [38].

The crystallographic structure of *A. vinelandii* FeSII [37] shows that this small dimeric protein is present in the crystal in two distinct states that differ only in the conformation of an extended loop in close proximity to the Fe-S cluster ("N-loop" from Gly59 to Pro96) whereas, in the "closed state", the cluster is shielded by a helix of the N-loop. When in the "open state", the helix moves away from the cluster, revealing a new protein surface and increasing the solvent accessibility of the cluster. Gel filtration analysis demonstrates that the oxidized form has a larger hydrodynamic radius than the reduced form. Thus, the "open form" was attributed to the oxidized form and the "closed form" to the reduced one. A model of the complex between FeSII and nitrogenase was built from each crystal structure and clearly, only the "open state" (oxidized) FeSII was able to insert into the cleft between the two components of nitrogenase, leaving the Fe-S cluster accessible to the solvent and to reduction. Thus, the oxidation of the cluster of FeSII protein induces a conformational change that allows the FeSII protein to bind reversibly to nitrogenase and to protect it from oxygen with a still ill-defined mechanism (simple induction of conformational switch of the complex or a more active role in the protection of nitrogenase) (Fig. 1). After stress relief, reduced FeSII dissociates from nitrogenase, the activity of which is restored.

### 2.2. Fe-S repair proteins: case of the NEET proteins

NEET proteins are present in all the kingdoms of life. They are characterized by the presence of at least one highly conserved 39-amino-acid motif called CDGSH Iron-Sulfur Domain (CISD) and involved in the coordination of a [2Fe-2S] cluster coordinated by 3 cysteines and one histidine. In mammals, this family is composed of three members: mitoNEET (CISD1), Miner1 (CISD2, ERIS, Noxp70 or NAF-1), and Miner2 (CISD3). MitoNEET and Miner1 are dimers that assemble one cluster per monomer whereas Miner2 is monomeric and assembles two clusters. In plants and bacteria, NEET orthologs are similar to mitoNEET/Miner1 and Miner2, respectively [39, 40].

MitoNEET is anchored to the mammalian outer mitochondrial membrane (OMM) by its N-terminus with the major part of the protein, including the C-terminal [2Fe-2S]-binding domain, located in the cytosol [41]. This is the first identified Fe-S protein of the OMM. Its biological activity is still debated [42], but several studies have shown that this protein is involved in the regulation of iron and ROS homeostasis [20, 41, 43, 44], in cell proliferation in human breast cancer [45], and in lipid accumulation in adipocytes without insulin resistance [43]. Miner1 is anchored by its N-terminus at the endoplasmic reticulum (ER) membranes and at contact sites between mitochondria and ER (MAM)[46]. Miner1 could be involved in neuronal development [47], in the regulation of autophagy through its interaction with Bcl2 [48] and of apoptosis [49], in the homeostasis of intracellular calcium [50], in the maintenance of the integrity of mitochondria and lifespan [51] and in the inflammatory response [52]. Recessive mutations in *CISD2* gene coding for Miner1 are the cause of Wolfram syndrome 2 (or WFS-2), a neurological disease characterized by at least optical atrophy and diabetes [53-55]. Finally, like mitoNEET, a strong link was noted between a high level of expression of Miner1 and fast proliferation of various cancer cells [56], which makes Miner1 a new marker of negative prognosis for various cancers [57]. Miner2 is less characterized than the two other NEET proteins. It seems to be mostly localized to the mitochondria, and it might also be involved in cellular proliferation [41, 58].

Crystallographic studies of the overexpressed soluble C-terminal domains of mitoNEET and Miner1 revealed a similar unique folding for both proteins. Both form dimers with two distinct domains, a β-cap and an Fe-S cluster binding domain. Both structures slightly differ in the β-cap domain. Each monomer accommodates one [2Fe-2S] cluster coordinated by three cysteines and one histidine [59-63] and they have very similar UV-visible absorption spectra. The cluster of mitoNEET is pH labile and redox-active with a midpoint redox potential of roughly 0 mV at pH 7 [62-65]. The reduced form, which is very likely present in quiescent mammalian cells holds a $[2Fe\text{-}2S]^{+}$ [44, 62, 66] and is

extremely stable and cannot lose its cluster. The latter can be reversibly oxidized by hydrogen peroxide and reduced by biological thiols implying a redox sensory function of mitoNEET [44, 67]. Recently, we have shown that the cluster of the oxidized mitoNEET is relatively stable and that oxygen strongly increases its lability [66].

*In vitro*, mitoNEET and Miner1 are both able to transfer their Fe-S cluster to an apo-protein receptor (an Fe-S protein that has lost its Fe-S cluster) of various origins [20, 42, 66, 68]. The His ligand is critical for cluster release and transfer [69-71]. *In cellulo*, mitoNEET can transfer its cluster to the human IRP-1/cytosolic Fe-S aconitase, a key regulator of cellular iron homeostasis after oxidative damage of its cluster. We proposed that mitoNEET is involved in the repair of oxidatively damaged IRP-1/aconitase and possibly in a more general repair pathway of damaged cytosolic Fe-S protein [20]. Initial studies on mitoNEET/Miner1 suggested that the transfer can occur only if the cluster is oxidized [69]. Recently, by decoupling the oxygen effect from the cluster redox effect, we have proven that only oxidized mitoNEET cluster triggers its transfer to a model receptor protein, independently of the presence or absence of oxygen and without major protein rearrangement between the two redox state of the protein [66].

Then, we proposed a model (Fig. 2) for a new Fe-S cluster repair pathway involving mitoNEET. In the absence of stress, mitoNEET would be reduced. Oxidative stress damages Fe-S clusters of proteins and oxidizes the mitoNEET Fe-S cluster. If the damaged Fe-S cluster protein is not degraded after loss of its cluster (i.e. IRP-1/c-aconitase), oxidized mitoNEET can then transfer its cluster to the damaged protein. This newly discovered repair pathway might allow very fast recovery of key Fe-S protein activity after stress.

### 2.3. DNA repair enzymes

#### *2.3.1. BER glycosylases*

Cellular DNA is constantly attacked by damaging agents including ROS. Cells have developed efficient DNA repair pathways in order to maintain genome integrity. BER is a highly conserved pathway from prokaryotes to eukaryotes involved in removing a large variety of DNA lesions including oxidation, deamination, depurination, alkylation and single-strand break. DNA glycosylases are key components of this repair pathway. They first search for and detect DNA damage among the vast excess of undamaged bases and then cleave the glycosidic bond between damaged base and sugar, leaving an abasic site that is further processed by downstream enzymes to restore the undamaged DNA [72]. Several BER glycosylases assemble a [4Fe-4S] cluster including the *E. coli* endonuclease III (Endo III) [73, 74] and MutY [75, 76], *Micrococcus luteus* UV endonuclease [77], *Methanobacterium thermoautotrophicum* G:T-specific thymine glycosylase [78], *Thermotoga maritima* methylpurine glycosylase [79], and uracil DNA glycosylases (UDG) from thermophile and hyperthermophilic organisms [21, 23, 80]. Until now, their human homologs have not been studied extensively but, clearly, mammalian hNTH1 and MUTYH, homologs of Endo III and MutY respectively, assemble a [4Fe-4S] cluster [81-84].

The first BER glycosylase reported as an Fe-S-containing protein is Endo III from *E. coli*. The purified enzyme contains a single $[4Fe\text{-}4S]^{2+}$ that cannot be reduced under physiological conditions or oxidized without cluster degradation. Thus, the Fe-S cluster was thought to be redox-inactive [73]. The apo-form of MutY is globally folded but is completely inactive and its activity can be restored by chemical cluster reconstitution [85]. Moreover, mutagenesis of the cluster ligands decreases their DNA-binding affinity [86]. The crystal structures of *E. coli* Endo III [87], *E. coli* MutY [75] and *Thermus thermophilus* UDG [88] show that the polypeptide forms a solvent exposed loop (iron-sulfur cluster loop, FCL) between two cysteines involved in the coordination of the cluster. This loop contains numerous positively charged residues. The crystal structures of these enzymes bound to DNA substrate reveal that the cluster is necessary to properly position conserved basic residues from the FCL to interact with the DNA phosphate backbone [89-91]. The importance of these residues of FCL in enzyme binding to substrate and activity was confirmed by site-directed mutagenesis [74, 92, 93]. Thus, the Fe-S cluster clearly plays, at least, a structural role in these Fe-S cluster-containing glycosylases. Interestingly, MutY lacks the [4Fe-4S] cluster in bacteria such as *Lactobacillaes* as well as in the protozoan genus *Entamoeba*. In these organisms, loss of the cluster is compensated for by bulky amino acids that stabilize the structure in a similar manner [94].

The pioneering work of Barton and David's laboratories has led to a reexamination of the role of the Fe-S cluster in these glycosylases [21, 22]. Using DNA-modified gold electrodes, they first showed that the binding of $[4Fe\text{-}4S]^{2+}$-MutY to DNA shifts the redox potential of the +3/+2 couple into the physiological range (roughly 80 mV vs. Normal Hydrogen Electrode, NHE) and makes the cluster more easily oxidized (redox active cluster) [95]. Thus, the binding of the protein to DNA allows the oxidation of its cluster and the release of an electron. Similar observations were made with Endo III and UDG [96]. Moreover, they observed that the DNA-binding affinity of the $[4Fe\text{-}4S]^{2+}$-MutY is much lower than that of the oxidized form (DNA-binding affinity controlled by the redox state of the cluster) [96].

Guanine radicals are frequent oxidative DNA lesions generated by ROS. Interestingly, by their very high redox potential, they facilitate the oxidation of DNA-bound MutY and initiate the binding of MutY in the vicinity of oxidized DNA [97]. The intact double helix structure of DNA, with $\pi$-stacked base pairs, allows charge transfer (CT) along DNA between two redox partners over at least 200 Å and displays sensitivity to mismatched or damaged base pairs such as abasic sites that disturb the $\pi$-staked array. Interestingly, MutY and EndoIII exhibit a diminished electrochemical signal with an electrode modified with DNA containing an abasic site [95, 96], suggesting that DNA-mediated CT is necessary for the redox-controlled activity of MutY and EndoIII through Fe-S clusters [95, 96,

98].
Taken together, a model for BER enzymes scanning DNA for damaged bases using DNA-mediated CT has been proposed (Fig. 3). A highly oxidizing guanine radical in DNA oxidizes a cluster of MutY and initiates binding of the enzyme to the DNA. The electron released during this oxidation process can reduce a distally bound protein using DNA-mediated CT by intact DNA between the two proteins. The newly reduced protein then dissociates from the DNA. As a consequence, local concentrations of MutY/EndoIII remain low where DNA is not damaged. If DNA is damaged, the CT cannot occur and both proteins remain bound to DNA on both sides of the lesion (higher local concentration of glycosylases around the DNA damage) and can slide along DNA using a processive mechanism without dissociation of the enzyme and, subsequently, repair the damage [99]. This mechanism allows glycosylases, only 30-500 copies of which are found per bacterial cell, to localize DNA damage rapidly and relocate in the surrounding vicinity [22, 100, 101].

*2.3.2. XPD/XPD-like helicases and AddB-type nucleases*

XPD is an ATP-dependent super-family 2 DNA helicase with 5'-3' polarity. In eukaryotes, it is a component of the transcription factor TFIIH complex and is involved in DNA repair by the Nucleotide Excision Repair (NER) pathway. XPD opens the DNA around the lesion in an ATP-dependent fashion and then additional NER factors are recruited. The presence of a [4Fe-4S] cluster was first shown in XPD from the archaea *Sulfolobus acidocaldarius* [102] then XPD-like helicases were further found in all kingdoms of life including Rad3 in yeast [103], *E. coli* DinG [104] and human Rtel1 [105]. Typically, the cluster is essential for the helicase activity and to couple ATP hydrolysis to DNA translocation [102-104, 106, 107]. Moreover, an Fe-S cluster was also found in AddB-type nuclease of the AddAB helicase-nuclease complex from *Bacillus subtilis* [106], as well as in the yeast bifunctional helicase-nuclease protein Dna2 involved in Okazaki fragment processing during DNA replication [107]. Crystal structures of archaeal XPD [108-111] and of bacterial AddAB bound to DNA [112] show that the cluster is involved in the formation of a FCL-type domain between two ligands of the Fe-S cluster similar to the one found in Fe-S cluster-containing glycosylases. Moreover, crystal structures revealed a four-domain organization of the helicases with the two motor domains (HD1 and HD2), ARCH and Fe-S domains with the HD1, ARCH and Fe-S domains forming a channel critical for ssDNA translocation during XPD helicase unwinding [113].
Thus, the cluster plays a structural role in maintaining local conformations that are critical for enzyme activity, but is also redox active (redox potential of the +2/+1 couple around 400 mV vs. NHE) [104, 105, 110]. Actually, *in vitro* reduction of the $[4Fe\text{-}4S]^{2+}$ cluster of *E. coli* DinG reversibly switches off its helicase activity [104]. Moreover, both the cluster and the helicase activity are resistant to high concentrations of hydrogen peroxide, whereas exposure to NO reversibly inactivates the enzyme *in vitro* by formation of a DinG-bound dinitrosyl iron complex (DNIC). Thus, these results suggest that the cluster might also act as a sensor of intracellular redox potential to modulate its helicase activity [104].
Finally, experiments using DNA-modified electrodes showed that DNA binding shifts the 3+/2+ redox potential of the helicase to roughly 80 mV, a redox potential similar to the one previously measured for the Fe-S cluster-containing glycosylases [114]. Moreover, redistribution of XPD around DNA damage, which inhibits CT, was observed [115]. Then, the model of DNA scanning and repair by Fe-S cluster-containing glycosylases using DNA-mediated CT was extended to XPD-type helicases and even to UvrC, a NER endonuclease containing an Fe-S cluster [116]. In this extended model, XPD-type helicase, EndoIII/MutY glycosylase and UvrC have similar redox potentials and cooperate at long range using DNA-mediated CT for repair protein redistribution around DNA damage [115, 117, 118]. This DNA-mediated signaling among different DNA repair pathway enzymes was also demonstrated to be critical to maintenance of the integrity of the bacterial genome under stress conditions [117].
Finally, it was shown that all replicative DNA polymerases (POLα, POLδ, POLε), but also the primase subunit PRIM2, assemble an Fe-S cluster [119, 120]. Preliminary studies showed that PRIM2 is able to also mediate DNA CT [121]. Thus, it was proposed that DNA-mediated CT might be a mechanism largely used by all these Fe-S cluster-containing DNA synthesis and repair enzymes to interrogate DNA integrity, to communicate rapidly and to coordinate their activities efficiently by modulating the redox state of their cluster [23].

### 2.4. Regulators of gene expression

*2.4.1.ThnY/ThnR, regulators of the tetralin degradation pathway*

In response to the presence of the organic solvent tetralin (1,2,3,4-tetrahydronaphthalene), the expression of the *thnA* genes of the Gram-negative *Sphingomonas macrogolitabida* strain TFA allows the utilization of this compound as a carbon and energy source (tetralin biodegradation) [122, 123]. The catabolism pathway involves the ferredoxin reductase ThnA4, the Rieske-type ferredoxin ThnA3, and the dioxygenase ThnA1/ThnA2 forming an electron transfer chain from the NAD(P)H to the tetralin substrate [123].
The regulation of the expression of the four *thnA* genes is complex and involves two proteins of the catabolic pathway, ThnA4 and ThnA3, the LysR-type transcriptional activator ThnR and ThnY. ThnY is an Fe-S flavoprotein containing an FAD-binding site and a plant ferredoxin-like [2Fe-2S] cluster, sharing homology with bacterial oxygenase-coupled NAD(P)H-dependent ferredoxin reductases (enzymes that play an important role in the degradation of aromatic compounds) [124-126]. Clearly, ThnR and ThnY are both essential for tetralin biodegradation [124, 126]. ThnR activates *thnA* gene transcription by binding to promoter

regions [126]. *In vitro*, oxidized ThnY binds to ThnR and enhances the DNA-binding affinity of ThnR by modifying the structure of the ThnR-DNA complex, most likely through direct interaction with ThnR [125]. Mutations of residues involved in the coordination of the Fe-S cluster or in the FAD-binding region of ThnY allow the transcription of the *thnA* genes in response to compounds other than tetralin (loss of the specificity of the pathway), suggesting that ThnY cofactors are important for tetralin response specificity [127]. Finally, it has been proposed that the redox state of the ThnY cluster might modulate the protein activity under the regulation of the ferredoxin ThnA3 [125]. Interestingly, similar poor substrate specificity was observed in a strain deleted for ThnA3, a protein of the catabolic pathway [127]. Moreover, in the absence of a good substrate for the dioxygenase or in conditions when reduced ThnA3 presumably accumulates, the expression of *thnA* genes is blocked, indicating communication between the regulatory and catabolic pathways [127, 128].

The whole regulatory cascade leading to the specific expression of *thnA* genes only in the presence of tetralin was recently described (Fig. 4) [129]. It is proposed that in presence of tetralin, the catabolic pathway is very efficient and that electrons from NAD(P)H are transferred to ThnA4, then to ThnA3 and finally to the dioxygenase ThnA1/ThnA2, which degrades the substrate. In this case, the substrate acts as an electron sink. ThnY is mostly oxidized, binds to ThnR and stimulates its binding to DNA. Conversely, at low tetralin concentration or in the presence of a non-optimal substrate for the dioxygenase, electrons from NAD(P)H that accumulate in ThnA3 are redirected towards ThnY (ThnY reduction) instead of the dioxygenase. The binding of reduced ThnY to ThnR is thus impaired and *thnA* gene expression is not induced by ThnR.

*2.4.2. The bacterial redox-sensor histidine kinase, AirS*

Bacteria frequently sense numerous environmental signals and respond rapidly through sophisticated information-processing two-component signaling (TCS) pathways that exhibit complex dynamics on different timescales. Typically, these pathways involve a sensor histidine kinase that autophosphorylates in response to a given stimulus, and then transfers its phosphate group to a cognate response regulator that initiates appropriate cellular responses [130]. Among them, the FixL/NreB $O_2$-sensor kinase family members are iron-containing proteins [131]. The FixL subfamily uses heme as sensor while the NreB subfamily, including NreB and AirS, uses an Fe-S cluster.

*Staphylococcus aureus* is a facultative anaerobe, which is able to invade almost every kind of tissue and to adapt to diverse hypoxic conditions, including the highly hypoxic environment at the onset of inflammation. Thus, bacteria have different sensing mechanisms, including TCS pathways, to adapt to oxygen availability and to use either nitrate respiration or carbohydrate fermentation [132, 133]. The AirS-AirR TCS of *S. aureus* (AirS is the kinase; AirR is the transcriptional regulator), also known as YhcSR, is essential for growth of the bacteria in culture [134] and for its pathogenesis [135]. It is a global key regulator that controls the transcription of more than 350 genes [136], including regulation of the nitrate respiratory pathway [137], of cell wall synthesis [138], and of various virulence factors [136]. It is also involved in the resistance to ROS induced by the host immune system by upregulation of the production of the antioxidant molecule staphyloxanthin [139] and, more generally, it contributes to bacterial survival in human blood [135].

*In vitro* studies demonstrate that the membrane-bound histidine kinase AirS coordinates a [2Fe-2S] cluster with four cysteine ligands acting both as an oxygen- and a redox-sensor [136]. Under anaerobic conditions (Fig. 5), AirS cluster is in the reduced state $[2Fe\text{-}2S]^{1+}$ and AirS kinase activity is low (low concentration of phosphorylated AirR). The unphosphorylated AirR represses transcription of genes involved in bacterial virulence, including the quorum-sensing TCS Agr, the virulence TCS SaeRS, and some other stress-associated factors [136]. Oxidation of the cluster to the $[2Fe\text{-}2S]^{2+}$ state leads to efficient AirS kinase activity (optimal phosphorylation of AirR). However, prolonged exposure to oxygen, intense host-generated ROS, or contact with reactive nitrogen species (RNS) leads to inactive AirS (Apo-AirS and DNIC forms for oxidative and NO stresses, respectively [136]), and accumulation of unphosphorylated AirR. Thus, AirS is capable of sensing oxygen and redox signals through its Fe-S cluster, which is essential for its kinase activity. This redox-dependent kinase activity of AirS allows AirRS TCS to play an important role in appropriately coordinating and rapidly synchronizing virulence determinant production in response to the host environment.

*2.4.3. SoxR*

The SoxRS system found in enteric bacteria such as *E. coli* is another two-part regulatory system [140, 141]. It is involved in a global response to exogenous oxidative and nitrosative stresses by controlling the expression of more than 100 genes involved in redox homeostasis, damage repair, multidrug resistance, and heavy metal detoxification [142, 143]. First, SoxR is activated in response to an oxidative stress in a superoxide-independent manner [144], and switches on the transcription of the global regulator SoxS, which activates the *soxRS* regulon [145, 146]. SoxS homologs do not exist in nonenteric bacteria such as *Pseudomonas aeruginosa* and *Streptomyces coelicolor*. Thus, in these antibiotic producer bacteria, SoxR is believed to regulate directly the transcription of a small set of genes that protect the bacterium against excreted endogenous redox-active antibiotics involved in quorum-sensing, inhibition of competitor bacteria, and bacterial virulence during infection [147-151].

SoxR is a small protein of the MerR family of transcriptional sensors that forms a dimer in solution and assembles a [2Fe-2S] cluster fully coordinated by cysteine residues in the C-terminal domain of each monomer [152, 153]. The crystal structure of SoxR alone or in complex with *soxS* promoter DNA showed a protein folding similar that of other MerR proteins (DNA-binding domain, sensor domain, and coiled-coil dimerization domain), but with

cluster irons exposed to the solvent with an asymmetric electrostatic environment [154]. The Fe-S cluster present in the sensor domain of SoxR can be found in the +1 and +2 oxidation states, with a midpoint potential around -285mV vs. NHE [155, 156]. SoxR can bind DNA with similar affinity independently of the absence (apo-form) or presence of the Fe-S cluster [152], and of its redox state (oxidized *vs.* reduced). Still, only the oxidized holo-SoxR can activate the transcription of SoxS [155, 156].

During normal aerobic growth, the SoxR cluster is clearly reduced in *E. coli* [157]. Using DNA-modified electrodes, it was proposed that the binding of SoxR to its cognate DNA shifts its redox potential to +200 mV [158]. As a consequence, only a strongly oxidizing environment would be able to oxidize the cluster quickly and to activate SoxR and the transcription of the *soxS* operon [144]. Interestingly, SoxR from different organisms exhibit various sensitivities to redox-cycling agents depending on the redox potential of their Fe-S cluster, which is affected by changes in a few residues around the cluster [159]. It has been also reported that SoxR can be activated by DNA-guanine radicals and DNA-mediated charge transfer [160], which can provide rational bases for rapid activation of SoxR. Finally, SoxR can be activated by exposure to macrophage-generated NO followed by nitrosylation of its Fe-S cluster resulting in SoxR-bound DNIC [161-163].

SoxR cluster oxidation induces a protein conformational change with interdomain reorganization [164] and significant distortion and underwinding of the SoxR-bound DNA [165] that improves RNA polymerase binding and further increases transcription [166, 167]. When the stress is over, the SoxR cluster is rapidly reduced by *rsxABCDGE* and *rseC* gene products [168] and SoxS is subject to proteolytic degradation [169], ensuring that the system's response remains dependent on the signal (Fig. 6).

Recently, it was proposed that the *Streptomyces venezuelae* NsrR homolog RsrR for Redox sensitive response Regulator assembles a redox active [2Fe-2S]. It is believed that its cluster controls its DNA-binding ability in a similar manner to SoxR (the oxidized form binds DNA more efficiently) and regulates NADH/NAD(P)H and glutamate/glutamine pathways [170].

## REMARKS AND CONCLUSION

Upon hyperactivation or insult, cells classically respond by activating different pathways that promote protection such as activation of the heat shock pathway or of the unfolded protein response pathway, and eventually, cell defense, survival and recovery. However, if this typical adaptive response fails, cell death programs are activated to eliminate damaged cells.

In this review, we have examined Fe-S proteins with different cluster nuclearity ([2Fe-2S] or [4Fe-4S]) from organisms as diverse as bacteria, yeast and mammals, with disparate cellular functions including enzyme protection (FeSII), Fe-S cluster repair (mitoNEET), DNA repair (BER glycosylases and helicases), and transcriptional regulation (ThnY, AirS and SoxR) (Fig. 7). Despite all these differences, a common point emerges: they are inactive when their Fe-S cluster is in the reduced state, which confers on the protein a state of dormancy. Upon sensing a signal (typically change of oxygen tension or oxidative stress), the cluster becomes oxidized and the protein turns to the "active state". This general mechanism appears to be a very efficient way for the cell to turn on survival pathways to recover quickly from stressful conditions. An emblematic example is given by the bacterial nitrogenase, which is irreversibly inactivated by oxygen due destruction of its Fe-S cluster. As developed above, by forming a reversible complex with nitrogenase during oxidative stress, FeSII allows fast recovery of nitrogenase activity by simple dissociation of the complex as the stress disappears. Likewise, in mammals, the cytosolic aconitase loses its Fe-S cluster after suffering from oxidative or nitrosative stress. In the absence of mitoNEET, the Fe-S cluster biogenesis pathway would have to synthesize a new cluster before supplying it to aconitase. Instead, the reduced cluster of dormant mitoNEET can benefit from oxidative stress to become oxidized and quickly to replenish Fe-S-damaged aconitase with an intact [4Fe-4S] cluster. These last two examples are illustrative of general situations in which cells keep protective responses silent, but are ready to be activated promptly as soon as a single electron is transferred to Fe-S cluster. Such rapid and efficient stress response systems underline once again how the exceptional electronic properties of Fe-S clusters are being used by living organisms and permit subtle tuning of Fe-S proteins properties. In conclusion, Fe-S cluster proteins acting as redox switch appear as new key actors of very efficient and rapid cellular reactions that help cells to restore their activity after a change in environment.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| BER | Base excision repair |
| CISD | CDGSH Iron-Sulfur Domain |
| CT | Charge transfer |
| DNIC | Dinitrosyl iron complex |
| Endo III | Endonuclease III |
| FCL | Iron-sulfur cluster loop |
| FNR | Fumarate and Nitrate reduction Regulator |
| IRP-1 | Iron-Regulatory Protein-1 |
| NER | Nucleotide Excision Repair |
| NHE | Normal Hydrogen Electrode |
| OMM | Outer mitochondrial membrane |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| TCS | Two-component signaling |
| UDG | Uracil DNA glycosylase |

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.


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## FIGURE LEGENDS

**Figure 1. Protection of bacterial nitrogenase by oxidized FeSII protein.** Composed of two Fe-S cluster-containing proteins (Fe and MoFe proteins), nitrogenase can be irreversibly inactivated by oxygen due to cluster oxidation. FeSII protein is involved in a pathway to protect nitrogenase against oxidative damage. In oxidative conditions, the [2Fe-2S] cluster of FeSII becomes oxidized. This change in the redox state of the cluster induces a major conformational change ("open conformation") that allows FeSII to form a protective but inactive complex with nitrogenase. After stress relief, FeSII returns to its reduced form ("closed conformation"), the complex dissociates, and nitrogenase activity is restored.

**Figure 2. Fe-S cluster repair process of cytosolic aconitase/IRP1 by mitoNEET after oxidative stress.** Under physiological redox conditions, mitoNEET is believed to accommodate a very stable [2Fe-2S]$^{+}$ cluster, and the cytosolic aconitase/IRP1 assembles a redox sensitive [4Fe-4S] cluster, which is essential for aconitase activity. When cells are exposed to an oxidative or nitrosative stress, the [4Fe-4S] is completely disassembled revealing the IRP1 regulatory function. At the same time, the mitoNEET [2Fe-2S] cluster is oxidized (+2 state). Upon cessation of oxidative stress, mitoNEET [2Fe-2S]$^{2+}$ transfers its clusters to apo-IRP1, which is converted back into aconitase. Unfolded apo-mitoNEET might be recycled into holo-mitoNEET through the iron-sulfur cluster (ISC) assembly machinery, so as to repeat the steps described above.

**Figure 3. Search for DNA lesions by DNA repair proteins using their Fe-S clusters and DNA-mediated charge transfer.** A reduced (2+ state) DNA repair enzyme (BER glycosylase or helicase) loosely binds DNA *(step 1)*. After transfer of one electron to a highly oxidizing guanine cation radical, the oxidized form (3+ state) tightly binds DNA *(step 2)*. Then, a second DNA repair enzyme of similar redox potential (2+ state) loosely binds to DNA *(step 3)*. If DNA is intact between the two proteins *(in the absence of DNA damage, step 4)*, the first enzyme can receive one electron (reduction of the first enzyme, 2+ state) from the second one *via* DNA-mediated charge transfer and dissociates from DNA. If DNA is damaged between the two enzymes, they cannot exchange electrons *(in the presence of damage to DNA, step 4)*. The second enzyme then transfers its electron to another enzyme localized in the distal side of the lesion *(step 5)*. Both enzymes are oxidized (3+ state), bind DNA efficiently, and can slide along DNA to find and repair the lesion.

**Figure 4. Regulation by ThnY of the catabolic pathway dedicated to the biodegradation of tetralin in *Sphingomonas macrogolitabida*.** Biodegradation of tetralin is performed by the dioxygenase ThnA1/ThnA2, which receives electrons from NAD(P)H *via* the ferredoxin reductase ThnA4 and the ferredoxin ThnA3. **(1)** In the presence of tetralin substrate, ThnA3 is mostly oxidized. The Fe-S cluster-containing ThnY remains oxidized, binds to ThnR, and the transcription of *thn* genes is activated. **(2)** In absence of tetralin, ThnA3 is reduced and transfers electrons to ThnY (accumulation of reduced ThnY). Therefore, ThnR unable to form a complex with reduced ThnY does not induce the transcription of *thn* genes.

**Figure 5. Regulation of *Staphylococcus aureus* virulence by the kinase AirS.** Under anaerobic conditions, the [2Fe-2S]-containing AirS is reduced and its kinase activity is low. In moderate oxidative conditions, the AirS Fe-S cluster is oxidized and exhibits efficient kinase activity. It phosphorylates AirR, a major transcriptional regulator of more than 350 genes involved in bacterial survival and virulence. However, under conditions of prolonged exposure to oxygen, ROS or RNS, the AirS cluster is lost (apo-form due to oxygen exposure or ROS) or modified (DNIC-modified form due to RNS), and AirS has no kinase activity.

**Figure 6. SoxR, a bacterial two-stage control system for cell survival under physiological stress.** The redox-sensor SoxR is a homodimer, which holds one [2Fe-2S] cluster per monomer. During normal aerobic growth, SoxR [2Fe-2S] clusters are reduced and the protein binds a specific promoter region of its target gene *soxS* and inactivates its transcription. During oxidative stress, the redox cycling compounds produced cause oxidation of SoxR [2Fe-2S] clusters, resulting in a conformational change of the protein. The active oxidized form of SoxR remodels the conformation of the *soxS* promoter in a way that allows RNA polymerase to initiate transcription of SoxS. The increased levels of SoxS protein then activate the various *soxRS* regulon genes.

**Figure 7. Fe-S cluster-containing redox switches are key components of efficient cellular responses to change in cellular environments.**

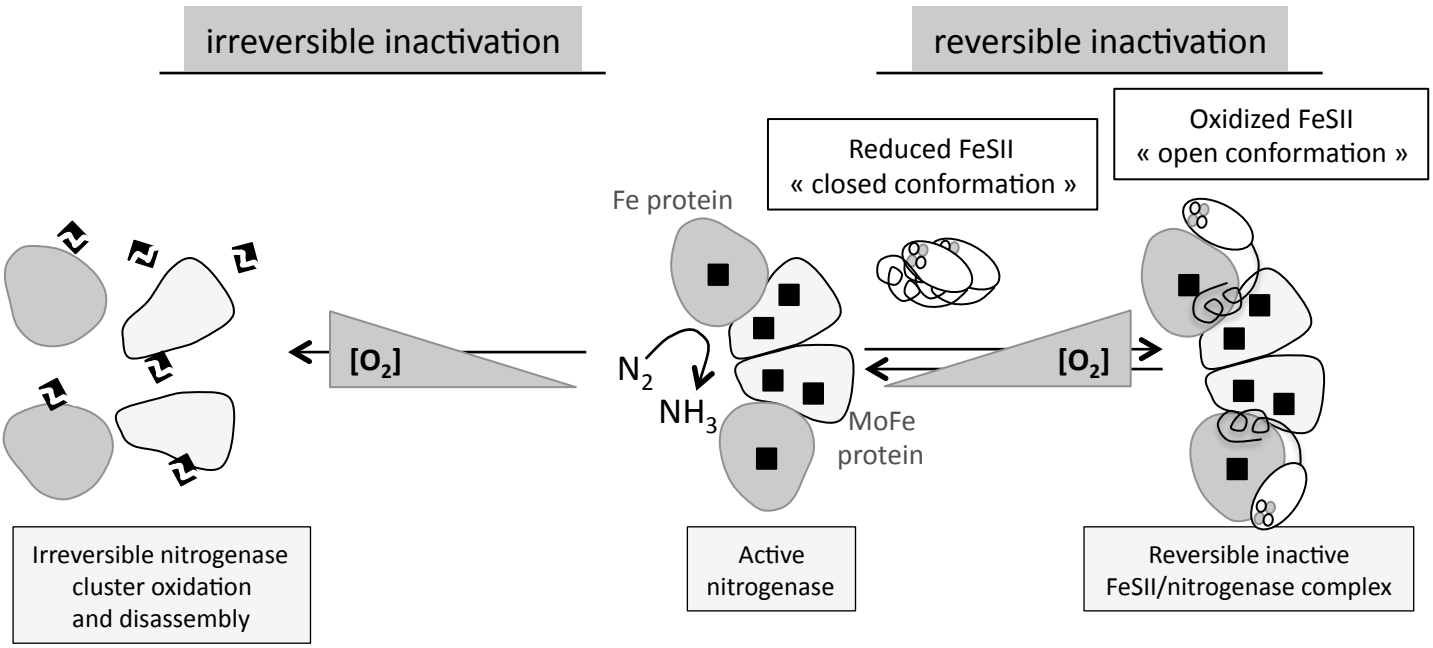

irreversible inactivation
reversible inactivation
Reduced FeSII
« closed conformation »
Oxidized FeSII
« open conformation »
Fe protein
[O2]
N2
NH3
[O2]
MoFe
protein
Irreversible nitrogenase
cluster oxidation
and disassembly
Active
nitrogenase
Reversible inactive
FeSII/nitrogenase complex

Figure 1

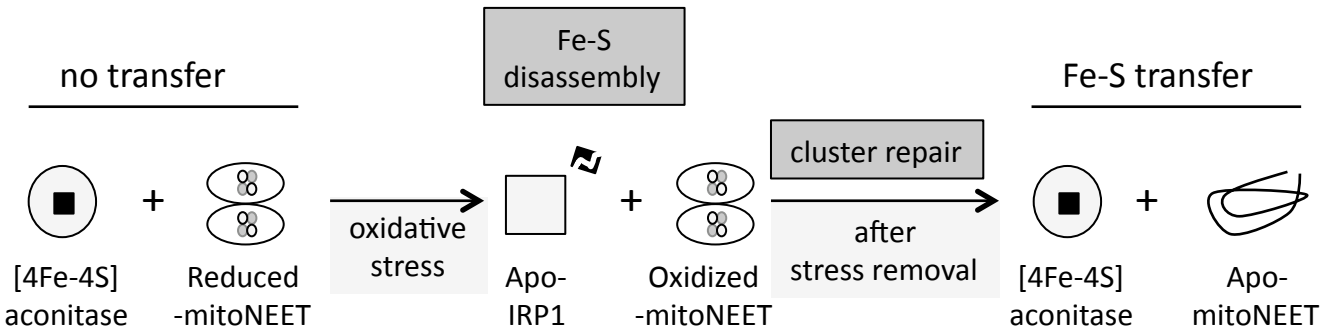

no transfer
Fe-S disassembly
Fe-S transfer
cluster repair
oxidative stress
after stress removal
[4Fe-4S] aconitase
Reduced -mitoNEET
Apo-IRP1
Oxidized -mitoNEET
[4Fe-4S] aconitase
Apo-mitoNEET

Figure 2

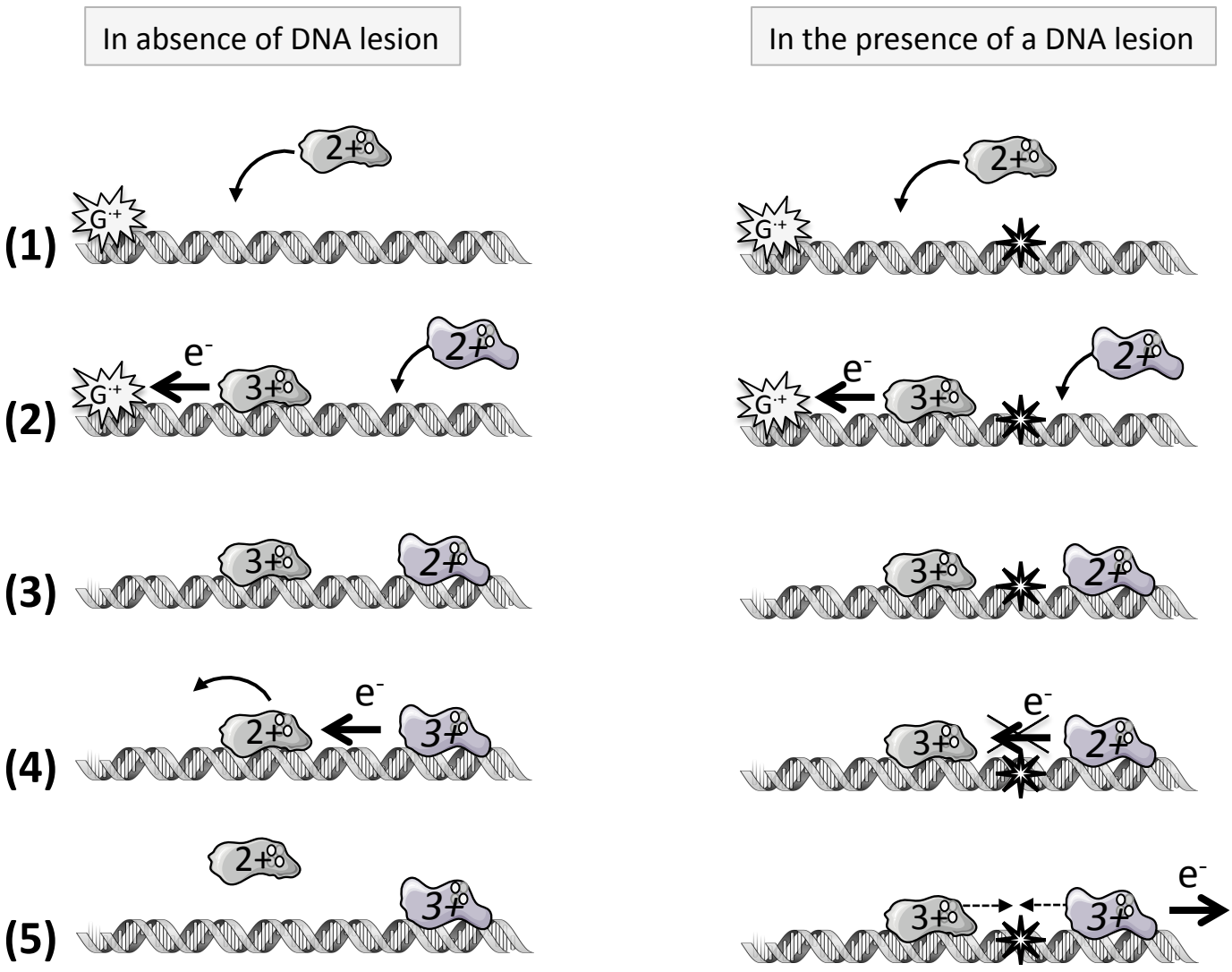
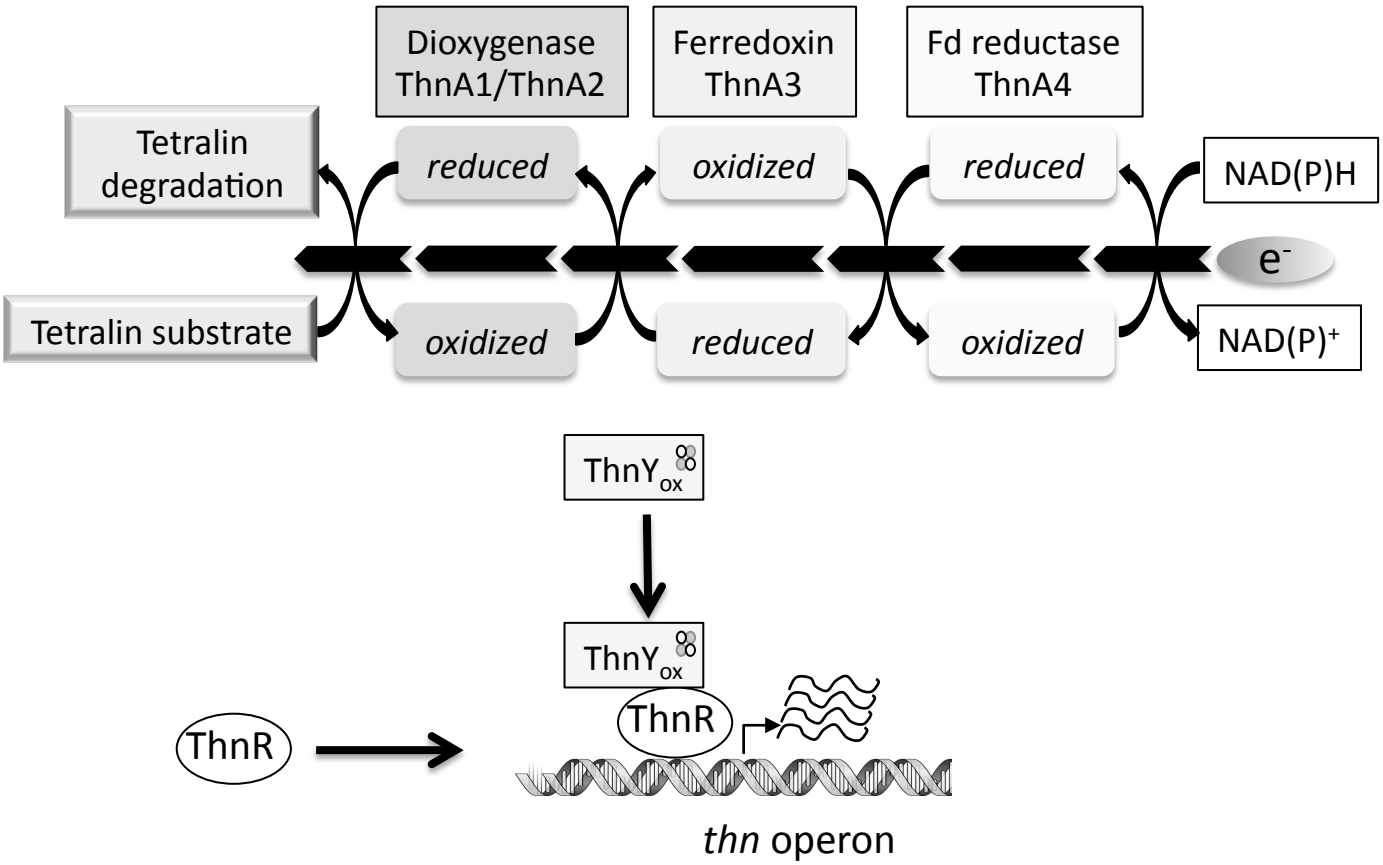

In absence of DNA lesion
In the presence of a DNA lesion
(1)
(2)
(3)
(4)
(5)
2+
3+
G+
e-

Figure 3

**(1)** In the presence of tetralin




**(2)** In the absence of tetralin

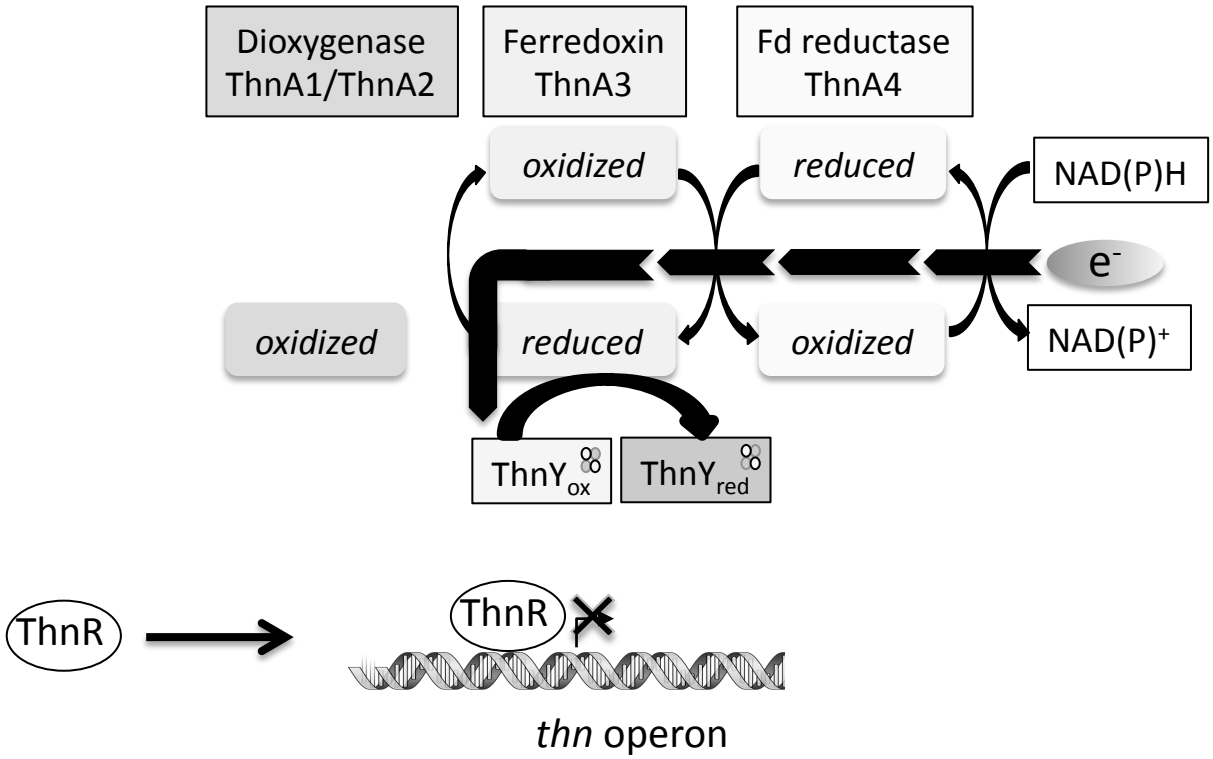
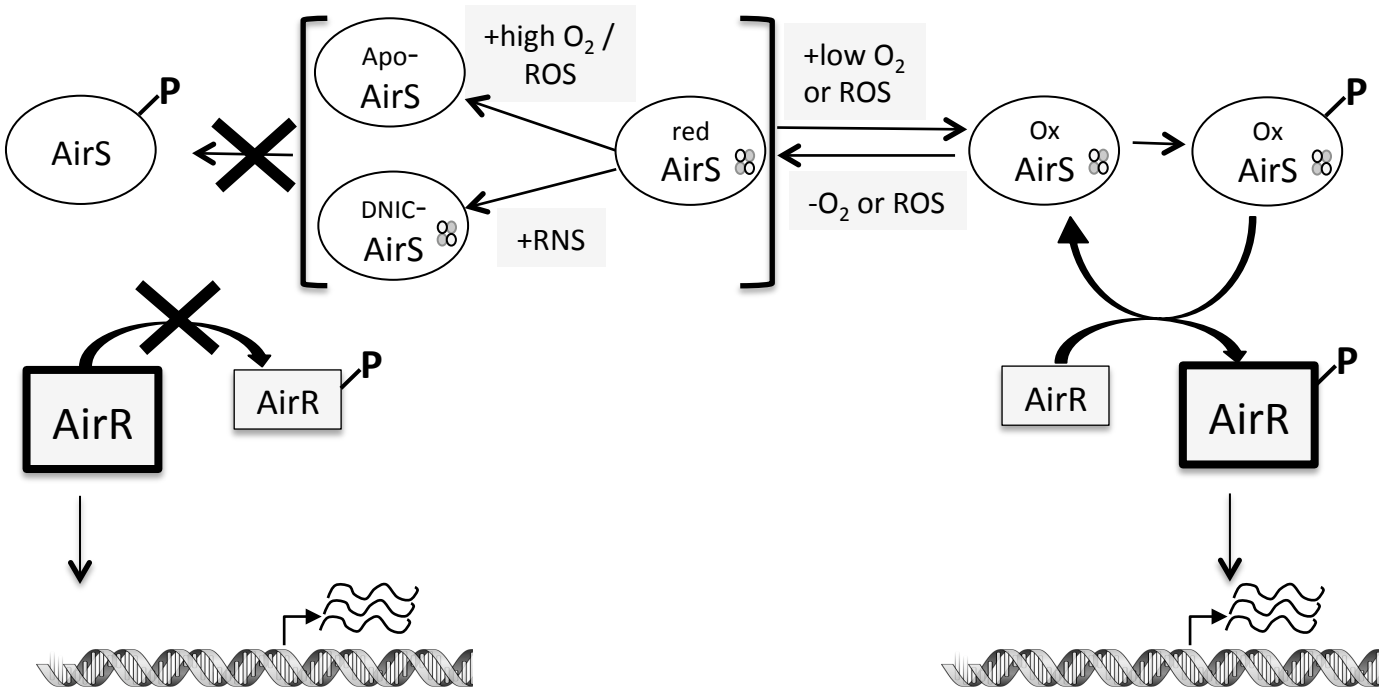



Figure 4




**Positive regulation :**
*Nre, nar*
**Negative regulation :**
*cap5A, sae, agr*

**Positive regulation :**
*lac, opuC*

Figure 5

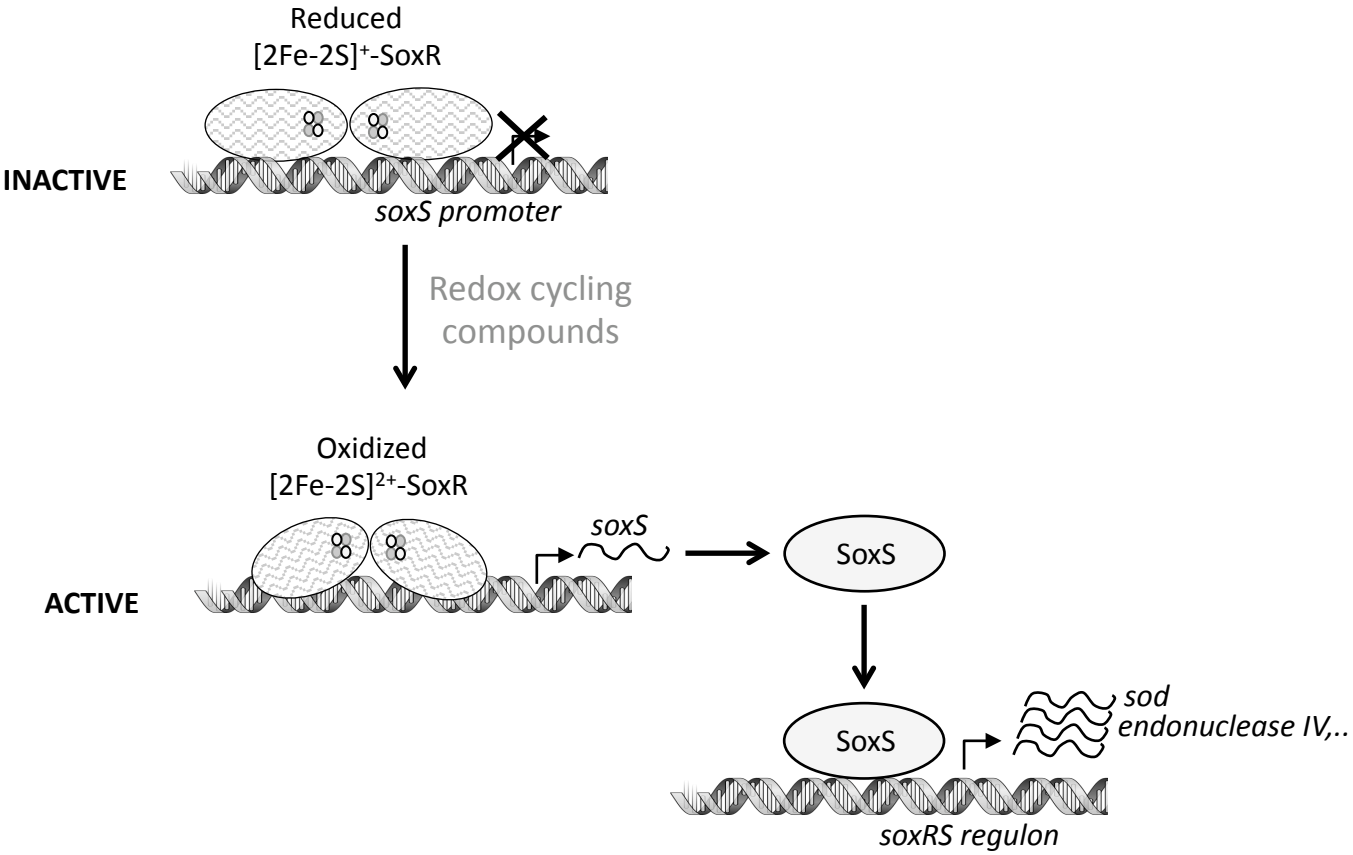

Reduced
[2Fe-2S]+-SoxR
INACTIVE
soxS promoter
Redox cycling
compounds
Oxidized
[2Fe-2S]2+-SoxR
ACTIVE
soxS
SoxS
SoxS
sod
endonuclease IV,..
soxRS regulon

Figure 6

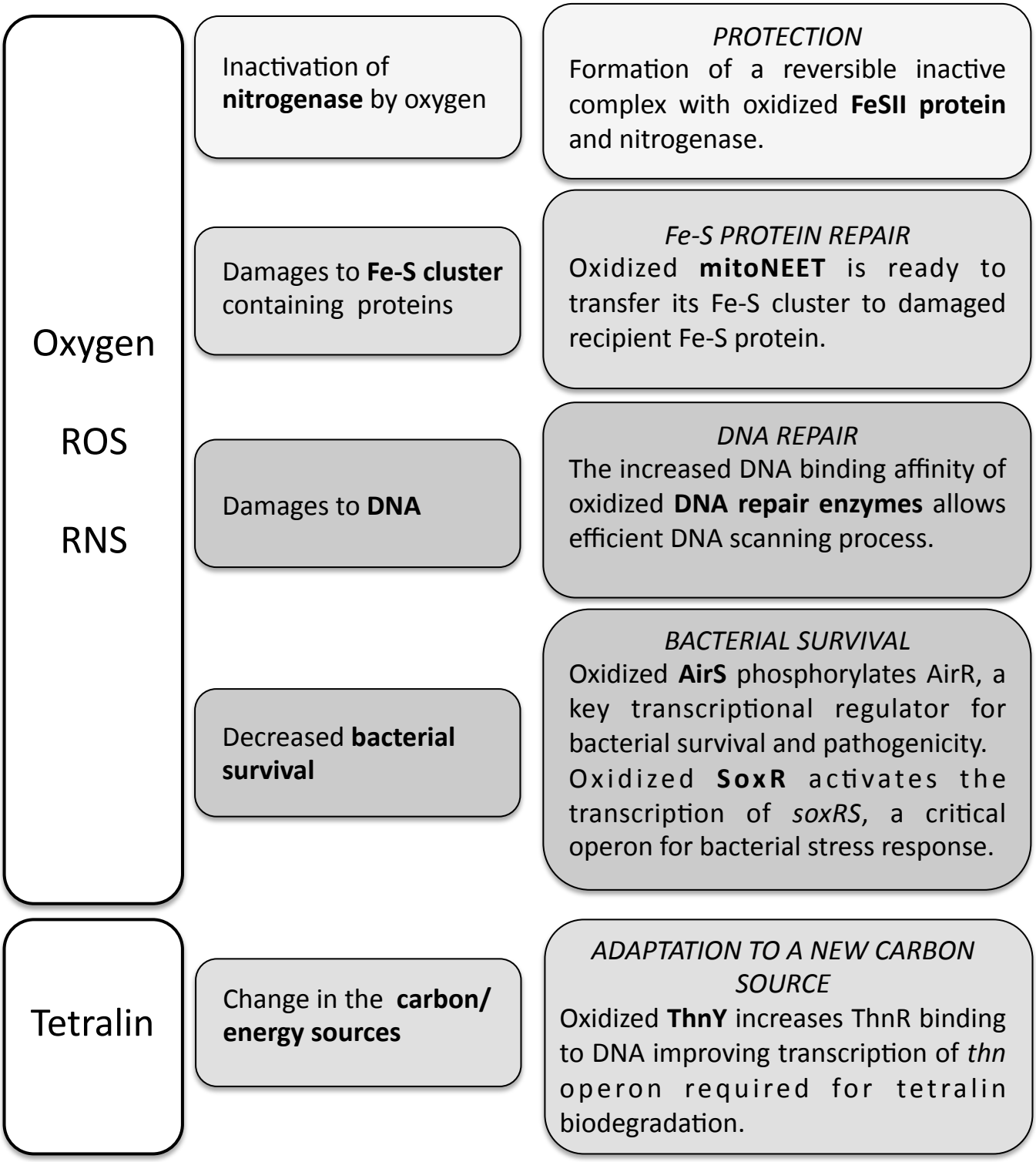

Oxygen
ROS
RNS
Inactivation of **nitrogenase** by oxygen
*PROTECTION*
Formation of a reversible inactive complex with oxidized **FeSII protein** and nitrogenase.
Damages to **Fe-S cluster** containing proteins
*Fe-S PROTEIN REPAIR*
Oxidized **mitoNEET** is ready to transfer its Fe-S cluster to damaged recipient Fe-S protein.
Damages to **DNA**
*DNA REPAIR*
The increased DNA binding affinity of oxidized **DNA repair enzymes** allows efficient DNA scanning process.
Decreased **bacterial survival**
*BACTERIAL SURVIVAL*
Oxidized **AirS** phosphorylates AirR, a key transcriptional regulator for bacterial survival and pathogenicity.
Oxidized **SoxR** activates the transcription of *soxRS*, a critical operon for bacterial stress response.
Tetralin
Change in the **carbon/ energy sources**
*ADAPTATION TO A NEW CARBON SOURCE*
Oxidized **ThnY** increases ThnR binding to DNA improving transcription of *thn* operon required for tetralin biodegradation.

Figure 7