Acta Biochimica Polonica

Vol. 53 No. 4/2006, 685–691

on-line at: www.actabp.pl

Review

Iron-sulfur cluster proteins: electron transfer and beyond★

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Received: 27 September, 2006; revised: 17 October, 2006; accepted: 30 November, 2006
available on-line: 04 December, 2006

Iron-sulfur clusters-containing proteins participate in many cellular processes, including crucial biological events like DNA synthesis and processing of dioxygen. In most iron-sulfur proteins, the clusters function as electron-transfer groups in mediating one-electron redox processes and as such they are integral components of respiratory and photosynthetic electron transfer chains and numerous redox enzymes involved in carbon, oxygen, hydrogen, sulfur and nitrogen metabolism. Recently, novel regulatory and enzymatic functions of these proteins have emerged. Iron-sulfur cluster proteins participate in the control of gene expression, oxygen/nitrogen sensing, control of labile iron pool and DNA damage recognition and repair. Their role in cellular response to oxidative stress and as a source of free iron ions is also discussed.

Keywords: IRP1 protein, aconitase, labile iron pool, DNA glycosylases

INTRODUCTION

Iron-sulfur clusters are ancient structures developed at early stages of life. In the most primitive archeobacteria ISC-containing ferredoxins are used in metabolic pathways, while NAD/NADP was substituted for the same functions in a later stage of evolution (Daniel & Danson, 1995). The extraordinary chemical properties of sulfur and iron place these structures among the most versatile enzymatic cofactors ever known. The different types of iron-sulfur clusters found in nature, from simple [Fe-0S] clusters found in ferredoxins to the complex [7Fe-8S] and [8Fe-8S] structures of bacterial nitrogenases are shown in Fig. 1. In addition to the large number of possible cluster structures, conversions between clusters or within a single cluster have also been observed. The consequences of intercluster conversion from [4Fe-4S] to [3Fe-4S] observed in mammalian ACO1/IRP1 protein will be discussed below.

In vitro, simple clusters, such as mononuclear ferredoxin-type cluster $[Fe(SR)_4]$ or the $[Fe_4(SR)_{10}]$ cage complex, are formed from ferric iron and thiols. With sulfide added, more complex clusters can be obtained, e.g. $[Fe_2S_2(SR)_4]$ or $[Fe_4S_4(SR)_4]$ (Holm, 1975). However, the biogenesis of complex clusters is a complicated, multistep process involving many proteins both in pro- and eukaryotes (reviewed in Rouault & Tong, 2005; Lill *et al*., 2006; Lill & Muhlenhoff, 2006). The key proteins in ISC assembly are indispensable for life and their absence or dysfunction cause growth defects, diseases or lethality (reviewed in Napier *et al*., 2005).

The function of ISC proteins was first recognized in ISC-containing ferredoxins as electron transfer. In this minireview, the recently discovered new functions of [4Fe-4S] cluster proteins, ranging far beyond electron transfer, will be discussed.

★Presented at the 41st Meeting of the Polish Biochemical Society, 12–15 September, 2006, Białystok, Poland.

Abbreviations: ACO1, aconitase 1; soluble, EC 4.2.1.3; BER, base excision repair; eIF4F, eukaryotic initiation factor-4F; DMT1, divalent metal transporter 1; Fpg, formamidopyrimidine DNA glycosylase, EC 3.2.2.23; FT, ferritin; GO, 7,8-dihydro-8-oxoguanine; HiPIP, high potential iron-sulfur proteins; IRE, iron responsive element; IREG1, iron regulated transporter 1; IRP1, iron regulatory protein 1; ISC, iron-sulfur cluster; LIP, labile iron pool; LY, L5178Y; MTP1, metal transporter protein 1; MUTYH, MutY homolog; Nth, DNA-(apurinic or apyrimidinic site) lyase, EC 4.2.99.18; OGG1, 8-oxoguanine DNA glycosylase; rpS3, ribosomal protein S3; SLC11A2, solute carrier family 11 member 2; SLC40A1, solute carrier family 40 member 1; UDGs, uracil DNA glycosylases; UTR, untranslated region; TDG, thymine DNA glycosylase; TFRC, transferrin receptor.

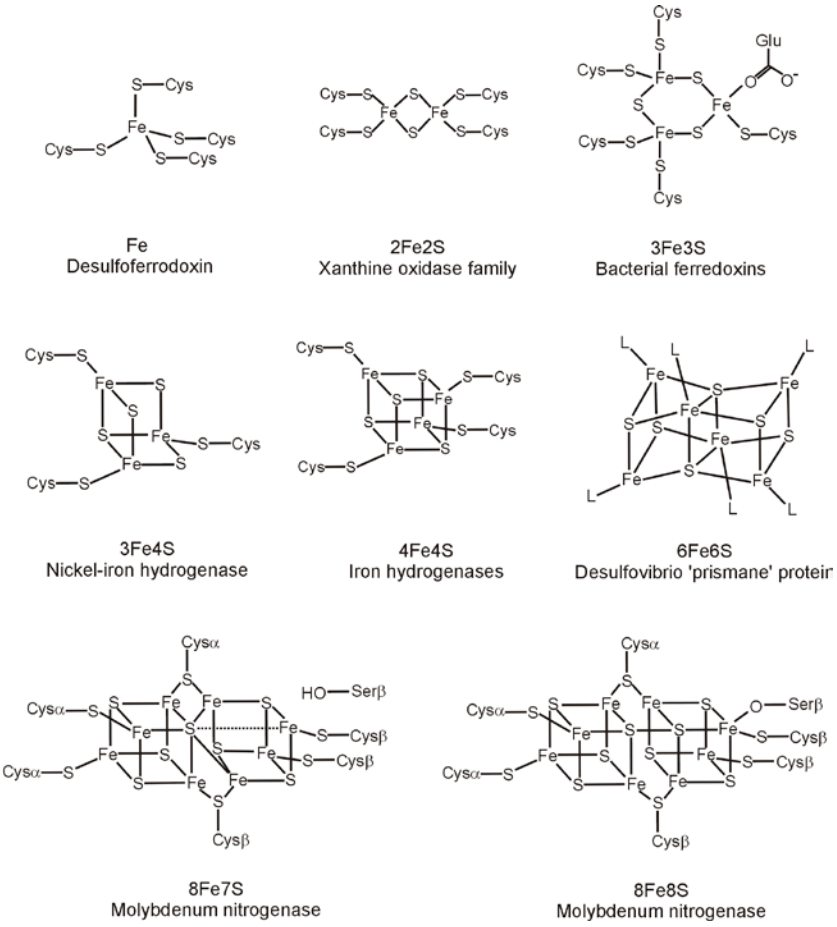

Figure 1. Structure of different types of Fe-S clusters known in nature.

ELECTRON TRANSFER — PRIMARY FUNCTION OF [4Fe-4S] CLUSTER PROTEINS

The first ISC proteins with a known function were ferredoxins, a ubiquitous group of ISC proteins involved in many fundamental processes of life, including photosynthesis, respiration and nitrogen fixation (reviewed in Sticht & Rosch, 1998). Ferredoxins are the main group of ISC proteins used for electron transfer and contain a variety of iron-sulfur clusters. [2Fe-2S] clusters are found in chloroplast ferredoxins acting as electron donors to enzymes, such as nitrite reductase and glutamate synthase (Knaff & Hirasawa, 1991), and in many animal tissues, acting as electron donors to cytochrome P450 (Nemani *et al*., 1989). [3Fe-4S] and [4Fe-4S] cluster ferredoxins were found in *Streptomyces griseolus* and *Desulfovibrio gigas*, respectively. Bacterial ferredoxins may also contain two [4Fe-4S] or [3Fe-4S] clusters or [4Fe-4S] [3Fe-4S] cluster combination, and act primarily as low potential electron transfer proteins (Sticht & Rosch, 1998). Exceptional among ferredoxins are high potential iron-sulfur proteins that contain a typical cubic [4Fe-4S] cluster. Whereas most ferredoxins are able to exchange electrons at a low potential (from –250 to –650 mV), HiPIP proteins are able to exchange electrons at potentials from +50 to +450 mV (Bian & Cowan, 1999). HiPIP proteins are found in purple photosynthetic bacteria and are likely a photosynthetic electron carrier between the cytochrome b–c_1 complex and reaction centers (Meyer & Cusanovich, 2003).

Another group of electron-carrying ISC proteins consists of membrane bound proteins involved in electron transfer within the respiratory chain of mitochondria, as well as in electron transfer in chloroplasts and bacteria. [2Fe-2S] cluster-containing Rieske proteins have been isolated from the chloroplast b6f and cytochrome b–c_1 complexes and some dioxygenases (Ferraro *et al*., 2005). Membrane-bound are also components of the photosystem I of plants and cyanobacteria. Three ISCs in the primary electron acceptor complex of photosystem I have been proposed to be [4Fe-4S]-type clusters (Saenger *et al*., 2002).

Although electron transfer seems to be the primary function of ISC proteins, many of them also have a catalytic function. These include redox and non redox catalysts, such as carbon monoxide dehydrogenases, iron hydrogenases and nitrogenases or aconitase, endonuclease III, fumarate dehydratase and ferrochelatase, respectively. These proteins usually contain [2Fe-2S] or [4Fe-4S] clusters cooperating with other metal centers, such as Mo, V or Ni (Bian & Cowan, 1999).

POSTTRANSCRIPTIONAL GENE REGULATION OR DUAL LIFE OF CYTOSOLIC ACONITASE

Aconitase 1 belongs to the lyases class of enzymes. It contains a [4Fe-4S] cluster and catalyses the conversion of citrate to isocitrate (Haile *et al*., 1992). Isocitrate is the substrate for NADP-dependent isocitrate dehydrogenase — an important producer of NADPH in the cytosol. Glutathione reductase uses NADPH to regenerate reduced gluthatione, which may be oxidized by glutathione peroxidase to remove H_2O_2 — an oxidative stress inducer (Lee *et al*., 2002). In this respect, production of isocitrate by ACO1 may be considered as a contribution to the cellular defense against oxidative stress.

When cellular iron level is low, ACO1 loses its [4Fe-4S] cluster and the apoprotein called iron regulatory protein 1 acquires the ability to bind specific RNA structures called iron responsive elements. IREs are conserved hairpin structures containing a six-nucleotide apical loop with the consensus sequence 5′-CAGUGN-3′, located in the UTRs of several mRNAs. Single IREs are located in the 5′ UTRs of mRNAs encoding iron-storage protein ferritin heavy and light chains, aminolevulinate, δ-synthase (the rate limiting enzyme in heme biosynthesis), mitochondrial aconitase 2 (a citrate cycle enzyme), SLC40A1 (also named IREG1, MTP1 or ferroportin1) (Haile *et al*., 1992; Hentze & Kuhn, 1996; Donovan *et al*., 2000; McKie *et al*., 2000). Binding of IRP1 to these IREs inhibits mRNA translation initiation by preventing the recruitment of the 43S pre-initiation complex to mRNA and its interaction with the cap binding complex eIF4F (Muckenthaler *et al*., 1998) (Fig. 2).

A different type of regulation occurs when IREs are located in the 3′-UTR. They have been found beyond the coding sequence of mRNAs encoding TFRC, SLC11A2, CDC14 cell division cycle 14 homolog A, CDC42-binding protein kinase α (alias myotonic dystrophy kinase-related Cdc42-binding kinase α) (Hentze & Kuhn, 1996; Gunshin *et al*., 2001; Cmejla *et al*., 2006; Sanchez *et al*., 2006). The interaction of IRP1 with IREs in the 3′-UTR does not affect mRNA translation, but enhances mRNA half-life by protecting it from the action of an yet uncharacterized RNase (Eisenstein & Ross, 2003) (Fig. 2).

Point mutations in IREs may significantly decrease the strength of IRP1–IRE interaction and affect regulation. In patients with hereditary hyperferritinaemia-cataract syndrome a point mutation in the IRE of ferritin light subunit mRNA leads to high serum FT level in the absence of an iron overload and to cataract formation. The direct molecular link between high FT level and cataract formation is unknown (Cazzola *et al*., 1997).

Although the IRP/IRE driven regulation of gene expression seems to be a precise and sensitive mechanism of iron homeostasis, regulation of some genes involved in iron turnover escapes this regulation due to alternative splicing. Several alternatively spliced mRNA molecules are produced from the primary transcript of *SLC11A2* gene, some of which lack the iron responsive element in their 3′-UTR (Hubert & Hentze, 2002). Alternative splicing forms of SLC40A1 that lack 5′-UTR IRE have also been found (Cianetti *et al*., 2005). Expression analyses show that alternative transcripts are differentially expressed in distinct tissues. Thus, alternative splicing may constitute another level of regulation of expression of proteins involved in iron homeostasis.

The best studied example of IRP1-mediated regulation and its effects on cellular iron homeostasis is regulation of labile iron pool through changes in FT and TFRC expression. LIP is a pool of accessible, "free" iron ions and constitutes the crossroads of the metabolic pathways of iron-containing compounds (Kruszewski, 2003). As mentioned above, when LIP level is low, IRP1 loses the [4Fe-4S] cluster and binds IREs in the 5′-UTR of FT mRNA and in the 3′-UTR of TFRC mRNA. Consequently, FT level decreases and less iron ions are sequestered by FT molecules. On the other hand, when TFRC expression is enhanced, more TFRC molecules are present on the cell surface which eventually leads to an increased uptake of transferrin-bound iron (Fig. 2).

Apart from the two major forms of IRP1, i.e. the [4Fe-4S] cluster-containing aconitase and the IRE-binding apoprotein, two additional totally inactive forms of IRP1 have been described. One of them is the oxidized form of apo-IRP1; its existence was shown both *in vitro* and in living cells (Schalinske & Eisenstein, 1996; Oliveira *et al*., 1999). It has been

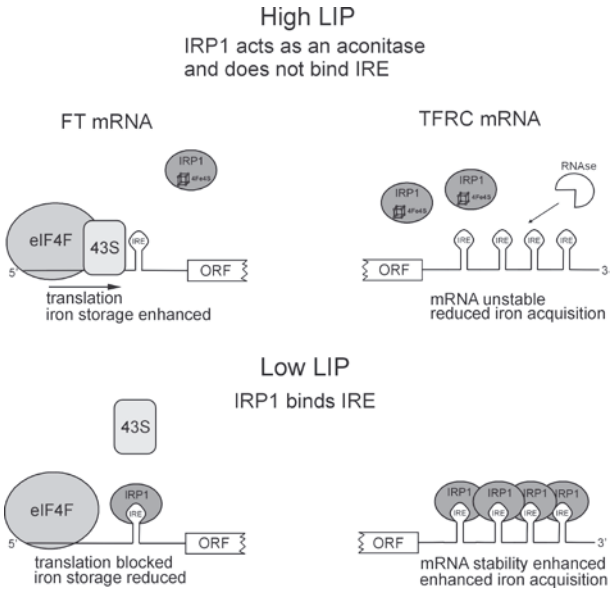

Figure 2. IRP1/IRE regulation of gene expression.

shown that oxidized apo-IRP1 forms in the reaction of peroxynitrite with the holo-protein. Peroxynitrite disrupts the cluster and promotes formation of disulfide bridges between cysteine residues in the vicinity of the IRE-binding domain, which prevent IRP1/IRE interaction. This oxidized form of apo-IRP1 can easily gain the IRE-binding activity in a suitable reducing environment (Bouton *et al*., 1997).

In vitro, a direct reaction of the holo-protein with hydrogen peroxide results in the release of a single ferrous ion from the [4Fe-4S] cluster and its conversion to the [3Fe-4S] cluster. The protein containing the [3Fe-4S] cluster is the second inactive form of IRP1. Brazzolotto *et al*. (1999) have shown that addition of ferrous sulfate can replenish iron at the cluster and restore the aconitase activity of the IRP1 protein. It has been proposed that these two latent forms that have neither aconitase nor IRE binding activity constitute a storage pool of the protein that could quickly turn back into the aconitase or regulatory form under suitable conditions (Bouton *et al*., 1997).

Interestingly, the IRE-binding by IRP1 *in vivo* is activated not only by iron shortage, but also by reactive oxygen species. However, the *in vivo* response of IRP1 towards oxidative stress is rather complex. While H_2O_2 and NO induce the mRNA binding activity, $O_2^{\bullet}$ and HOCl have been shown to block it. Activation of IRP1 by H_2O_2 likely occurs *via* an unknown signaling pathway. It has been shown that the response of IRP1 to H_2O_2 requires ATP, GTP, and a phosphorylation event (Mueller, 2005). The significance of IRP1 regulation by oxidative stress is still a matter of debate. As mentioned above, activation of the IRE-binding activity leads to increased LIP within the cell. During acute inflammation it may reduce iron availability to invading pathogens and may be beneficial for the host. However, in the case of chronic inflammation it reduces iron availability for erythropoiesis, which may eventually lead to severe diseases such as anemia of chronic disease (Mueller, 2005). Moreover, accumulation of labile iron within cells increases generation of highly reactive hydroxyl radicals *via* Fenton chemistry and may result in tissue damage (Kruszewski & Iwanenko, 2003).

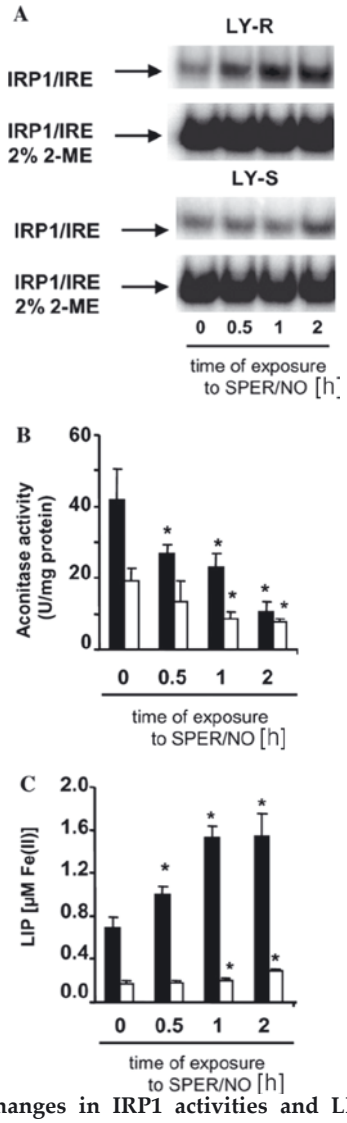

Figure 3. Changes in IRP1 activities and LIP levels in two mouse lymphoma cell lines, LY-R and LY-S, during exposure to an NO donor, SPER/NO.
LY cells were incubated for the indicated times with 25 μM SPER/NO. (**A**) IRP1 RNA-binding activity by EMSA. (**B**) IRP1 aconitase activity. (**C**) LIP levels in LY-R (solid bars) and LY-S (open bars) cells. Reprinted from Lipinski *et al*. (2005) with permission from Elsevier.

[4Fe-4S] CLUSTER PROTEINS AS A SOURCE OF FREE IRON

The intracellular paths of iron traffic and their contribution to labile iron pool are poorly understood. One possible source of free iron ions may be [4Fe-4S]-cluster containing proteins. It is likely that iron ions released from [4Fe-4S] proteins after disruption of the cluster enter the LIP. Indeed, inhibition of aconitase activity (i.e. [4Fe-4S] cluster disassembly) by NO in mouse lymphoma cells is accompanied by an increase in the LIP level as shown by Lipinski *et al*. (2005) (Fig. 3). This increase of LIP level in response to NO is not associated with decreased iron sequestration and increased iron uptake, since no changes in heavy and light FT subunits nor TFRC expression have been found during a 2 h challenge with NO (Lipinski *et al*., 2005).

The cellular chelatable iron pool is not restricted to the cytosol. It can also be detected in other cellular compartments (Petrat *et al*., 2001). The nuclear LIP subfraction is of great importance, as iron ions are necessary for *in vivo* DNA damage induction by H_2O_2 (Meneghini, 1997). Iron ions released after oxi-

dative stress from nuclear proteins containing [4Fe-4S] clusters probably increase labile iron level within the nucleus and in consequence, hydroxyl radicals generation *via* Fenton chemistry, which leads to induction of DNA damage.

THE ROLE OF [4Fe-4S] PROTEINS IN DNA DAMAGE REPAIR AND RECOGNITION

An oxidatively damaged single DNA base is preferentially repaired by BER, the repair system that directly removes damaged base with or without nicking the adjacent sugar backbone (Dizdaroglu, 2005). The key enzymes in this process are DNA glycosylases, enzymes that cut the N-glycosidic bond between the damaged base and sugar, thus initiating the first step of repair. Most glycosylases cut out the damaged base from the DNA leaving an abasic site or a single-strand break. There is one exception known so far. Bacterial MutY protein and its mammalian homolog MUTYH cut out undamaged adenine opposite damaged guanine in the A:GO mismatch (Zhang *et al*., 1998). Interestingly, MutY/MUTYH may have both anti- and promutagenic action depending on nucleotide context. A damaged GC pair is preferentially repaired by bacterial Fpg (OGG1 in mammals) protein that cuts GO from the GO:C base pair. However, if some damage escapes the repair, in the next replication round GO preferentially pairs with adenine. The GO:A mismatch leads to the G:C→T:A transversion in the subsequent replication round. The enzymatic activity of MutY/MUTYH glycosylase prevents DNA mutation by cutting out the mispaired A in the A:GO mismatch. However, in the case of an AT pair, misincorporation of GO during replication followed by MutY action will result in an T:A→G:C tranversion. The MutY protein contains a [4Fe-4S] cluster, but in contrast to ACO1, the ISC in MutY protein does not participate in catalysis and rather has a structural function.

The MutY/MUTYH glycosylase is not the only DNA glycosylase containing ISCs. Other known DNA glycosylases containing ISC are shown in Table 1. Nth shows a structural similarity to the MutY protein, but repairs a wide variety of oxidized pyrimidines in DNA. *Archaeoglobus fulgidus*, *Pyrobaculum aerophilus* and *Thermotoga maritima* UDGs belong to the family-4 UDGs that is present mostly in thermophilic bacteria. BER enzymes in thermophiles face a greater challenge than in normophiles in their duty to eliminate base damage, as cytosine deamination, the main process of uracil production in DNA, is greatly enhanced at high temperatures. However, it is not yet known whether the [4Fe-4S] clusters in these enzymes enhance the efficacy of repair.

Table 1. DNA glycosylases containing [4Fe-4S] clusters.

Enzyme	Substrate/Activity*
MutY/MUTYH	**A**:GO
Nth (EndoIII)/hNTH1	Oxidized pyrimidines; **T-T**
Family-4 UDGs	**U**:A/G
Methanobacterium thermoautotrophicum TDG	**T**:G
rpS3 (UV-endonuclease III)	**T-T**; C:**GO**; AP-lyase

*Removed base is written in boldface and underlined.

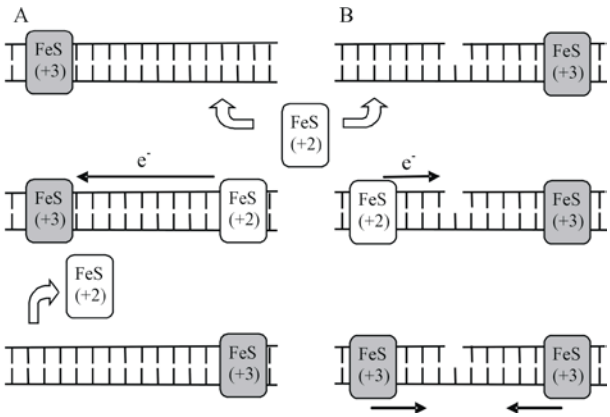

Figure 4. DNA damage recognition by ISC-containing DNA glycosylases.

Recently, an additional role of ISC-containing endonucleases has been proposed. Based on the observation that ISCs of DNA glycosylases change the redox state upon binding to and detaching from DNA, Boon *et al.* (2003) proposed that the [4Fe-4S] cluster in MutY glycosylase can function in DNA-mediated electron transfer. This was further confirmed for other ISC-containing glycosylases (Boal *et al*., 2005). On binding DNA, ISC becomes oxidized and releases an electron in a DNA-mediated reaction. The released electron, through DNA-mediated electron transfer, reduces an alternate ISC-containing DNA glycosylase and facilitates its dissociation from DNA (Fig. 4A). DNA break, base damage or mismatch inhibit DNA-mediated electron transfer resulting in local procession of the DNA glycosylase towards the damage site (Fig. 4B). This model, so far based only on *in vitro* experiments, offers a reasonable explanation of the mechanism of recognition of DNA damage that does not induce large distortion in DNA structure, such as single base damage, mismatch or single-strand break.

CONCLUSION

In spite of the structural limitations due to the presence of the [4Fe-4S] cluster, the chemical properties of sulfur and iron atoms allowed phylogenic development of multifunctional proteins with a wide range of activities. Their role in biological systems extends far beyond simple electron transfer reactions

and has expanded to: redox and nonredox catalysis, gene transcription regulation, sensing of iron and oxidative stress, DNA damage recognition and repair.

Acknowledgements

The work was supported by the Ministry of Science and Higher Education grant No. N30104031/1298.

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