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Editorial

Iron–Sulfur Clusters: Assembly and Biological Roles

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Iron–sulfur (Fe-S) clusters are critical to a wide range of biological processes, from DNA repair and transcriptional regulation to mitochondrial respiration and enzymatic catalysis [1–4]. Composed of iron and inorganic sulfur, Fe-S clusters are ubiquitous cofactors, with the most common types being rhombic [2Fe-2S] and cubane [4Fe-4S] structures [5]. Their unique ability to facilitate electron transfer, catalyze reactions involving organic radicals, and stabilize protein structures makes them indispensable across all domains of life. While the exact role of [4Fe-4S] clusters in most enzymes remains unclear, in the over 100,000 known radical S-adenosyl-L-methionine (RS) enzymes [6,7], including those that repair or modify nucleic acid substrates, [4Fe-4S] cofactors, coordinated by three cysteines, cycle between oxidation states to generate the potent aliphatic 5′-deoxyadenosyl radical, which drives subsequent reactions [6,8–15]. The relevance of RS enzymes to human health is paramount, with gene variants linked to diseases like molybdenum cofactor deficiency [16], lipoic acid deficiencies (reviewed in [2,16]), type 2 diabetes [17,18], and motor neuron degeneration in ALS [19]. Pathogenic variants in Fe-S domains of DNA helicases, like XPD, FANCJ, RTEL1, DDX11, and glycosylases, such as NTHL1 and MUTYH, have been linked to several cancers and compromised DNA repair activity [20–39]. Additionally, an increasing number of human conditions are being identified through exome sequencing, which are caused by loss of function in the components of the Fe-S biogenesis machinery [2,40–42].

Gaining a deeper understanding of Fe-S cluster assembly and the roles of proteins that ligate these cofactors is crucial for elucidating cellular functions and their implications for human health and disease development. The articles in this Special Issue, entitled "Iron–Sulfur Clusters: Assembly and Biological Roles", present primary research studies and in-depth reviews that significantly advance our knowledge of these essential cofactors. They explore the intricate processes of Fe-S cluster biogenesis, the vulnerabilities of these cofactors, and the sophisticated techniques used to study them. This collection also highlights the roles of Fe-S clusters in various biological processes, including the sensing of oxygen and nitric oxide levels, cellular homeostasis, and, as recently emerged, viral pathogenesis [43–48]. Together, these contributions emphasize the importance of continued investigation in this field.

SantaMaria and Rouault [49] provide a comprehensive review on how cells utilize Fe-S clusters to sense and regulate intracellular environments. They delve into the ancient and ubiquitous nature of Fe-S clusters, highlighting their role as biological sensors across diverse forms of life, from unicellular bacteria to complex humans. This review includes a timeline illustrating the pace of discovery of Fe-S enzymes and offers a thorough examination of the mechanisms by which Fe-S clusters maintain cellular homeostasis. The authors also explore unanswered questions in the field, such as the potential of undiscovered Fe-S cluster-containing proteins, the role of these clusters in viruses beyond SARS-CoV-2, and the mechanisms enabling iron sensing in archaea and plants. Additionally, they emphasize the importance of understanding how Fe-S proteins switch between their apo and holo forms, how Fe-S clusters are regained after loss, and the balance between cluster stability and turnover, highlighting these as critical areas for future research.

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Ogunkola and colleagues [50] address the controversy surrounding the Fe-S dependency of the *E. coli* MnmA protein. The wobble nucleoside at position 34 of tRNAs universally carries thiomodifications that enhance codon binding and are crucial across all life forms [51]. These modifications, particularly the sulfur-based s^2 and mnm^5 modifications at uridine 34, stabilize tRNA structures, improve interactions with aminoacyl-tRNA synthetase, and ensure accurate mRNA decoding. In *E. coli*, a sulfur-relay system involving several proteins, including MnmA, facilitates these modifications [52]. While the mnm^5s^2U34 modification in bacteria was believed to be Fe-S cluster-independent, recent reports have suggested otherwise. This study aims to resolve these contradictions by investigating the activity of Fe-S cluster-containing MnmA under specific conditions. Through meticulous experimentation, the authors demonstrate that the presence of Fe-S clusters inhibits tRNA thiolation, thereby establishing MnmA as an Fe-S cluster-independent protein. These finding challenges previous assumptions and add a new dimension to our understanding of tRNA modifications in prokaryotes.

Heffner and Maio [53] uncover the emerging roles of Fe-S clusters in viral proteins. Highlighting the competition for iron resources between host cells and viruses, the authors discuss how viral proteins exploit Fe-S clusters for replication and survival. This work opens up new avenues for research into viral pathogenesis and the potential for targeting Fe-S cluster interactions in antiviral strategies.

Raza and colleagues [54] review the advanced techniques required to study Fe-S proteins, with a focus on those recently discovered in viruses [43–47]. They highlight the challenges posed by the oxygen sensitivity of Fe-S clusters and the specialized equipment required to preserve their integrity. This review offers an in-depth examination of the methods used to characterize the stoichiometry and oxidation state of Fe-S clusters, including UV–Vis absorption, NMR, X-ray crystallography, EPR and Mössbauer spectroscopies, and electrochemical techniques. By elucidating the unique redox transitions of specific Fe-S clusters, this review offers essential insights into how these transitions might influence enzyme function and interactions, providing a valuable resource for researchers investigating Fe-S proteins.

Quist and colleagues [55] focus on the enigmatic hepatitis B virus X protein (HBx), a critical factor in HBV-induced hepatocellular carcinoma (HCC). They describe how they uncovered the nature of the Fe-S cofactor in HBx despite its sensitivity to oxygen [56]. Their work revealed that HBx binds a [4Fe-4S] cluster, contrary to earlier assumptions of Zn ion binding. Through detailed spectroscopic analyses, including Mössbauer and EPR spectroscopies, they demonstrated that HBx can switch between [2Fe-2S] and [4Fe-4S] clusters depending on oxygen availability [45]. This finding suggests potential roles for the cluster interconversion in HBV pathogenesis and oncogenesis. The study also underscores the broader significance of Fe-S clusters in viral biology, challenging previous assumptions and paving the way for new research into viral metalloenzymes and their interactions with the host–cell machinery.

Crack and colleagues [57] examine the differential reactivity of the *E. coli* fumarate and nitrate reduction (FNR) regulator in response to oxygen (O_2) and nitric oxide (NO). Their study reveals how FNR, which plays a key role in managing the shift between aerobic and anaerobic respiration, responds differently to these signaling molecules. They demonstrate that the L28H variant of FNR shows enhanced stability under aerobic conditions, a result attributed to a cation–π interaction between His28 and Arg184 that reduces the flexibility of the Cys20-Cys29 loop. This increased stability affects the reactivity of the protein to O_2, while its response to NO remains largely unchanged. These findings offer valuable insights into the mechanistic basis for improved O_2 resistance and selective response to NO of the L28H variant, enhancing our understanding of how Fe-S cluster regulators discern between different gaseous signals and influence cellular responses to environmental changes.

Aubert and colleagues [58] provide a thorough review comparing bacterial Fe-S protein biogenesis factors with their eukaryotic counterparts. They focus on Mrp and SufT, bacterial homologs of eukaryotic Cytoplasmic Iron–Sulfur Assembly (CIA) components,

and their roles in Fe-S cluster assembly. By examining both the unique and shared features of these systems, this review offers a detailed overview of the evolutionary conservation and divergence in Fe-S cluster biogenesis pathways. The parallels between bacterial Mrp and SufT proteins and their eukaryotic CIA counterparts suggest that further research could enhance our understanding of Fe-S cluster assembly in higher eukaryotes. Future studies on the Mrp/Nbp35 cycle may reveal new regulatory factors involved in Fe-S cluster assembly and transfer, potentially leading to novel strategies for addressing diseases associated with Fe-S cluster deficiencies and for targeting Fe-S biogenesis in pathogenic bacteria.

The papers in this Special Issue highlight the central role of Fe-S clusters in a diverse array of biological processes. The contributions explore the fundamental mechanisms of Fe-S cluster biogenesis and their implications for cellular function, from DNA replication and repair, to translation and viral replication. Key insights include the regulatory roles of Fe-S clusters in different environmental and metabolic conditions, the importance of these cofactors for viral pathogenesis, and the advanced techniques used for studying Fe-S proteins. The comparative analysis of bacterial and eukaryotic Fe-S cluster assembly systems reveals both conserved mechanisms and potential novel regulatory factors. These findings could pave the way for future studies that leverage Fe-S clusters to develop therapeutic strategies for diseases related to their deficiencies and to target pathogenic organisms. As our understanding deepens, integrating these discoveries promises to significantly enhance our ability to address critical biological and medical challenges.

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