Structural and Functional Properties of Iron–Sulfur Cluster Assembly Proteins: A Mini Review of IscS, IscU, and CyaY

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Iron–sulfur (Fe–S) clusters are multifunctional cofactors involved in a broad variety of biological processes, including respiration, DNA repair, and redox regulation. Their formation requires a complex network of proteins, including IscS, IscU, and CyaY. This mini review gives an overview of structural and functional information on these key proteins of the Fe–S cluster assembly pathway, drawn from the literature between 2010 and 2025. We cover their roles in different cellular systems, ranging from plants to mammals, their iron and sulfur coordination, and their interaction within the global Fe–S cluster biosynthetic machinery. This short synthesis aims to present background information for students and beginning researchers with interests in mitochondrial biology, redox biochemistry, and disease mechanisms involving Fe–S proteins.

Introduction

Fe–S clusters are evolutionary cofactors composed of iron and inorganic sulfur, generally in the form of [2Fe-2S] or [4Fe-4S] clusters. They are involved in electron transport, enzyme function, and regulation. Assembly of clusters is carried out by highly characterized protein machineries, the ISC (iron–sulfur cluster), SUF (sulfur mobilization), and CIA (cytosolic iron– sulfur protein assembly) pathways. Mitochondrial ISC machinery initiates the process in eukaryotes, while CIA regulates cytosolic and nuclear maturation of Fe–S proteins [1].

The ISC pathway of mitochondria and bacteria includes IscS (a cysteine desulfurase), IscU (a scaffold protein), and CyaY (a frataxin homolog). IscS catalyzes activation of sulfur from cysteine to produce an intermediate persulfide that is delivered to IscU, in which it loads iron to give nascent Fe–S clusters. These are subsequently delivered to recipient apoproteins by chaperone systems [2].

The SUF machinery, both in plant and bacterial plastids, is oxidative-stress resistant and uses a unique scaffold (SufBCD complex) to possess a flavin redox cofactor important for the stabilization of sulfur intermediates and oxidative environment resistance [3].

Plant systems are more complex since ISC and SUF pathways are located within chloroplasts and mitochondria, respectively. These pathways employ different but functionally homologous proteins, which allow specialized metabolic processes and stress responses [4].

Interestingly, Fe–S cluster assembly occurs in two broad phases: one is de novo cluster assembly on scaffold proteins like IscU, and the other is transfer and insertion of preassembled clusters into recipient apoproteins. Both phases are tightly regulated and evolutionarily conserved [5]. The CIA pathway, indirectly not being regulated with IscS or IscU, is dependent on ISC- derived precursors and helps in the Fe–S cluster incorporation into nuclear and cytosolic proteins in general cellular metabolism [6]. Familiarity with these pathways provokes a basis upon which to deconvolute the physiological and biochemical function of Fe–S clusters in a variety of biological contexts. These things are very important in cellular metabolism. It can be seen by their conservation through species and evolution.

Structural Features of IscS, IscU, and CyaY

IscS is a cysteine desulfurase that transfers sulfur into clusters when being assembled. IscS prefers to exist as a homodimer and bears the cofactor pyridoxal phosphate (PLP). IscU is a kinetic scaffold on which initial cluster assembly takes place. CyaY is a frataxin structural homolog with the best capacity to bind iron and to modulate iron availability when a cluster assembles. Structural analysis shows how these proteins interact transiently to enable effective iron and sulfur transfer [5]. Sulfur transfer and cysteine are attached by the active site of IscS, while conformational change in IscU is enabled to stabilize intermediate clusters. CyaY has a conserved β-sheet structure and negatively charged surface regions suitable for iron coordination. The SufBCD complex, despite being a member of the Isc system, has given structural insights into alternative scaffolds and shown the function of flavin redox cofactors in Fe–S assembly with functional similarity to the IscU function [3].

Functional Roles in Fe–S Cluster Biogenesis

IscS catalyzes cysteine-to-alanine conversion through a persulfide sulfur intermediate that is passed on to IscU. The reaction is strictly regulated because an excess or lack of Fe– S clusters can cause oxidative stress or metabolic inhibition [1]. Isc machinery functions in a linear manner with assembly and targeting to acceptor proteins being extremely faithful. Intermediates must be strongly regulated to avoid cytotoxicity due to free iron or aberrant cluster assembly. In plant organisms, functional diversification of Fe–S cluster proteins like orthologs of IscU and IscS indicates plastid and mitochondrial compartment adaptation. The proteins are involved in redox balance and help in photosynthesis and respiration [4]. Coordination between nuclear-encoded and organelle-targeted proteins also illustrates the complexity of Fe–S assembly in multicellular organisms, where complex regulation is required to coordinate developmental and environmental cues.

Protein–Protein Interactions and Regulatory Mechanisms

IscS, IscU, and CyaY are a transient complex whose stability is controlled by the availability of cellular iron. Frataxin (bacterial CyaY) can act as an iron donor or regulatory inhibitor depending on redox and iron status. Coordination among these proteins avoids free iron toxicity and ensures correct cluster transfer [7]. For example, regulation of iron loading on IscU by frataxin is dependent on binding to IscS and regulating the overall rate of cluster biosynthesis.

Fe–S cluster reactivity with reactive molecules like oxygen and nitric oxide reveals both their potential to serve as regulators and their sensitivity. Reactivity also indicates the necessity of having a particular cluster assembly in order to prevent unwanted oxidative reactions [8]. Through feedback control mechanisms, iron sensors and redox-sensitive transcription factors play an important role in regulating Fe–S cluster homeostasis under a variety of biological circumstances.

Fe–S Clusters in Cellular Processes and Disease

Apart from electron transport, Fe–S clusters also play a role in enzyme catalysis and DNA metabolism. Cluster biosynthesis and iron homeostasis can be stopped by frataxin loss. This causes defects in cluster assembly which can lead to mitochondria to malfunction and have disorders [9].

In mammalian cells, carrying the cluster from IscU to recipient protein is done by specialized systems. If there is a defect in these processes, cellular metabolism and viability can be affected by it [2].

Recent findings reveal the role of new assembly factors in cluster maturation and transport in mammalian cells, including GLRX5 and HSC20. This helps us better understand the molecular handoff processes in the clusters within mammalian cells [6].

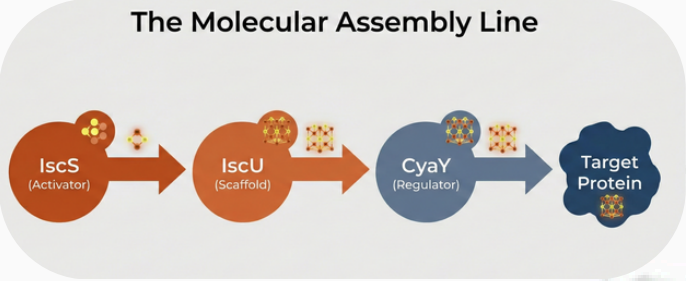
The role of Fe–S clusters in disease has also been explored for neurodegenerative and metabolic diseases. If clusters are defective, these can cause iron to not be distributed correctly and therefore increase oxidative damage [10].

Emerging Research and Perspectives

Recent evidence shows that [4Fe–4S] cluster assembly needs specialized complexes in mitochondria that include scaffold, transfer, and regulatory components [11]. Besides, the connection between genome stability and cluster assembly has been established by evidence of Fe–S cluster requirements in polymerases and DNA repair proteins. For example

MMS19 has been described as a protein that connects cytosolic Fe–S cluster assembly and DNA metabolism and impacts genome integrity [12]. Furthermore, studies have indicated that the formation of active eukaryotic DNA polymerase complexes is dependent on the incorporation of an Fe–S cluster, which is required for their catalytic function [13]. Furthermore, defects in Fe–S cluster biogenesis have also been implicated in Friedreich's ataxia pathogenesis, a neurodegenerative disease characterized by iron overload, oxidative stress, and mitochondrial dysfunction [14]. These findings as a whole show the ubiquitous role of Fe–S clusters in functions outside of their established metabolic roles and propose their involvement in cell homeostasis and resistance to genetic instability.

Finally, novel roles for Fe–S cluster function in vascular biology have been discovered with regulatory roles in oxygen sensing and redox signaling, adding further to their biological significance [15].



Conclusion

Assembly of Fe–S clusters depend on IscS, IscU, and CyaY, which together enable the correct synthesis, delivery, and regulation of these critical cofactors. Further research on these clusters is required to completely understand their interactions, dynamics, and disease significance. This background is necessary to fully understand the general significance of Fe–S clusters in cellular life and human disease.

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