Mammalian iron–sulphur proteins: novel insights into biogenesis and function

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Abstract | Iron–sulphur (Fe–S) clusters are inorganic cofactors that are found in nearly all species and are composed of various combinations of iron and sulphur atoms. Fe–S clusters can accept or donate single electrons to carry out oxidation and reduction reactions and to facilitate electron transport. Many details of how these complex modular structures are assembled and ligated to cellular proteins in the mitochondrial, nuclear and cytosolic compartments of mammalian cells remain unclear. Recent evidence indicates that a Leu-Tyr-Arg (LYR) tripeptide motif found in some Fe–S recipient proteins may facilitate the direct and shielded transfer of Fe–S clusters from a scaffold to client proteins. Fe–S clusters are probably an unrecognized and elusive cofactor of many known proteins.

Iron–sulphur (Fe–S) proteins, which are found in all three kingdoms of life, are increasingly being recognized for their importance in cellular biochemistry and the maintenance of energy homeostasis (BOX 1). Fe–S clusters are inorganic cofactors that typically bind to cysteinyl ligands of an Fe–S protein. Common Fe–S clusters include a rhomboid cluster composed of two iron atoms and two inorganic sulphur atoms ([2Fe–2S]), which often functions as a building block for more complex Fe–S clusters, and a cubane form composed of four iron and four sulphur atoms ([4Fe–4S]) (FIG. 1). Much larger and more complex stoichiometries of Fe–S clusters are found in enzymes such as hydrogenase and nitrogenase, which often contain other metals, such as molybdenum.

In bacteria, the Fe–S biogenesis machinery supplies Fe–S clusters to client proteins in a single cellular compartment, whereas in mammalian cells the synthesis and distribution of Fe–S clusters are more complicated. Mitochondria are a major site of Fe–S cluster biogenesis in mammalian cells; the 12 Fe–S clusters of respiratory chain complexes I–III, as well as those of the citric acid cycle enzymes aconitase and succinate dehydrogenase (SDH; complex II), are incorporated into their respective complexes from initial scaffold to specific recipient proteins. These mitochondrial proteins acquire Fe–S clusters is a subject of controversy, as there is little evidence that intact Fe–S clusters can cross the inner mitochondrial membrane to exit from mitochondria. The mammalian proteins involved in initial assembly of Fe–S clusters have been detected and have been shown to function in the cytosolic and nuclear compartments, but they are not abundant in these compartments. Moreover, most of these proteins have not been found in the cytosolic or nuclear compartment of the model organism *Saccharomyces cerevisiae*, in which an alternative pathway of Fe–S cluster biogenesis is proposed to function. Thus, it is not yet clear whether mammalian cells depend on the mitochondria to generate simple Fe–S components that are the original building blocks of Fe–S clusters of cytosolic and nuclear proteins.

During the cellular biogenesis of Fe–S clusters, nascent clusters are initially assembled on a dedicated scaffold protein contained within a multimeric complex. Subsequently, the scaffold protein is bound by a chaperone–co-chaperone complex and transfers its Fe–S clusters to initial Fe–S scaffold–co–chaperone complex and transfers its Fe–S clusters from the initial scaffold to specific recipient proteins. Advances in our understanding of mammalian Fe–S protein biogenesis have resulted from the biochemical characterization of a multimolecular complex devoted to initial Fe–S cluster assembly in bacteria, guided by structural characterizations of bacterial assembly complexes and their counterparts in mammalian cells. After the initial synthesis and transfer of a cluster-bearing scaffold to a chaperone–co–chaperone system, ATPase activity probably provides energy to drive conformational changes in the scaffold to facilitate transfer of the Fe–S clusters.
Iron–sulphur (Fe–S) clusters are protein cofactors with unusual electronic configurations that enable them to readily accept or donate single electrons and to engage in several types of complex chemical reaction. Fe–S proteins commonly mediate simple transfer reactions involving single electrons, such as that mediated by ferredoxin in steroid biosynthesis. In complex I of the mitochondrial respiratory chain, eight Fe–S clusters relay electrons from NADH to ubiquinol, releasing energy that is used to generate the proton gradient required for ATP synthesis. Fe–S electron-relay chains are also important in succinate dehydrogenase, xanthine oxidase, and Fe–S clusters function as active sites of enzymes, such as mitochondrial aconitase, in which three Cys residues ligate a cubane Fe–S cluster, leaving one iron atom free to bind to a hydroxyl group and a proton from the substrate, citrate; aconitase inactivates the bound hydroxyl and proton to generate isocitrate. Fe–S clusters facilitate free-radical-dependent chemistry implemented by enzymes of the radical SAM family. In lipoyc acid synthase (LIAS), an iron atom from one of its two [4Fe–4S] clusters cleaves SAM to generate a highly reactive deoxyadenosine radical that oxidizes two carbons of an octanoyl carbon backbone. The other [4Fe–4S] cluster of LIAS donates two sulphur atoms to positions C6 and C8 of the octanoyl carbon backbone and thereby generates lipoic acid, which is crucial for the function of some protein complexes, such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Mutations that interfere with Fe–S cluster acquisition by LIAS cause severe acidosis in infants.

Numerous DNA metabolism proteins contain Fe–S clusters, and these have a role in DNA-mediated signalling, which indicates that the Fe–S clusters are functionally important. Fe–S proteins can also function as sensors of iron and oxygen, as exemplified by iron regulatory protein 1 (IRP1), which switches from functioning as a cysteolic aconitase in mammalian iron-replete cells to an RNA-binding regulator that restores iron homeostasis in iron-depleted cells.

Changes that facilitate the release of nascent Fe–S clusters to recipient proteins are crucial for the mammalian co-chaperone HSC20 (known as J-type accessory chaperone 1 (Jac1) in yeast and HscB in bacteria) guides the specificity of Fe–S cluster acquisition by recipient proteins; was recently discovered: the tripeptide motif Leu–Tyr–Arg (LYR) was identified as a specific site in recipient proteins to which HSC20 binds, in association with the Fe–S–containing scaffold protein, ISCU, and the chaperone, HSPA9. These studies have elucidated important aspects of how SDH subunit B (SDHB) incorporates its Fe–S–containing scaffold protein, as well as how the discovery of a role of LYR motifs in Fe–S recipient proteins may lead to the identification of many new Fe–S proteins in the nucleus and cytosol (BOX 2). In addition, we detail several recently described human diseases that are attributable to defects in Fe–S cluster biogenesis and that offer potential insights into the specific roles of affected proteins in the biogenesis of these clusters.

Chemical properties of Fe–S clusters

Many Fe–S proteins depend on the ability of Fe–S clusters to accept or donate single electrons to carry out complex enzymatic reactions, whereas non-metal-containing prosthetic groups, such as NAD+, are less versatile and are limited to accepting electron pairs. Fe–S proteins have often eluded identification, but they carry out numerous crucial functions. The ability of Fe–S clusters to accept and directionally shuttle a single electron results from blending of the orbital electron structure of the transition metal iron — which usually contains five or six unpaired electrons within its atomic d orbitals — with the orbitals of sulphur, which can expand its outer electron valence shell to form chemical bonds with up to six other atoms. This feature enables Fe–S clusters to assume multiple stoichiometries and shapes that can be further modified on ligation to proteins. Sulphur atoms are well suited to binding iron as bridging ligands, partly because the relatively small nucleus of sulphur fits within a wide range of Fe–S cluster geometries. The presence of iron, which is stabilized by bridging sulphur atoms, makes it possible for cubane [4Fe–4S] clusters to accept single electrons into overlapping blended orbitals in which a single extra electron can be delocalized between two iron atoms. Importantly, many Fe–S clusters, including the common cubane cluster, can accept and donate single electrons without substantially reorganizing their internal geometries and protein contacts. The fact that electron transfer can occur without undergoing energetically costly protein reorganization is one of the main reasons that Fe–S proteins can rapidly transfer electrons between different metal centers in large protein complexes, such as mitochondrial complex II, or between multiple clusters in a single protein, such as SDHB. In complex I of the mitochondrial respiratory chain, eight Fe–S clusters are positioned near one another in a quasi-linear arrangement through the portion of complex I that extends into the mitochondrial matrix. The ascending reduction—oxidation (redox) potentials enhance electron transfer from NADH, a strong electron donor in the mitochondrial matrix, to the electron acceptor and carrier of the inner mitochondrial membrane, ubiquinone.

Initial biosynthesis of the Fe–S cluster

Initially, researchers thought that Fe–S clusters assembled spontaneously in vivo, given that chemists were able to synthesize various Fe–S clusters in vitro using elemental iron and sulphur compounds as building blocks. These clusters were found to be accurate analogues of comparable protein-bound Fe–S clusters, and their remarkable ability to interconvert their stoichiometries and shapes, and to transfer from one protein to
Figure 1 | Examples of various Fe–S clusters. a | Rhombic iron–sulphur ([2Fe–2S]) clusters are common and are found in many reducing proteins, such as ferredoxins and glutaredoxins. The ability of two rhombic [2Fe–2S] clusters to coalesce to form a cubane [4Fe–4S] cluster has been documented in vitro and in vivo. The versatile binding characteristics of sulphur are exemplified by its ability to bridge two metal (iron) sites in rhombic [2Fe–2S] clusters, three metal sites in cubane [4Fe–4S] clusters and up to six metal sites for the central sulphur of the complex P-cluster of nitrogenase. The two iron atoms in the top plane of the cubane Fe–S cluster share a blended orbital, in which a single electron is delocalized such that each iron atom has a functional charge of 2.5+, instead of one iron having a charge of 3+ while the other monopolizes a single electron to reduce its charge to 2+. Similar delocalization of an electron is present in the bottom plane (not shown). Delocalization of the added electron between paired iron atoms in a cubane Fe–S cluster is energetically very favourable because the Fe–S cluster does not need to substantially reorganize its components and ligands to share the electron. Part c from Beinert, H., Holm, R. H. and Munck, E. Iron–sulfur clusters: nature’s modular, multipurpose structures. Science 277, 653–659 (1997). Reprinted with permission from AAAS.

another, supported the conclusion that the Fe–S clusters are modular and fairly robust structures. Further studies showed that the coalescence of two [2Fe–2S] clusters to form a single [4Fe–4S] core is a reversible process that occurs with relative ease in synthetic clusters and in physiological settings. However, the discovery of a bacterial operon (nif) that contains genes associated with synthesis of the Fe–S clusters of nitrogenase in the bacterial plant symbiont Azotobacter vinelandii revolutionized studies of cellular Fe–S cluster biogenesis. In bacterial DNA, genes that function in a single pathway are often grouped together in an operon that facilitates the co-expression of functionally related proteins, such as the nif operon of A. vinelandii and two related operons of Escherichia coli, the iron–sulphur cluster (isc) and sulphur formation (suf) operons. The roles of most of the encoded proteins of the nif operon were subsequently determined. As bacteria lack organelles, such as mitochondria, bacterial Fe–S biogenesis takes place in a single cellular compartment encompassed by the bacterial inner membrane.

One of the main challenges in synthesizing an Fe–S cluster is to mobilize inorganic sulphur for incorporation into the cluster. In many in vivo synthetic reactions, desulphurization of soluble Cys generates persulphide (R–S–SH) groups, which function as the major source of sulphur. The bacterial Cys desulphurase encoded by the nif operon was designated NifS. Homologues of this important enzyme have subsequently been discovered in other non-nitrogen-fixing bacteria (such as E. coli) and numerous other related bacteria, in which the enzyme is called Fe–S cluster sulphide generator (IscS) or sulphur formation S (SufS) and in eukaryotes (including S. cerevisiae (Nfs1) and humans (NFS1)).

Structural studies of bacterial proteins have shown that IscS dimerizes along its central domain, which harbours a pyridoxal phosphate (PLP) cofactor and a Cys substrate-binding pocket, to create a large central platform on which multiple reactions take place. Free Cys donates its sulphur to the PLP cofactor, which in turn donates its sulphur to a Cys residue in an unstructured loop of IscS to form a Cys persulphide (R–S–SH). Recent studies in bacteria indicate that a ferredoxin encoded by the isc operon, known as Fdx, directly binds to IscS and donates an electron to stabilize the Cys-bound persulphide, however, further studies are required to determine how and when additional electrons are introduced during nascent cluster formation. The persulphide-bearing IscS loop can move more than 14 Å across the complex away from the PLP cofactor towards the scaffold protein, IscU. IscU binds in a monomeric form at opposite ends of the IscS dimer and contains reactive Cys residues that may bind to the persulphide sulphur.

The source of iron for the Fe–S cluster assembly remains unresolved but has been the subject of much discussion. Recent studies indicate that the iron source in bacteria may be a protein encoded by iscX, a member of the isc operon that has a preference for binding reduced ferrous iron and that can form a ternary complex with IscU and IscS. IscX binds to IscU only when IscS contains iron. After donating reduced iron to the nascent Fe–S cluster and possibly donating a second electron from iron to the inorganic sulphur atom, IscS seems to move to a binding site on IscS where the apo, non-iron-bound form of IscX can block further Cys desulphurase activity of IscS. At this point, rearrangements of iron and sulphur atoms at the Fe–S cluster assembly site of IscS could enable the iron atom from IscX to bind to the reduced inorganic sulphur and cysteinyl ligands of IscU. A second cycle of sulphur and iron donation may be initiated when Fdx displaces IscS from its binding site on IscS, and repetition of the cycle could generate the nascent [2Fe–2S] clusters that were recently structurally characterized. The order of events is unclear, although several studies previously concluded that IscU binds to the persulphide sulphur first. It is probable that IscU with a bound [2Fe–2S] cluster is subsequently displaced from the IscS–IscU complex.
<table>
<thead>
<tr>
<th>Human disease</th>
<th>Affected step</th>
<th>Human protein mutated</th>
<th>Yeast orthologue</th>
<th>Putative functions</th>
<th>Incidence</th>
<th>Affected tissues and/or symptoms</th>
<th>Mutation</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friedrich's ataxia</td>
<td>Initial Fe–S cluster synthesis</td>
<td>Frataxin</td>
<td>Yfh1</td>
<td>Derepressor for initial Fe–S cluster synthesis; Cys desulphurase activity; iron donor; potential allosteric modifier</td>
<td>1 in 50,000</td>
<td>Dorsal root ganglia, cerebellum, heart and pancreas; ataxia, myopathy and diabetes</td>
<td>GAA expansion in first intron</td>
<td>102–104</td>
</tr>
<tr>
<td>ISCU myopathy</td>
<td>Initial Fe–S cluster synthesis</td>
<td>ISCU</td>
<td>Iscu1 and Isu2</td>
<td>Scaffold for initial Fe–S cluster synthesis</td>
<td>25 patients known in northern Sweden</td>
<td>Skeletal muscle; muscle weakness</td>
<td>Splicing error leads to inclusion of intronic sequence in mature transcript</td>
<td>105, 106</td>
</tr>
<tr>
<td>ISCU myopathy and cardiomyopathy</td>
<td>Initial Fe–S cluster synthesis</td>
<td>ISCU</td>
<td>Iscu1 and Isu2</td>
<td>Scaffold for initial Fe–S cluster synthesis</td>
<td>2 known patients</td>
<td>Skeletal and heart muscle; muscle weakness and heart failure</td>
<td>Splicing error and missense mutation</td>
<td>107</td>
</tr>
<tr>
<td>Combined oxidative phosphorylation deficiency</td>
<td>Initial Fe–S cluster synthesis</td>
<td>ISD11</td>
<td>Isd11</td>
<td>Part of the Cys desulphurase complex</td>
<td>2 known patients</td>
<td>Primarily skeletal muscle and liver</td>
<td>Homozygous missense mutation</td>
<td>108</td>
</tr>
<tr>
<td>Skeletal muscle myopathy</td>
<td>Initial Fe–S cluster synthesis</td>
<td>FDX2</td>
<td>Yah1</td>
<td>Electron donor for initial Fe–S cluster formation</td>
<td>1 known patient</td>
<td>Skeletal muscle</td>
<td>Mutation of ATG initiation codon to TTG</td>
<td>109</td>
</tr>
<tr>
<td>Multiple mitochondrial dysfunction syndrome, type 1</td>
<td>Fe–S cluster delivery to subset of Fe–S proteins, including LIAS</td>
<td>NFU</td>
<td>Nfu1</td>
<td>Intermediate scaffold</td>
<td>10 known patients</td>
<td>Many tissues affected; neonatal lactic acidosis and hyperglycinaemia</td>
<td>Missense and splice donor mutations</td>
<td>53,54</td>
</tr>
<tr>
<td>Multiple mitochondrial dysfunction syndrome, type 2</td>
<td>Fe–S cluster delivery to subset of Fe–S proteins, including LIAS</td>
<td>BOLA3</td>
<td>Aim1</td>
<td>Intermediate scaffold</td>
<td>3 known patients</td>
<td>Many tissues affected; neonatal lactic acidosis and hyperglycinaemia</td>
<td>Missense mutation or premature stop codon</td>
<td>53,55</td>
</tr>
<tr>
<td>Multiple mitochondrial dysfunction syndrome, type 3</td>
<td>Fe–S cluster delivery to LIAS and to respiratory chain complexes</td>
<td>ISAb7</td>
<td>Isb7</td>
<td>Delivery of Fe–S cluster to LIAS and to respiratory chain complexes</td>
<td>2 known patients</td>
<td>Many tissues affected; neonatal lactic acidosis and hyperglycinaemia</td>
<td>Missense mutation</td>
<td>52</td>
</tr>
<tr>
<td>Non-ketotic hyperglycinaemia and severe neurodegeneration</td>
<td>Fe–S cluster delivery to subset of Fe–S proteins, including LIAS</td>
<td>BOLA3</td>
<td>Aim1</td>
<td>Intermediate scaffold</td>
<td>3 known patients</td>
<td>Central nervous system, heart and eye</td>
<td>Premature stop codon</td>
<td>56</td>
</tr>
<tr>
<td>Non-ketotic hyperglycinaemia and severe neurodegeneration</td>
<td>Fe–S cluster delivery</td>
<td>GLRX5</td>
<td>Grx5</td>
<td>Intermediate scaffold</td>
<td>3 known patients</td>
<td>Spinal cord and optic nerves</td>
<td>Premature stop codons</td>
<td>56</td>
</tr>
<tr>
<td>Sideroblastic anaemia</td>
<td>Fe–S cluster delivery</td>
<td>GLRX5</td>
<td>Grx5</td>
<td>Intermediate scaffold</td>
<td>1 known patient</td>
<td>Erythroid cells</td>
<td>Splicing mutation</td>
<td>79</td>
</tr>
<tr>
<td>X-linked sideroblastic anaemia with cerebellar ataxia</td>
<td>Export of unknown substrate from mitochondrial matrix to cytosolic and nuclear compartments</td>
<td>ABCB7</td>
<td>Atm1</td>
<td>Exporter of Fe–S cluster building block or regulatory signal</td>
<td>23 known patients</td>
<td>Central nervous system and erythroid cells</td>
<td>Missense mutations in or near transmembrane domains of exporter</td>
<td>110, 111</td>
</tr>
</tbody>
</table>

ABCB7, ABC transporter B family member 7; Aim1, altered inheritance of mitochondria 1; Atm1, ABC transporter of the mitochondrion 1; BOLA3, BolA-like 3; FDX2, ferredoxin 2; Fe–S, iron–sulphur; GLRX5, glutaredoxin-related protein 5; Grx5, monothiol glutaredoxin 5; LIAS, lipoic acid synthase.
Box 2 | The difficulty with identifying Fe–S proteins

The identification and recognition of iron–sulphur (Fe–S) proteins, which were discovered in 1960 (REFS 88,89), have lagged behind the study of many other metal cofactors, partly because Fe–S proteins often lack a distinctive visible colour. By contrast, haemoglobin, which has the distinctive red colour of the haem moiety, was described in 1940 (REF. 90) and structurally analysed in 1960 (REF. 91). Another group of metal cofactor proteins that drew early attention were the blue copper proteins, including ceruloplasmin (first described in 1944)92, which is bright blue because it contains copper bound to a Cys residue and two His residues in a distorted geometry. The unusual proximity of Cys to copper promotes movement of an electron from Cys to an orbital in copper, driven by visible light absorption in a process that imparts visible colour to many metalloproteins93. In addition to lacking a distinctive colour, Fe–S proteins are often sensitive to oxygen, and Fe–S clusters are frequently degraded during standard purifications. These characteristics enabled Fe–S proteins to evade attention for the most part until 1960, when experimental advances in electron paramagnetic resonance (EPR) techniques showed that proteins such as succinate dehydrogenase have magnetic properties and therefore contain metals94,95. In the mid-1960s, using Mössbauer spectroscopy96 and iron and sulphur assays, researchers realized that some iron proteins contain multiple interacting iron atoms97. Techniques that can definitively identify Fe–S clusters, such as EPR spectroscopy98 and Mössbauer spectroscopy99, are difficult to use with mammalian proteins because they require large amounts of purified protein, and purifications often require anaerobic conditions100. We propose that screening primary peptide sequences for the Leu–Tyr–Arg (LYR) motif101 will facilitate the discovery of candidate Fe–S proteins.

Hydrophobic residues in the carboxyl terminus of HscB (Jac1 in yeast and HSC20 in humans) bind to IscU in bacteria102 (or its homologues in yeast103 and humans104). HSC20 is a member of the J class of proteins, which contain the tripeptide His-Pro-Asp in their amino termini that activates the ATPase activity of the cognate chaperone (for example, HSPA9). ATPase activity probably drives a conformational change of the complex that displaces the nascent Fe–S cluster from the IscU scaffold to recipient proteins105,106,107. This proposed sequence of events is suggested in the yeast model system by the fact that the co-chaperone Jac1 (HscB in bacteria and HSC20 in humans) competes with Nfs1 (IscS in bacteria and NFS1 in humans) to bind to Isu (IscU in bacteria, and ISCU1 and ISCU2 in humans)108. The displaced Fe–S–Isu–Jac1 complex can then recruit the cognate chaperone Ssq1 (REF. 42) (HscA in bacteria and HSPA9 in humans) to form an Isc–chaperone–co-chaperone complex. The chaperone that is involved in all species discussed has a nucleotide-binding domain and a substrate-binding domain, which binds to a peptide from ISCU that contains the essential sequence Pro-Val-Lys (PVK)109,110 (FIG. 3).

Transfer of the Fe–S cluster to a recipient protein

Until recently, few molecular details were known about how Fe–S clusters are transferred to specific client proteins in cells. As Fe–S clusters are known to move readily between proteins that contain permissive binding sites (usually accessible combinations of four Cys residues) when mixed in vitro111, flaws in experimental design can lead to incorrect assignments of donor–recipient relationships. One way to minimize such mistakes is to identify direct physical interactions that occur between Fe–S proteins in living cells, although this method is also subject to limitations that are dependent on the transience of the interaction and other factors, such as the requirement for carrier molecules. When components of the complexes involved in Fe–S cluster assembly and transfer have been identified and biochemically analysed in cells, further analyses can be completed in reconstituted systems using purified proteins in vitro, in which kinetics and stoichiometries can be studied using complex analytical and spectroscopic techniques112, such as those used to analyse the Fe–S protein lipoyl acid synthase (LIAS)113.

Formation of an Fe–S transfer complex

On dissociation from the IscS (NFS1 in humans) Cys desulphurase complex, the Fe–S–bearing IscU scaffold is protected by the formation of a new complex with the co-chaperone–chaperone pair HscA–HscB in bacteria114 (Ssq1–Jac1 in yeast115 or HSPA9–HSC20 in humans116).
and eliminate its dependence on an IscS ligand. Part sulphur atoms apparently enables IscU to fully ligate the newly formed \([2\text{Fe}–2\text{S}]\) cluster of IscS ligate the nascent \([2\text{Fe}–2\text{S}]\) cluster. Ultimately, reorganization of the iron and sulphur atoms appears to ligate an \([2\text{Fe}–2\text{S}]\) cluster and eliminate its dependence on an IscS ligand. Part a adapted from REF. 10. Part b adapted from Marinoni, E. N. et al. (IscS-IscU), complex structures provide insights into Fe\(_S\) biogenesis and transfer. Angew. Chem. Int. Ed. Engl. 51, 5439–5442 (2012), John Wiley & Sons. Copyright © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 2 | Initial Fe–S cluster assembly in bacteria. a | Iron–sulphur (Fe–S) cluster assembly occurs on a complex composed of a Cys desulphurase (iron–sulphur cluster sulphide generator (IscS)) dimer to which a monomeric scaffold protein, IscU, binds at each carboxyl terminus of IscS. The Cys desulphurase, IscS, uses the pyridoxal phosphate (PLP) cofactor to extract sulphur from free Cys and transfer it to a highly reactive Cys residue of IscS to generate a persulphide (R–S–SH) group. An unstructured loop of IscS (shown here as a monomer) contains the highly reactive Cys328 residue (C328; yellow) that carries the sulphur-containing persulphide group (R–S–SH). The flexible C328-containing loop subsequently moves the persulphide over a distance of 14 Å from near the PLP cofactor to the vicinity of IscU to contribute sulphur to the nascent cluster forming on IscU (direction of movement indicated by the dashed arrow in the magnification box). Formation of a nascent \([2\text{Fe}–2\text{S}]\) cluster on IscU in an archaeal structure\(^\text{11}\) indicates that three Cys residues of IscU and one Cys residue from the flexible loop of IscS ligate the nascent \([2\text{Fe}–2\text{S}]\) cluster. Ultimate reorganization of the iron and sulphur atoms apparently enables IscU to fully ligate the newly formed \([2\text{Fe}–2\text{S}]\) cluster.

Interference from bulky neighbouring residues. Studies of proteins that contain the Lyr motif are ongoing, and the exact role of the motif in ISD11 of the initial Fe–S assembly complex is not yet known. Future studies will reveal whether most proteins that contain a permutation of the Lyr motif recruit an Fe–S cluster either to their primary peptide sequence or to a partner in a protein complex.

Studies of Fe–S cluster acquisition by the recipient protein SDHB, which acquires three Fe–S clusters, revealed that the motif closest to the N terminus, Ile–Tyr–Arg, facilitates insertion of the first cluster, a \([2\text{Fe}–2\text{S}]\) cluster ligated by four Cys residues. Insertion of additional Fe–S clusters probably occurs subsequently when the second motif, Lyr, engages the HSC20 transfer apparatus. A model that could explain how Fe–S clusters are inserted into nascent polypeptides is depicted in FIG. 4; the model suggests that Fe–S clusters are inserted as the primary peptide translocates into the mitochondrial matrix, which enables the Fe–S transfer complex to bind to unstructured peptide sequences. This probably allows the primary peptide sequence to envelop the Fe–S clusters, driven by the strong energetics of cysteinyl–iron bond formation.

The mature SDH complex is composed of four subunits, designated SDHA, SDHB, SDHC and SDHD in humans. SDHA and SDHB form a soluble complex that then associates with two membrane components of the complex, subunits SDHC and SDHD. Proper folding of the mature tetrameric form depends on the activity of SDH assembly factor 1 (SDHAF1) and SDHAF2. SDHAF1 also contains a Lyr motif that binds to HSC20 (REF. 47). In addition, SDHAF1 has an independent binding site for SDHB, which raises the possibility that this assembly factor facilitates maturation of the SDH complex by providing \([2\text{Fe}–2\text{S}]\) clusters to SDHB during assembly\(^\text{19}\). In addition to its \([2\text{Fe}–2\text{S}]\) cluster, SDHB also incorporates a \([4\text{Fe}–4\text{S}]\) cluster and a \([3\text{Fe}–4\text{S}]\) cluster. Therefore, it might be necessary for two \([2\text{Fe}–2\text{S}]\) clusters to coalesce on the recipient protein to generate the \([4\text{Fe}–4\text{S}]\) cluster, and it is possible that this occurs when two transfer complexes are bound simultaneously by the Lyr motif in SDHB and the Lyr motif in SDHAF1. Importantly, the Lyr motifs are highly conserved in SDH in bacteria and plants, which indicates that mechanisms for generating specificity in the acquisition of Fe–S clusters evolved early in evolution and have been highly retained\(^\text{49}\).

Before these recent studies, the Lyr family of proteins had been identified through informatics (annotated as Conserved Domains accession number cl05087), but the role of these proteins was unknown\(^\text{49}\). It is possible that most of these annotated Lyr-motif proteins bind to either HSC20 or the full transfer complex to acquire Fe–S clusters or to facilitate the transfer of Fe–S clusters to a partner protein, although this requires further study. In addition to SDHB, a second Lyr motif protein involved in the assembly of complex III, Lyr motif-containing 7 (LyrM7), was identified in the initial yeast two-hybrid screen for HSC20-binding partners and was shown to bind HSC20 in vivo. Two more members of the Lyr motif family, LyrM3 and LyrM6, are subunits of the Fe–S-rich mitochondrial respiratory chain complex I\(^\text{49}\). These observations suggest that Lyr-motif proteins may be crucial for the proper insertion of Fe–S clusters in respiratory complexes I–III and that the other Lyr-motif proteins might also participate in Fe–S cluster transfer processes.

Indirect transfer through intermediate donors. The co-chaperone HSC20 also binds to glutaredoxin 5, perhaps through a Lys-rich motif at its C terminus\(^\text{50}\), which is important because glutaredoxin 5 is thought to form an Fe–S-containing cluster that distributes Fe–S clusters to downstream recipient proteins\(^\text{49}\). Other potential intermediate Fe–S cluster carriers include IscA\(^\text{48}\), IBA57 (REFS 51,52), NFU1 (REFS 53,54) and BolA-like 3 (BOLA3)\(^\text{55,56}\). NFU1, IBA57 and BOLA3 seem to be particularly important for activity of the Fe–S cluster-containing enzyme LIAS, which generates the flexible and mobile lipoyl group required for activity of
multisubunit enzymes, including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched-chain amino acid degradation and glycine-cleavage complexes.

In many cases, the conclusion that an intermediate carrier protein for Fe–S clusters has specific downstream recipients is based on broad inferences, as the effects on putative recipients may not be direct, and many experimental manipulations can alter the activity of Fe–S proteins indirectly by interfering with general mitochondrial function. Another pitfall is to assign a protein as an intermediate Fe–S cluster carrier when an Fe–S protein or protein complex is adversely affected by its dysfunction. For example, in plants, the deletion of iron–sulphur or protein complex is adversely affected by its dysfunction. Another pitfall is to assign a protein as an Fe–S ligand indirectly by interfering with general mitochondrial function. Another pitfall is to assign a protein as an Fe–S ligand indirectly by interfering with general mitochondrial function.

In summary, it seems that all Fe–S clusters are formed initially by the Cys desulphurase activity of the ISCU scaffold complex, but recipient proteins may acquire their Fe–S clusters directly from the ISCU–chaperone–co-chaperone complex or indirectly from intermediate carriers, which probably acquire their initial clusters from the ISCU transfer complex but then deliver these clusters to only the subset of Fe–S proteins with which they specifically interact. Whereas the early steps in biogenesis of Fe–S proteins are nearly universally shared, later steps may confer great specificity, thereby preventing the transfer of Fe–S cofactors to potential acceptor proteins that should not have an Fe–S cluster, such as zinc-finger proteins, which contain Cys residues that could potentially function as Fe–S ligands.

Cytosolic and nuclear assembly of Fe–S proteins

Fe–S clusters are highly prone to oxidative degradation because molecular oxygen can readily capture unpaired electrons from transition metals such as iron, whereas it cannot seize pairs of electrons from most organic molecules. As a result of their vulnerability, Fe–S clusters are highly prone to oxidative degradation because molecular oxygen can readily capture unpaired electrons from transition metals such as iron, whereas it cannot seize pairs of electrons from most organic molecules. Therefore, it is thought that intact Fe–S clusters are unlikely to be exported from the mitochondria for use in the cytosolic and nuclear compartments in eukaryotic cells, although small-molecular-weight complexes in which Fe–S clusters are ligated by the tripeptide glutathione have been suggested as candidate complexes for mitochondrial export. There are two main potential pathways by which Fe–S protein biogenesis could occur in the cytosolic and nuclear compartments. One pathway is based on the notion that Fe–S cluster assembly in the cytosolic and nuclear compartments incorporates an export product of the mitochondrial Fe–S cluster biogenesis machinery for use in initial Fe–S cluster assembly. In the yeast model system, the initial assembly proteins for Fe–S clusters were found...
Co-translational acquisition of Fe–S clusters

Figure 4 | A model to explain how Fe–S clusters can be correctly positioned deep within proteins. Experiments have demonstrated that the Leu-Tyr-Arg (LYR) motif of the iron–sulphur (Fe–S) cluster recipient protein succinate dehydrogenase subunit B (SDHB) is a binding site for HSC20, which forms a complex with holo-ISCU (containing the intact [2Fe–2S] cluster) and the chaperone, HSPA9 (REF 19). On hydrolysis of ATP, the nascent Fe–S cluster may directly transfer from ISCU into a neighbouring region of the bound SDHB polypeptide. In this scenario, cysteiny1 ligands in the primary peptide sequence can rapidly tether and enshroud the Fe–S cluster. Moreover, formation of strong cysteiny1–iron bonds with the Fe–S cluster can drive conformational changes. a | In co-translational acquisition of Fe–S clusters, initial translocation of the SDHB polypeptide into the mitochondrial matrix exposes the Ile-Tyr-Arg (IYR) motif near the amino terminus to which the Fe–S transfer complex binds. As translocation continues, the Fe–S transfer complex may donate a [2Fe–2S] cluster that ligates to Cys residues (C) in the N terminus and thereby drives folding. A second LYR sequence can again engage the Fe–S transfer complex on entry into the matrix to aid acquisition of the second of three clusters. Ultimately, the Fe–S cluster can be deeply buried within the folded secondary structure of the protein, where it is relatively inaccessible to oxidants and toxins. b | Fe–S clusters could also be incorporated during translation of polypeptides in the cytosol. This is the probable mechanism in bacteria, which do not have mitochondria. These hypothetical models address the complex question of how Fe–S proteins acquire and correctly position their Fe–S clusters deeply within fully folded proteins.

Recently, this pathway is postulated to depend on export of a special form of sulphur from mitochondria, which is transferred to a tetrameric scaffold composed of two members of the P-loop NTPase family — cytosolic Fe–S cluster-deficient 1 (Cfd1; NUBP2 also known as CFD1) in humans) and nucleotide-binding protein 35 (Nbp35; NUBP1 also known as NBP35) in humans) — which ligate bridging [4Fe–4S] clusters. These clusters obtain electrons from Tah18 (NADPH-dependent diflavin oxidoreductase 1 (NDOR1) in humans) and Dre2 (CIAPIN1 also known as anamorsin) in humans), and are transferred to nuclear architecture-related 1 (Nar1; IOP1 also known as NARFL) in humans), which is thought to mediate interactions between early and late steps of the CIA transfer machinery. Nar1 is then proposed to transfer its Fe–S clusters to targeting complexes composed of Cia1 (also known as Asf1), Fam96b (also known as Cia2B and methyl methanesulphonate-sensitivity 19 (Mms19)), which in turn transfer Fe–S clusters to proteins involved in DNA replication, repair and glycosylation, as well as chromosomal segregation and telomeric stability. In another proposed branch of the pathway, the mammalian homologue of Nar1, IOP1, is thought to transfer its Fe–S clusters to FAM96A (also known as CIA2A), which then transfers its Fe–S clusters to the mammalian iron regulatory protein 1 (IRP1), which is a regulator of cytosolic iron homeostasis.

The CIA pathway is one potential mechanism by which Fe–S clusters can be donated to cytosolic and nuclear proteins, but many of its conclusions are based on the assumption that proteins of the initial Fe–S assembly complex are absent in the mammalian cytosolic and nuclear compartments. However, there is little doubt that NFS1 is present in the nucleus of yeast and as well as in the cytosolic and nuclear compartments of mammalian cells, where it modifies tRNAs, contributes to formation of the molybdnenum cofactor, and may participate in de novo Fe–S cluster assembly. Mammalian NFS1 is more highly expressed in mitochondria, but it is detectably expressed in the nucleus and cytosol. Moreover, a cytosolic form of the major human scaffold protein ISCU forms a functional complex with cytosolic NFS1. More recently, the eukaryotic NFS1 partner ISD11 was found in both the mitochondrial matrix and the nucleus of mammalian cells. HSC20 is also present in the cytosolic and nuclear compartments, and HSPA9 has been detected in most compartments of mammalian cells. Thus, it is entirely possible that a second pathway for the biogenesis of cytosolic and nuclear Fe–S proteins in mammalian cells involves de novo synthesis of Fe–S clusters in these compartments.

Among the most important newly discovered recipients of an Fe–S cluster is the cytosolic and nuclear protein MMS19, which facilitates the transfer of Fe–S clusters to numerous proteins of DNA metabolism. The transfer of Fe–S clusters from MMS19 to multiple DNA replication and repair proteins, which was discovered through the identification of binding partners by mass spectrometry, greatly expands the number of known mammalian Fe–S proteins. The role of Fe–S clusters was once thought to be purely structural in bacterial
Box 3 | Are Fe–S cluster components exported from mitochondria?

Early studies in Saccharomyces cerevisiae used cells that were genetically manipulated to lack ABC transporter of the mitochondrial 1 (Atm1; ABC transporter B family member 7 (ABCB7) in humans); these yeast developed mitochondrial iron overload and had low levels of activity of the cytosolic iron-sulphur (Fe–S) protein Leu1, which is part of the Leu biosynthetic pathway of yeast. However, selective inactivation of Atm1 was achieved by using a plasmid that re-introduced functional Leu2 into a Leu2-null strain. It is known that when Leu biosynthesis is low owing to defects in either Leu1 or Leu2, the regulatory gene Leu3 upregulates the transcription of genes encoding both Leu1 and Leu2 to correct for Leu deficiency. Thus, transcript levels of Leu1 were extremely high in the Leu2-deficient strain that expressed normal Atm1 (REF. 98), and Leu1 enzymatic activity was accordingly high. Higher Leu1 activity in the Atm1-sufficient cells was interpreted to mean that Atm1 exports the Fe–S cluster that is incorporated into cytosolic Leu1. However, when yeast strains were re-examined with matching Leu2 genes, Leu1 activity was not influenced by Atm1 overexpression or depletion. Thus, the indirect evidence that suggested that Atm1 exports Fe–S clusters from mitochondria should be analysed with caution. The idea that initial Fe–S cluster assembly cannot proceed without the presence of mitochondria has gradually been modified to the suggestion that Fe–S cluster synthesis cannot proceed without the export of a special type of sulphur from mitochondria. Despite the fact that crystal structures of Atm1 from yeast and the bacterium Novosphingobium aromaticivorans were recently solved, it remains unclear what this transporter exports. A glutaredoxin complex has emerged as a potential candidate, as have heavy metals such as silver and mercury in the bacterial system. As loss of ABCB7 results in mitochondrial iron overload in humans, it is possible that the exported molecule is generated by intact Fe–S cluster synthesis and is involved in the regulation of mitochondrial iron homeostasis.

Fe–S cluster biogenesis and human disease

Given the established roles of Fe–S proteins in the essential processes of mitochondrial respiration and maintenance of DNA integrity (BOX 1), impairment of the proteins involved in Fe–S cluster biogenesis would be likely to cause death or disease in humans. Indeed, ten human diseases are now attributed to defects in Fe–S cluster biogenesis. For proteins involved in the first crucial assembly steps of a nascent Fe–S cluster, diseases tend to result from markedly reduced activity of the protein rather than from complete loss of function, which would probably be lethal. In the first identified disease, Friedreich’s ataxia, it is still unclear what role the poorly expressed frataxin protein has in Fe–S cluster assembly, and it is not known why the cerebellums, dorsal root ganglia and hearts of patients are much more severely affected than other tissues. In ISCU myopathy, skeletal muscles are much more adversely affected than other tissues by a splicing defect that markedly reduces ISCU expression, perhaps because abnormal splicing is more pronounced in skeletal muscles and because there are smaller reserves of functional ISCU in skeletal muscle (the bursts of oxidative stress associated with skeletal muscle work quickly degrade the Fe–S clusters of ISCU). In multiple mitochondrial dysfunction syndromes 1–3 (caused by mutation of the intermediate Fe–S cluster carriers NFU1, BOLA3 and IBA57, respectively), enzyme complexes that depend on the acquisition of a flexible lipoyl group have reduced function as a result of LIAS not acquiring its two [4Fe–4S] clusters. The functions of respiratory chain complexes are also adversely affected in patients with these syndromes, which indicates that NFU1, BOLA3 and IBA57 may have additional recipient proteins. Loss of function of the putative intermediate Fe–S cluster carrier glutaredoxin-related protein 5 (GLRX5) can apparently manifest as several distinct diseases, perhaps depending on how much residual function is present. It is possible that GLRX5 acquires its Fe–S cluster directly from the HSC20 transfer complex. Other diseases (TABLE 1) have not yet contributed to the elucidation of the various roles of proteins involved in mediating Fe–S cluster transfer to client proteins, although it is clear that loss of NFU1 (REF. 80) prevents Fe–S cluster transfer to mitochondrial aconitase. Malfunction of many of the Fe–S cluster assembly proteins results in mitochondrial iron overload (reviewed in REF. 7). For example, in Friedreich’s ataxia, ISCU myopathy, X-linked sideroblastic anaemia with ataxia, and sideroblastic anaemia with glutaredoxin 5 deficiency (TABLE 1). Interestingly, none of the CIA proteins has been identified as a human disease-associated gene, whereas mutations in NFU1, ISD11, ISCU, FDX2, NFU1, BOLA3, GLRX5 and IBA57 cause human diseases that present with a wide range of phenotypes. Advances in genetics and sequencing should lead to the identification of other diseases that can be attributed to problems with Fe–S cluster biogenesis, and perhaps to a better understanding of pathways of Fe–S cluster transfer in mitochondria and in the cytosolic and nuclear compartments.

Summary and conclusions

Fe–S clusters are unique cofactors that enable associated proteins to carry out redox reactions and that facilitate complex chemical transformations. In addition, Fe–S proteins are involved in the regulation of mammalian iron homeostasis, and Fe–S cofactors are thought to be involved in sensing DNA damage. The biogenesis mechanism of Fe–S clusters is highly evolutionarily conserved.
In eukaryotes, the Cys desulphurase (NFS1 in humans) requires an additional binding partner (ISD11), and frataxin probably activates Fe–S cluster synthesis on the scaffold protein ISCU. A co-chaperone binds to and displaces ISCU from the initial assembly complex, and it recruits a chaperone and Fe–S recipient proteins into a complex. The co-chaperone, HSC20, binds to the active motif in Fe–S recipient proteins or their partners. The discovery of the role of the LYR motif in engaging the Fe–S transfer complex has the potential to revolutionize the identification of potential Fe–S recipient proteins. There are probably many more mammalian Fe–S proteins that have not yet been recognized because of the instability of Fe–S clusters and the previous lack of identifying sequence elements. The key to how Fe–S clusters become buried in proteins may depend on co-translational (in the cytosol) or co-translational (in the mitochondrial matrix) binding of the HSC20 complex to LYR motifs of the primary peptide sequence and on transfer of the nascent cluster to accessible cysteiny l antigens. Accessory factors identified as being important in the assembly of respiratory chain complexes may contribute directly to Fe–S cluster assembly and ligation. Understanding the regulation of Fe–S cluster biogenesis is an important research frontier. Furthermore, understanding the role of the newly discovered Fe–S clusters in various proteins, such as DNA replication and repair proteins, holds promise for revolutionizing our understanding of DNA metabolism and numerous metabolic pathways of mammalian cells.


