Building Fe–S proteins: bacterial strategies

Béatrice Py and Frédéric Barras

Abstract | The broad range of cellular activities carried out by Fe–S proteins means that they have a central role in the life of most organisms. At the interface between biology and chemistry, studies of bacterial Fe–S protein biogenesis have taken advantage of the specific approaches of each field and have begun to reveal the molecular mechanisms involved. The multiprotein systems that are required to build Fe–S proteins have been identified, but the *in vivo* roles of some of the components remain to be clarified. The way in which cellular Fe–S cluster trafficking pathways are organized remains a key issue for future studies.

The abundance of iron and sulphur in the prebiotic Earth, together with the structural versatility and chemical reactivity of Fe–S clusters, facilitated the selection of the Fe–S cluster as a cofactor for many proteins, allowing these proteins to acquire new functions. It is therefore no surprise that proteins containing Fe–S clusters (referred to as Fe–S proteins) are present in all living organisms and have prominent roles in multiple important cellular processes, including respiration, central metabolism, gene regulation, RNA modification, and DNA repair and replication[1–3] (FIG. 1). Over the past 15 years, the mechanisms by which Fe–S clusters are built and inserted into apoproteins have been the subject of intense investigations in several organisms, including the model organisms *Escherichia coli*, *Saccharomyces cerevisiae* and *Arabidopsis thaliana* as well as *Azotobacter vinelandii*, *Erwinia chrysanthemi*, *Salmonella enterica*, *Synechocystis* sp. PCC 6803, *Synechococcus* spp. and humans[4–10].

Fe–S clusters endow proteins with diverse biochemical abilities, including: electron transfer, because Fe–S clusters can access various redox states; redox catalysis, because Fe–S clusters can reach very low redox potentials and thereby reduce redox-resistant substrates; non-redox catalysis, because Fe–S clusters allow small compounds to bind to accessible ferric sites with extensive Lewis acid properties; and the ability to regulate gene expression, because the reversible interconversion of Fe–S clusters makes them exquisite sensors of several redox- or iron-related stresses[1–3].

In general, Fe–S clusters arise as rhombic, [2Fe–2S], or cubic, [4Fe–4S], types (FIG. 1). Iron can change oxidation states from Fe^{2+} to Fe^{3+}, whereas sulphur is always present in the S^{2-} oxidation state. The recruitment of Fe–S clusters by biological macromolecules proved to be highly valuable in the development of life. However, the increase in oxygen after the emergence of photosynthesis created a threat to Fe–S proteins and, consequently, to the organisms relying on them[11]. In particular, Fe–S clusters in dehydratases can react directly with univalent oxidants, leading to inactivation of the dehydratase and concomitant loss of Fe^{2+} (REFS 12–16). This loss can fuel Fenton chemistry, which produces highly toxic reactive oxygen species (ROS) (FIG. 2). This process constitutes the basis of the deleterious effects of univalent oxidants such as H_2O_2 towards DNA and other macromolecules[16–18]. Similarly, the toxic effects of some bactericidal antibiotics have been attributed to ROS-mediated destabilization of Fe–S clusters in dehydratases[19]. This underscores the dual nature of the role of this class of Fe–S clusters, which are essential for the functioning of key metabolic enzymes but create an 'enemy within' situation.

Organisms have developed multicomponent systems that promote the biogenesis of Fe–S proteins while protecting the cellular surroundings from the potentially deleterious effects of free Fe^{2+}, Fe^{3+} and S^{2-} ions[4–10]. The advent of whole-genome sequencing revealed that such systems are widespread and highly conserved in Bacteria and Eukarya, although the situation in Archaea necessitates a thorough investigation of archaeal genome content. Subsequent genetic investigations in model organisms found several components that are particularly important for organisms to safely exploit Fe–S cluster-based biology in the presence of environmental stress. Several excellent recent reviews have covered the mechanistic and physiological aspects of Fe–S cluster biogenesis[4–10]. Here, we discuss the basic requirements for a bacterial cell to build and insert Fe–S clusters into apoproteins and summarize our current knowledge and understanding of this process *in vivo*.

Laboratoire de Chimie Bactérienne, UPR-CNRS 9043, Institut de Microbiologie de la Méditerranée, Aix-Marseille Université, 31 Chemin Joseph Aiguier, 13009 Marseille, France.
Correspondence to F.B.
e-mail: barras@ifr88.cnrs-mrs.fr
doi:10.1038/nrmicro2356

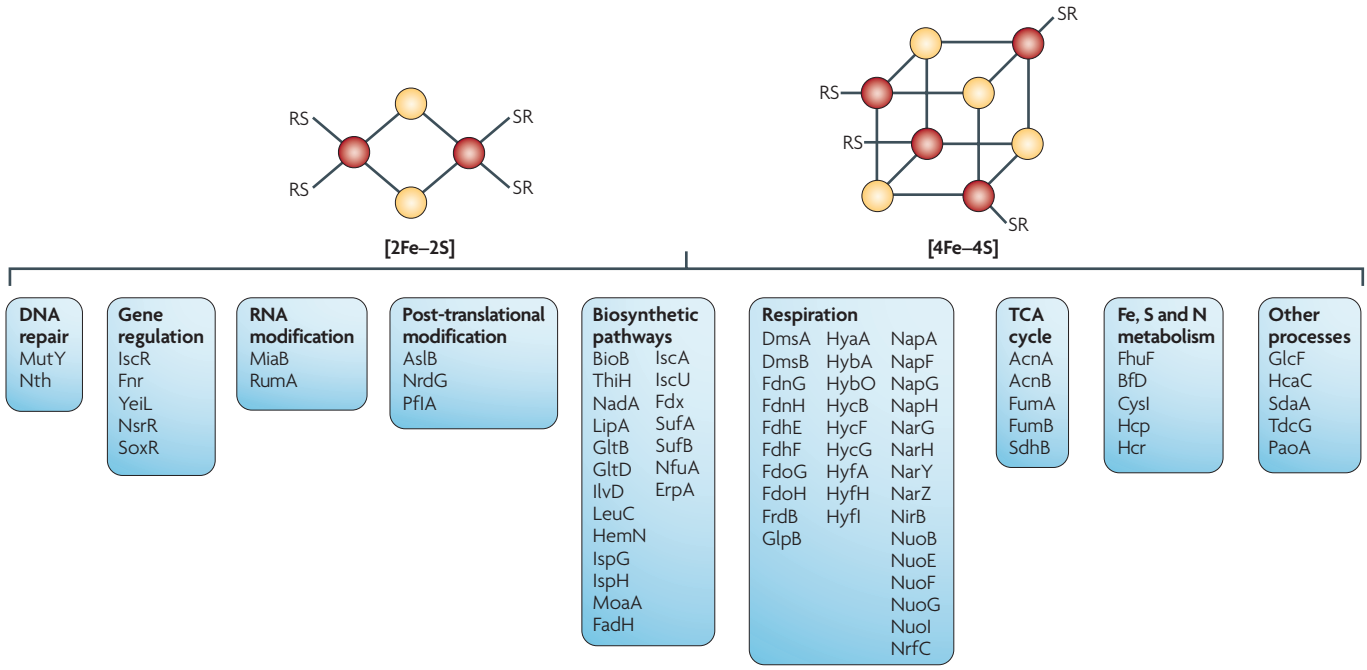


Figure 1 | **Fe–S clusters: structure and function.** In most cases, the iron (red) of Fe–S proteins is bound to the sulphur atom from cysteine residues in the peptide backbone (SR) and to inorganic sulphur in the prosthetic group (yellow). Residues such as histidine, arginine, aspartate and serine can also serve as the iron ligand. More complex clusters comprising additional metals can be found in nitrogenases or hydrogenases[1,2]. In *Escherichia coli*, nearly 80 Fe–S proteins have been described and are listed in the figure; numerous putative Fe–S-proteins have also been identified and are listed in Supplementary information S2 (box). TCA, tricarboxylic acid cycle.

Fe–S cluster biogenesis systems

A search for nitrogenase maturation factors in *A. vinelandii* led to the discovery of the nitrogen fixation (*nif*) operon and, subsequently, the Fe–S cluster (*isc*) operon[20,21]. Components of the Isc system are present in *E. coli*, *S. cerevisiae* and humans, supporting the idea that this system is ubiquitous. In *E. coli*, the *isc* genes form an operon that encodes, in this order, a regulator (IscR), a cysteine desulphurase (IscS), a scaffold (IscU), an A-type protein (IscA), a DnaJ-like co-chaperone (HscB), a DnaK-like chaperone (HscA) and a ferredoxin (Fdx). Subsequent studies in *E. coli* and *E. chrysanthemi* identified another operon related to Fe–S cluster biogenesis, components of which are also found in chloroplasts and cyanobacteria[9,10,22–25]. In *E. coli*, the sulphur mobilization (*suf*) operon encodes an A-type protein (SufA), a heterodimeric cysteine desulphurase (composed of SufS and SufE) and a pseudo-ABC (ATP-binding cassette)-transporter (composed of SufB, SufC and SufD) that could act as a scaffold. Current knowledge on each of these components and their interaction is detailed below.

Bacterial genome analyses revealed that the number and type of these operons varies from one microorganism to another. For example, *A. vinelandii* contains the *nif* and *isc* operons, *Helicobacter pylori* has only a simplified *nif* operon, *E. coli* encodes the *isc* and *suf* operons, and *E. chrysanthemi* has the *nif*, *isc* and *suf* operons (a multiplicity that seems to allow this plant pathogen to infect several different plant hosts)[23,26–30]. The components encoded by each operon also vary. For example, *Mycobacterium tuberculosis* and *Bacillus subtilis* have atypical *suf* operons that contain a combination of *suf*-like and *iscU*-like genes[31]. It is expected that the increasing availability of genome sequence information will provide other examples of such mix-and-match systems, blurring the clear separation between the Isc, Suf and even Nif systems. Moreover, genome sequence data have identified new Fe–S cluster biogenesis factors that are encoded by isolated genes that do not belong to the *isc*, *suf* or *nif* operons. Interestingly, most of these genes are involved in the delivery of Fe–S clusters to apoprotein targets, indicating that an important part of Fe–S cluster biogenesis *in vivo* is the trafficking of ready-made Fe–S clusters from their site of formation to the site of delivery. Fe–S cluster biogenesis comprises two major steps: assembly and traffic. Each of these steps relies on a variable number of modules, the networking of which can differ in complexity as a function of environmental conditions or target specificity (FIG. 3).

The source of the elements

The source of sulphur. The sulphur atoms in Fe–S clusters are sourced from L-cysteine, as a result of the action of cysteine desulphurases[32]. These pyridoxal-phosphate (PLP)-dependent enzymes degrade L-cysteine into L-alanine and sequester the released sulphur atom, in the form of a persulphide, on a specific cysteine residue (FIG. 4). This provides an excellent mechanism for making sulphur atoms available without releasing them in solution. The *A. vinelandii* NifS enzyme was the first cysteine desulphurase to be discovered and seems to be specific for nitrogenase maturation[33]. *E. coli* synthesizes three cysteine desulphurases, IscS, SufS and the cysteine sulphinate

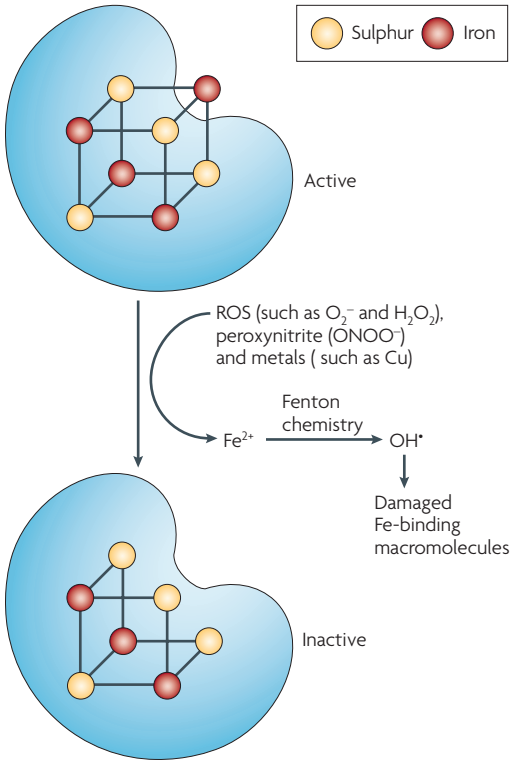


Figure 2 | **Fe–S proteins and oxidative stress.** Cluster degradation by univalent oxidants such as reactive oxygen species (ROS), peroxynitrite and copper converts the exposed $[4Fe–4S]^{2+}$ cluster of dehydratases to the unstable $[4Fe–4S]^{3+}$ oxidation state[10,15], which results in the release of Fe^{2+} and the formation of an inactive enzyme containing a $[3Fe–4S]^{1+}$ cluster. Further degradation of the cluster has been observed both *in vivo* and *in vitro*. Fe^{2+} fuels Fenton chemistry, which produces the highly reactive hydroxyl radical that reacts near the site of its formation and can oxidize molecules that bind Fe^{2+}, such as DNA. Modified, with permission, from REF. 16 © (2003) Annual Reviews.

desulphinase A (CsdA)[34–36]. The contribution of IscS and SufS to Fe–S biogenesis as part of the Isc and Suf systems is well established[21–25,28,29,36]. IscS also provides sulphur for pathways that are unrelated to Fe–S cluster biogenesis, such as thiamine or molybdopterin biosynthesis and tRNA modification pathways[32,37–40]. The activity of SufS is thought to be restricted to Fe–S cluster biogenesis, although its contribution to other pathways has not been thoroughly investigated, in particular under the stress conditions in which the Suf system is thought to operate[22,23,25,41–45]. CsdA also seems to have a dual role, as it can assist Fe–S cluster biogenesis when it is overproduced, but it may also participate in a sulphur transfer pathway that is unrelated to Fe–S cluster biogenesis[46,47]. IscS directly transfers the sulphane sulphur (S°) to the scaffold component (see below), and SufS and CsdA transfer it to the sulphur acceptors SufE and CsdE (also known as YgdK), respectively[48–54].

The source of iron. The identification of the iron source used to build Fe–S clusters has lagged behind the identification of the sulphur source. A clear constraint is that iron ions must be provided to the system through a tightly controlled, shielded pathway that prevents any leakage of harmful free iron. A close interaction between the iron donor and the components of the Fe–S cluster biogenesis system is therefore expected. In this respect, the CyaY protein is a likely iron donor, as it interacts with the sulphur donor IscS and the scaffold protein IscU[55–57]. The eukaryotic homologue of CyaY, frataxin, has been the subject of multiple studies, as frataxin dysfunction is responsible for Friedreich's ataxia, a neurodegenerative disease, and perturbs mitochondrial iron homeostasis[58]. CyaY has a low affinity for iron, which it binds through an exposed carboxylate-rich region[59–61]. However, *E. coli* and *S. enterica cyaY* mutants show no drastic loss of Fe–S enzyme activities[62–64]. Moreover, a thorough structural and biochemical study led to the controversial proposal that CyaY should be considered not as an iron donor but rather as an iron-sensing regulator, because iron-loaded CyaY binds to IscS and prevents Fe–S cluster formation on IscU[56].

IscA, which will be discussed in greater detail below, has been proposed to act as an iron donor on the basis of *in vitro* studies[65–68]. However, the situation is opposite to that for CyaY, as IscA binds iron with high affinity but has not been shown to interact with cysteine desulphurases or scaffold proteins. Again, *in vivo* phenotypic analysis has failed to provide any strong evidence favouring a role for IcsA in cellular iron homeostasis[69].

It is difficult to think of the Fe–S cluster biogenesis systems having evolved in total independence of the so-called iron storage proteins, such as ferritins (FtnA and FtnB), bacterioferritin (Bfr) and the stress-response DNA binding protein (Dps). However, experimental evidence for the roles of these storage proteins remains scarce, and no unifying picture has emerged from other organisms[14,70–72].

Where Fe and S meet: the scaffold concept

The scaffold concept emerged from early studies on the acquisition of an Fe–S cluster-containing MoFe-protein cofactor by nitrogenase[73,74]. This concept has since been used in most examples of cofactor insertion during protein maturation. In the Fe–S cluster field, a scaffold protein is expected to: interact closely with a cysteine desulphurase and an iron source; provide a chemical and structural environment that facilitates the formation of Fe–S clusters; and transfer the Fe–S cluster to apoprotein targets. Below, we discuss IscU, for which the most convincing evidence of a scaffold function has been provided *in vivo* and *in vitro*[75,76].

IscU receives sulphur directly from IscS, and the IscU–IscS interaction has been demonstrated both *in vitro* and *in vivo*. When incubated with $FeCl_3$, L-cysteine and IscS *in vitro*, IscU forms and binds an Fe–S cluster in a sequential manner[77]. The process starts with a dimeric form of IscU containing one [2Fe–2S] cluster, then two [2Fe–2S] clusters and, finally, one [4Fe–4S] cluster[77] (FIG. 5). The [4Fe–4S] cluster-bound form can be converted back to a [2Fe–2S] cluster-bound form after exposure to O_2 (REF. 78). Whereas the one

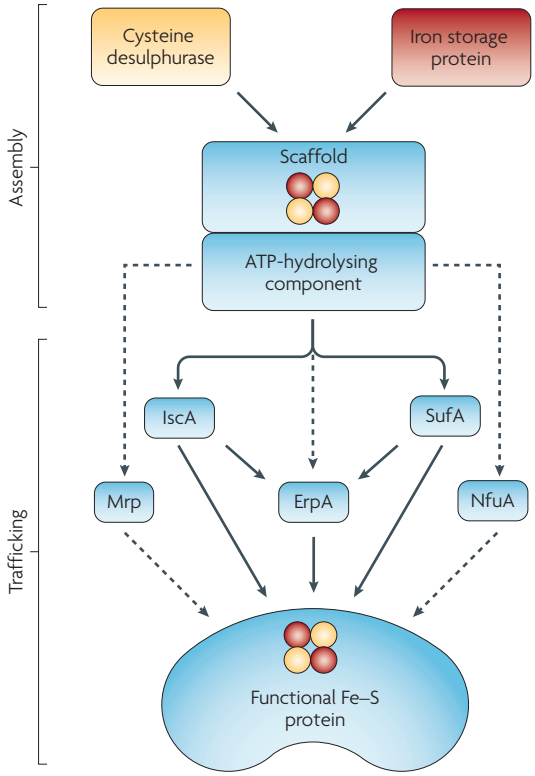


Figure 3 | **The main steps in the *Escherichia coli* Fe–S cluster biogenesis pathway.** Fe–S cluster biogenesis involves two main steps: first, the assembly of the Fe–S cluster on the scaffold protein, and second, the trafficking of the formed Fe–S cluster from the scaffold to the apoprotein target. In the first step, the scaffold protein receives the sulphur from the cysteine desulphurase and the iron from a donor protein, the identity of which is still under debate. Release of the Fe–S cluster from the scaffold involves an ATP-hydrolysing component. The Fe–S cluster is then trafficked by the Fe–S carrier proteins (in *Escherichia coli*, these are Fe–S cluster protein A (IscA), ErpA, sulphur mobilization protein A (SufA), Mrp and NfuA). The routes followed by the Fe–S cluster to reach its apoprotein target are expected to vary depending on the receiver apoprotein and on the environmental conditions influencing cellular demand. Dashed lines represent pathways that still need to be fully established *in vivo*.

[2Fe–2S] cluster-bound form is stable, the two [2Fe–2S] cluster-bound form seems to be a short-lived intermediate in the formation of the [4Fe–4S] cluster-bound form. This conversion can be achieved by reductive coupling using either dithionite or Fdx (the product of the last gene in the *isc* operon) as electron donors[78]. Together, these facts indicate that there is a dynamic equilibrium between the [2Fe–2S] and [4Fe–4S] cluster-bound forms of IscU (FIG. 5).

Sequence comparison and genetic, spectroscopic and structural analyses suggest that IscU residues Cys37, Cys63, His105 and Cys106 are necessary for its scaffold and coordination functions in the formation of Fe–S clusters[26,75,79]. Moreover, an invariant Asp residue (Asp39 in IscU from *A. vinelandii*) has been identified that seems to have an important role in cluster delivery. Indeed, an Asp39Ala mutant acts as a dominant-negative protein, because it forms a tight complex with IscS, leading to a drastic reduction in the activity of this cysteine desulphurase[26,76]. This observation fulfils, *in vivo*, one of the criteria of a bona fide scaffold: a close interaction with the cysteine desulphurase[75,76,79]. Also, the IscU Asp39Ala mutant contains a [2Fe–2S] cluster that it cannot transfer to apoprotein targets[80]. This shows that the binding of Fe–S clusters and their transfer to apoprotein targets are two distinct properties that can be separated genetically. Spectroscopic analysis concluded that Asp39 can act as a transient ligand and is needed to labilize the Fe–S cluster before it can be transferred to the apoprotein target[76,77,81].

The ATP-hydrolysing components

Results obtained in *H. pylori* showed that a sulphur donor and a scaffold are the only components required to build an Fe–S cluster[45]. However, the Isc and Suf systems include general ATP-hydrolysing components, such as DnaK-like and DnaJ-like chaperones (namely, HscA and HscB) and a pseudo-ABC-transporter (SufBCD). The contribution of these components to Fe–S cluster biogenesis is crucial, as indicated by analysis of mutant phenotypes, but their *in vivo* functions remain unclear[23,25,28,29,41,69]. A possible *raison d'être* of these ATP-hydrolysing components might be to allow cells to carry out Fe–S cluster biogenesis under unfavourable conditions.

The HscA and HscB chaperones. In *E. coli*, the IscU scaffold is the bona fide substrate of the HscBA chaperones, and a conserved motif, LPPVK, was identified in IcsU as the HscA recognition site[82]. In the reaction cycle, ATP binding and hydrolysis are coupled to the transient formation of a ternary HscB–HscA–IscU complex that eventually allows delivery of the IscU-bound Fe–S cluster to apoprotein targets[83,84] (FIG. 5). Interestingly, the addition of HscA or HscB does not enhance the transfer rate of the [4Fe–4S] cluster from IscU to aconitase A, raising the possibility that HscA and HscB are involved in the maturation of [2Fe–2S] proteins only[80]. In this context, it is worth noting that [2Fe–2S] proteins include the superoxide sensor SoxR, the Fe–S homeostasis sensor IscR and possibly the N_2O sensor, NsrR. Whether this putative connection between HscA, HscB and stress regulators points to the existence of a dedicated 'stress assembly line' will require *in vivo* analysis of the maturation pathways of these regulators.

SufBCD: a pseudo-ABC-transporter. The characteristics of SufC were puzzling at first, as it has the sequence hallmarks of the ATPase subunit of ABC transporters, but no potential membrane partner was predicted among the other Suf proteins[41]. Evidence from several biochemical and molecular analyses points to the existence of a cytosolic SufB–$SufC_2$–SufD complex[41,52,85]. Interestingly, crystallographic data revealed that the structural determinants of the SufC–SufD and SufC–SufB interactions are highly related to the classic interaction between the ATPase and the inner-membrane components in orthodox ABC transporters[86].

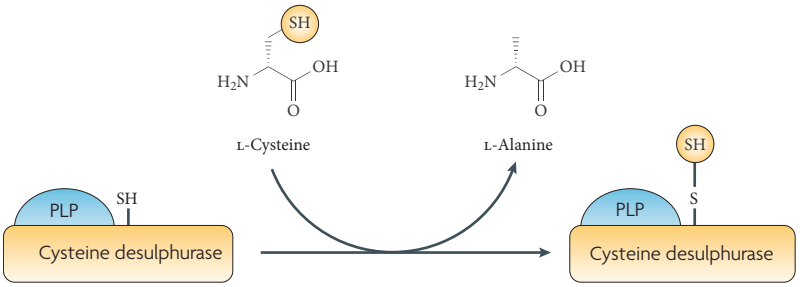


Figure 4 | **The source of sulphur.** The sulphur atom for Fe–S cluster biogenesis is provided by the pyridoxal-phosphate (PLP)-dependent cysteine desulphurase that catalyses the conversion of L-cysteine into L-alanine and sulphane sulphur. The active site contains a conserved catalytic cysteine residue (Cys328 in Fe–S cluster protein S (IscS), Cys364 in sulphur mobilization protein S (SufS), and Cys358 in cysteine sulphinate desulphinase A (CsdA) from *Escherichia coli*) to which the sulphur atom of the PLP-activated cysteine substrate is transferred to generate a persulphide (SSH)-intermediate form. The sulphur of IscS is transferred directly to the scaffold protein, whereas in the case of SufS and CsdA, a second protein-bound persulphide intermediate, on Cys51 of SufE and Cys62 of CsdE, is involved.

The SufBCD complex was proposed to channel sulphur or iron to a scaffold or apoprotein target under sulphur- or iron-poor conditions[25]. The fact that SufBCD activates the cysteine desulphurase activity of SufSE supports this hypothesis[52]. Moreover, recent *in vitro* reconstitution experiments showed that SufB receives sulphur from SufS, either directly or via SufE, and binds and transfers a [4Fe–4S] cluster to SufA, an A-type carrier (discussed below), or to aconitase[53,87] (M. Fontecave, personal communication). Therefore, the current view of the SufBCD complex is that it might be the Suf system's assembling centre for Fe–S clusters, with SufB as the scaffold. In keeping with the idea that the SufBCD complex has retained some transport-related properties, one can speculate that SufD might be involved in channelling iron from iron sources. However, the role of SufC as an ATPase remains unclear, as ATPase activity is essential for Fe–S cluster biogenesis *in vivo*, whereas it is not required for Fe–S cluster formation or for Fe–S cluster transfer *in vitro*[41,87,88]. However, by analogy with classical ABC transporters, one can predict that ATP binding and hydrolysis by SufC induces major conformational rearrangements in its partner, SufB, thereby triggering Fe–S cluster delivery from SufB to the downstream components of the Fe–S cluster biogenesis pathway.

Delivery of Fe–S clusters: the traffic step

In this section, we focus on a series of factors that are likely to operate downstream of the Fe–S cluster assembly step, in pathways that allow the delivery of Fe–S clusters to apoprotein targets.

The ATC proteins. Phylogenomic studies revealed that the A-type carriers (ATCs) IscA, SufA and ErpA emerged in the last common ancestor of bacteria, are conserved in most bacteria and were acquired by eukaryotes and a few archaea through horizontal gene transfer[89]. On the basis of *in vitro* studies, ATCs were initially proposed to act as scaffolds, because they bind Fe–S clusters and eventually transfer them to a wide range of [2Fe–2S] protein and [4Fe–4S] protein targets[90–92]. But, ATCs do not interact with cysteine desulphurases, and this disqualifies them from being scaffold proteins. ATCs were also proposed to act as iron chaperones (see above). However, ATCs could be purified in a form containing Fe–S clusters [93,94], thus the most widely accepted view is that ATC proteins carry ready-made Fe–S clusters from the scaffold to the apoprotein targets. Moreover, the fact that Fe–S clusters can be transferred from IscU to IscA, whereas the reverse is not possible, supports the view that IscA acts downstream of IscU as an intermediate Fe–S cluster carrier[95].

Most organisms synthesize more than one ATC. Genetic studies in *E. coli* showed how this is exploited by the cell to select the most appropriate Fe–S cluster delivery pathway depending on the cellular demand for Fe–S clusters, which in turn depends on the growth conditions[89]. *E. coli* encodes three ATCs (IscA, ErpA and SufA), which are biochemically interchangeable but not fully redundant *in vivo*[89,96,97]. For example, an *erpA* mutation is lethal under aerobic conditions but not under anaerobic conditions when *iscA* is present[89,92]. Also, a double *iscA sufA* mutant is lethal under aerobic conditions but not under anaerobic conditions owing to the presence of *erpA*[89]. This redundancy can be controlled by genetic regulation, as a constitutive mutation in the *sufA* promoter that results in *sufA* expression at levels that are similar to those seen under stress conditions compensates for a lack of *iscA* and *erpA*. The efficiency of the partnerships between ATCs and other components of the Fe–S cluster assembly and delivery pathways also varies as a function of the growth conditions. Under anaerobiosis, Fe–S clusters are thought to traffic from the IscU scaffold to either ErpA or IscA and, eventually, to the target apoprotein, whereas under aerobic conditions Fe–S clusters are expected to traffic from IscU to IscA and then ErpA; under stress conditions, Fe–S clusters would traffic through the SufBCD–SufA route. A similiar conditional phenotype has been reported in *A. vinelandii*, in which an *iscA* mutation is lethal at high oxygen concentration[26].

It should be noted that these conclusions were obtained by studying the maturation of the IspG and IspH proteins that are required for the synthesis of isopentenyl diphosphate[89]. Analysis of a different target apoprotein might uncover different trafficking pathways. For example, under aerobiosis, the maturation of the [4Fe–4S] protein fumarate and nitrate reduction regulatory protein (Fnr) seems to be under the control of IscA only, whereas the [2Fe–2S] proteins Fdx and ferric-iron-reductase protein (FhuF) require only ErpA[96,97].

NfuA: module recruitment. NfuA is necessary for adaptation to oxidative stress and iron starvation in *E. coli* and for aconitase maturation in *A. vinelandii*[98,99]. This protein is a fusion between a degenerate ATC domain and an Nfu domain. The amino-terminal domain is degenerate, because its sequence is similar to that of an ATC but does not possess the three cysteine residues that are probably used as ligands for Fe–S clusters. The carboxy-terminal Nfu domain is found either alone (as in *Synechococcus* sp. PCC7002, in which it is essential and can assemble a [4Fe–4S]

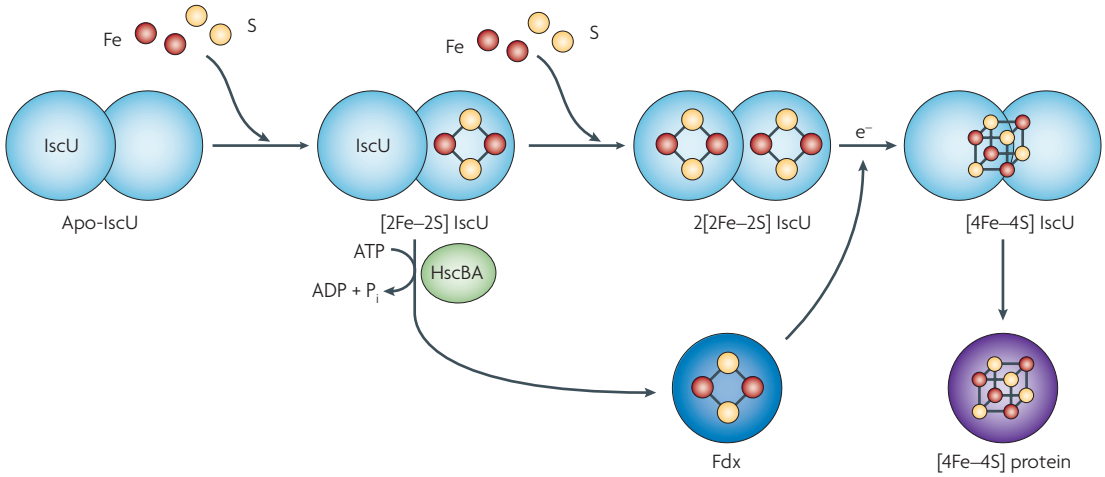


Figure 5 | **Model for Fe–S cluster assembly on Fe–S cluster protein U (IscU) and subsequent transfer to apoproteins.** Fe–S cluster formation on Fe–S cluster protein U (IscU) requires the sequential formation of IscU forms containing one [2Fe–2S] cluster per dimer, two [2Fe–2S] clusters per dimer and, finally, one [4Fe–4S] cluster per dimer. In this model, the ATP-hydrolysing component HscBA (comprising the DnaJ-like co-chaperone (HscB) and the DnaK-like chaperone (HscA)) is used only for maturation of [2Fe–2S] cluster-containing proteins, such as ferredoxin (Fdx) and the stress transcriptional regulators IscR and SoxR. Fdx-dependent formation of the [4Fe–4S] cluster-containing form of IscU is specifically involved in the maturation of [4Fe–4S] proteins, which include most of the known Fe–S proteins in *Escherichia coli*. Apo, apoprotein; P_i, inorganic phosphate.

cluster onto a component of photosystem I *in vitro*) or as part of multi-domain proteins (as in the paradigmatic NifU scaffold)[100–102]. Deletion analyses indicate that both domains are required for *in vivo* NfuA activity, and the importance of the cysteine residues in the carboxy terminus was established by site-directed mutagenesis[98,99]. One possible evolutionary scenario is that NfuA arose from two events: a fusion between two Fe–S-binding proteins — an ATC and an Nfu-like protein — and the concomitant loss of the functionally essential cysteine residues in the ATC domain. Preliminary investigations support the hypothesis that the degenerate ATC domain allows NfuA to be targeted to specific proteins and the Nfu domain allows the transfer of Fe–S clusters to these targets. Together with the importance of *nfuA* under stress conditions, this suggests that NfuA is an Fe–S carrier protein that is involved in the maturation of a specific subset of proteins under stress conditions.

ApbC: a carrier with ATPase activity. The *S. enterica* ApbC protein (the *E. coli* homologue of which is Mrp) belongs to the ATPase ParA-like family, the members of which contain a Walker A motif with two lysine residues instead of one (GKXXXKG(S/T))[103,104]. The bacterial ApbC-like proteins share sequence similarity with several eukaryotic proteins that have well-documented roles in Fe–S cluster biogenesis[7]. ApbC has ATPase activity, and it binds and transfers a Fe–S cluster. ATPase activity is required for its function *in vivo*, but ATP hydrolysis is not necessary for Fe–S cluster binding and transfer *in vitro*. Data suggest that the function of ApbC might be partly redundant with that of IscU[105]. However, this does not fit with data obtained for the yeast mitochondrial homologue of ApbC, which was shown to act downstream of the IscU-like Isu1 scaffold[106], and so, for now, it seems prudent to view ApbC proteins as a new class of ATPase Fe–S cluster transporters rather than as new scaffold proteins.

Repair of damaged Fe–S clusters

Fe–S proteins can be damaged by oxidative stress, and the observed reversibility of such damage *in vivo* is consistent with the existence of repair systems[12,14,69]. A likely candidate for a repair function is the YtfE protein, which is a member of the RIC (repair of iron centre) family, along with a group of related proteins[107,108]. YtfE is important for the virulence of the human pathogen *Haemophilus influenzae*[109]. The *E. coli ytfE* mutant exhibits decreased aconitase, fumarase and 6-phosphogluconate dehydratase activities[110]. Recovery of the fumarase activity can be achieved by the addition of purified YtfE, and a scavenging role for YtfE has been ruled out[110]. Consistent with a role for YtfE in repair, expression of this protein is induced by NO treatment, and the cognate mutant is sensitive to hydrogen peroxide stress[110,111]. Biochemical and spectroscopic characterization of the RIC proteins indicate that they are iron-binding proteins containing a non-haem dinuclear iron centre[112,113]. Thus, RIC proteins are proposed to recruit and integrate the Fe^{2+} needed for the repair of [3Fe–4S] clusters found in proteins that have been damaged by exposure to either H_2O_2 or NO. Interestingly, a role in Fe–S cluster repair was shown for the iron storage proteins bacterioferritin (Bfr), FtnA and FtnB[14,70–72]; the repair role of FtnB has been linked to virulence in *S. enterica*[71]. Another candidate for an Fe–S cluster repair or protection function is YggX, but conclusive evidence is still required[63,64,114].

Fe–S clusters and genetic regulation

Owing to their redox-sensing properties, Fe–S clusters have been recruited by a series of transcriptional regulators, such as Fnr (an O_2 sensor), SoxR (which responds to superoxide stress) and NsrR (which responds to nitrosative stress) in *E. coli*[3,115]. IscR was thought to be dedicated to the regulation of genes and operons involved in Fe–S cluster biogenesis, but it has recently emerged as a pleiotropic regulator.

IscR is the product of the first gene in the *isc* operon[116]. In the Fe–S cluster-bound (or 'mature') form it represses the expression of the *iscRSUA–hscBA–fdx* operon, and as an apoprotein, without the Fe–S cluster, it activates the *suf* operon[43,44,116,117] (BOX 1). Thus, IscR senses the capacity of the cell to produce mature Fe–S proteins: low levels of mature IscR reflect a reduced efficiency of Fe–S cluster biogenesis, and this is corrected by synthesizing both Isc and Suf proteins. However, transcriptional profiling studies have considerably broadened our understanding of the role of IscR in cellular physiology[117]. In addition to regulating the synthesis of several factors involved in Fe–S cluster biogenesis (namely, the Isc and Suf systems as well as ErpA and NfuA), IscR regulates the synthesis of Fe–S cluster-containing anaerobic complexes such as periplasmic hydrogenase 1 (Hyd1; encoded by the *hya* operon), periplasmic hydrogenase 2 (Hyd2; encoded by the *hyb* operon) and periplasmic nitrate reductase[117]. Thus, IscR seems to be able to control the synthesis of both the producers and the recipients of Fe–S clusters, thereby balancing the Fe–S cluster biosynthesis capacity with demand. Furthermore, IscR controls the expression of genes involved in various cellular processes, such as adaptation to oxidative stress (*soxS* and the superoxide dismutase gene *sodA*), C_4-dicarboxylate transport (*dctA*), arginine biosynthesis (the acetylornithine deacetylase gene, *argE*) and surface structure formation (the type 1 fimbriae regulatory gene, *fimE*, the *fimAICDFGH* operon and *flu*)[117,118]. The connection between the last three cellular processes and Fe–S cluster biogenesis remains to be established.

The mechanism of action of IscR is highly versatile. Based on DNase I footprinting of six IscR-regulated promoters, two IscR-binding sites have been inferred: a 25-base-pair type 1 site (ATASYYGACTRWWWYAGTCRRSTAT) and a 26-base-pair type 2 site (AWARCCCYTSNGTTTGMN GKKKTKWA)[117] (where S = G or C, Y = C or T, R = A or G, W = A or T, M = A or C, and K = T or G). The type 1 site is found in the *iscR* promoter, the activity of which is repressed by mature IscR. Type 2 sites are found in the promoters of *sufA* and the *hya* operon[117,119]. A detailed analysis of the type 2 site of the *hya* promoter revealed that, surprisingly, both holo-form and apo-form IscR bind specifically to this site[119]. This challenges the simple view that a conversion from holo-form IscR to apo-form IscR triggers the IscR-mediated regulation of type 2 promoters. In *Synechocystis* sp. PCC 6803, an IscR-like regulator, SufR, controls expression of the *suf* operon[120]. In this context, it will be of great interest to see how far *E. coli* IscR can serve as a model for SufR and, in particular, whether SufR is endowed with a similar pleiotropic role in *Synechocystis* spp. gene expression.

Perspectives

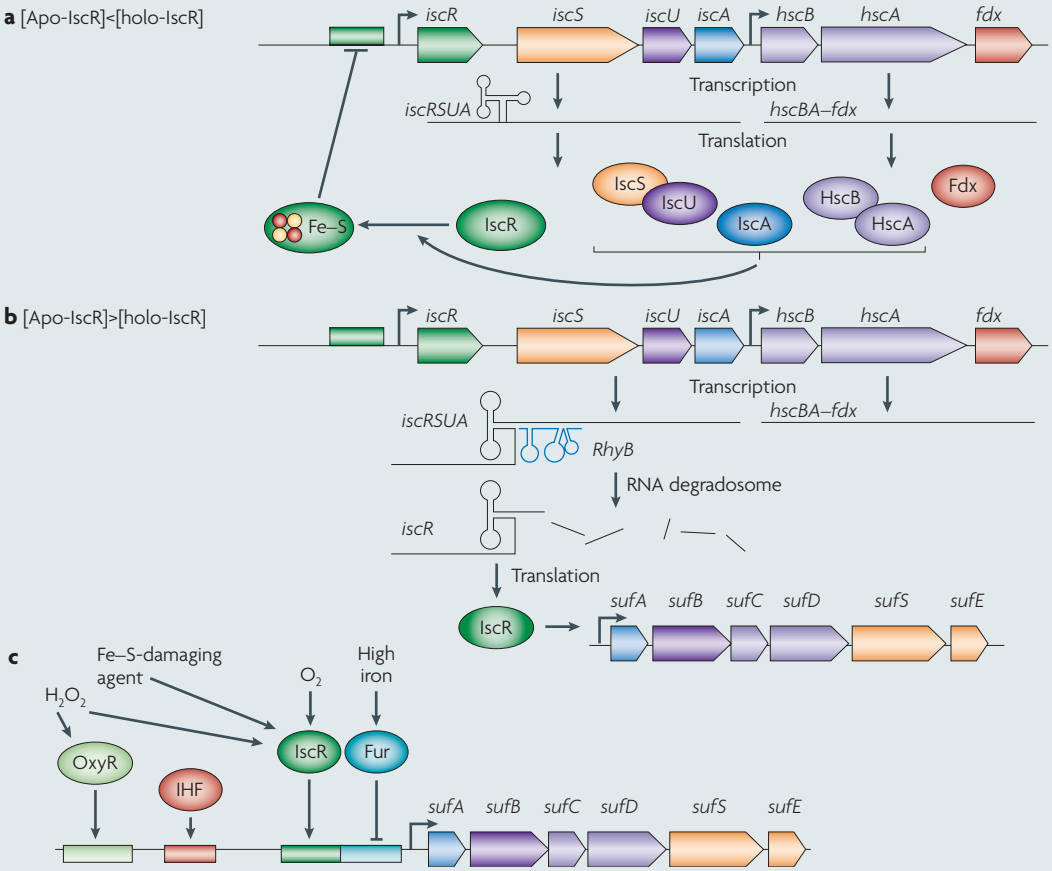
During the past decade, the combination of chemistry, structural biology, biochemistry, biophysics and genetics has greatly extended our understanding of Fe–S cluster biogenesis in cells, and we now know that this process involves two main steps.

The first step, the assembly of the Fe–S clusters, is carried out by multiprotein complexes, including cysteine desulphurases, scaffold proteins and, possibly, ATP-hydrolysing components. However, Fe–S clusters can be assembled *in vitro* by mixing components that are unlikely to work together *in vivo*. Therefore, the specificity of the interactions between these components must be investigated *in vivo*. In addition, the role of ATP and the use of the energy that is delivered by its hydrolysis are still far from understood, at least in the Suf system. However, the most important issue for researchers currently working on Fe–S cluster biogenesis is to identify the iron source and the way in which iron is brought to the assembly machinery. In this context, it was recently reported that the SufBCD complex contains $FADH_2$, which allows the complex to reduce ferric ion *in vitro* (M. Fontecave, personal communication). The hypothesis that SufBCD uses $FADH_2$ as a redox cofactor for mobilizing iron during assembly of its own cluster will be an exciting issue to investigate.

The second step, the trafficking of Fe–S clusters, remains a considerable challenge to untangle, as the delivery of Fe–S clusters to apoprotein targets seems to involve a large number of isolated components with similar biochemical properties and potential functional redundancy. Recent results revealed that both IspG and IspH could be matured by alternative pathways as a function of variations in environmental conditions[89]. But will such plasticity be true for all apoprotein targets? Will certain traffic-associated components be of particular importance under specific growth conditions? And what will the influence be of the apoprotein target itself? All of these questions will have to be tackled by introducing perturbations to the network, either by genetic methods or by analysing the maturation of a given target under a series of different conditions. Systems biology approaches might also be of great help in understanding the dynamics of protein circuits in Fe–S cluster biogenesis. Furthermore, once all of the components — and their conditions of action — have been identified, a new avenue of research must investigate whether Fe–S cluster insertion *in vivo* arises co- or post-translationally. One might expect that maturation of proteins with buried Fe–S clusters follows different rules from those with surface-exposed clusters.

Global proteomic and genetic approaches in *E. coli* have identified potential new candidates involved in Fe–S cluster metabolism[121–123]. Some are anticipated to participate directly in Fe–S cluster biogenesis, including GrxD, a monothiol glutaredoxin for which a role in Fe–S cluster biogenesis has already been documented in eukaryotes[7,123,124], and FdhD, which was found to interact with IscS and, therefore, might be a specific maturation factor for the Fe–S cluster-containing formate dehydrogenase

Box 1 | **Regulation of the Fe–S cluster (*isc*) and sulphur mobilization (*suf*) operons**



Expression of both the Fe–S cluster (*isc*) and sulphur mobilization (*suf*) operons is regulated by oxidative stress and iron limitation. However, the molecular mechanisms involved are different, as the *isc* operon is regulated by changes in Fe–S cluster homeostasis, whereas the *suf* operon is regulated by external stress situations. It is important to emphasise, however, that both operons are co-regulated by the same transcription factor, IscR.

Regulation of the *isc* operon is shown (see the figure, part **a**). The apoprotein form of IscR (apo-IscR) is matured by the Isc pathway and, in its holoform (holo-IscR), it represses expression from the *iscR* promoter[116,117]. Most studies on the regulation of the genes encoding the Isc pathway have focused on expression of the *iscRSUA* operon. Little information is available about the regulation of the genes located downstream of the *iscRSUA* operon. The DnaK-like chaperone (*hscA*), DnaJ-like co-chaperone (*hscB*) and ferredoxin (*fdx*) genes might be transcribed separately from the *iscRSUA* genes, from promoters upstream of *hscA* and *hscB*[127,128]. Interestingly, expression of *hscA* is induced by cold shock[128].

Regulation of the *isc* operon in low-iron conditions is also illustrated (see the figure, part **b**). It was recently demonstrated that the small regulatory RNA *RhyB*, which is regulated by ferric-uptake-regulation protein (Fur), causes differential degradation of the polycistronic *iscRSUA* mRNA[129]. Under low-iron conditions, *RhyB* is expressed and pairs with the 5′ untranslated region of *iscS*. This promotes recruitment of the RNA degradosome and degradation of the mRNAs encoding *iscS*, *iscU* and *iscA*, whereas the strong secondary structure at the 3′ end of *iscR* protects this transcript from degradation. Expression of *RhyB* leads to increased expression at the *iscR* promoter owing to a defect in the maturation of IscR that is probably caused by the unbalanced ratio of IscR and other Isc components.

Regulation of the *sufABCDSE* operon (see the figure, part **c**) is controlled by IscR and the global regulators OxyR, which senses H_2O_2, and Fur, which senses iron availability[23,25,42–44,117]. Induction of expression at the *sufA* promoter by OxyR requires integration host factor (IHF)-mediated DNA bending, as the OxyR-binding site (between positions –236 and –196 from the transcription start site) lies at an atypically large distance from the RNA polymerase-binding sites. IscR activates the expression of the *suf* genes by binding directly to the type 2 IscR-binding site, which is found between positions –56 and –35 from the transcription start site, close to the initiation complex. The Fur-binding site is centred between the –10 and –35 promoter elements.

complex[121,122,125]. Equally interesting are *ygfZ* and *folB*, which encode proteins that are related to folate; the inactivation of these genes yielded aggravating phenotypes when combined with *isc* mutations[123,126]. Further analysis might lead to the discovery of an unexpected link between folate and Fe–S cluster metabolisms.

Finally, the distribution and conservation of the factors involved in Fe–S cluster biogenesis in all living organisms (see Supplementary information S1 (table)) makes these proteins suitable for phylogenomic investigations. Several phylogenomic questions are of interest, such as the history of individual Fe–S cluster biogenesis

factors, their co-evolution and their recruitment into 'machineries'. Searching the genomes of non-model organisms might also assist in identifying new genes and strategies for building Fe–S proteins. Phylogenomic analysis might be a way to assess the theory that, since the appearance of oxygen, Fe–S clusters have been either selected against or managed by dedicated machineries. One prediction is that machineries such as the Isc or Suf systems began to be selected by organisms when the Earth's atmosphere became aerobic.

1. Beinert, H. Iron-sulfur proteins: ancient structures, still full of surprises. *J. Biol. Inorg. Chem.* **5**, 2–15 (2000). **A review from a pioneer in the Fe–S protein field.**
2. Fontecave, M. Iron-sulfur clusters: ever-expanding roles. *Nature Chem. Biol.* **2**, 171–174 (2006).
3. Kiley, P. J. & Beinert, H. The role of Fe-S proteins in sensing and regulation in bacteria. *Curr. Opin. Microbiol.* **6**, 181–185 (2003).
4. Ayala-Castro, C., Saini, A. & Outten, F. W. Fe-S cluster assembly pathways in bacteria. *Microbiol. Mol. Biol. Rev.* **72**, 110–125 (2008).
5. Fontecave, M., Py, B., Ollagnier de Choudens, S. & Barras, F. From iron and cysteine to iron-sulfur clusters: the biogenesis protein machineries. *Ecosal* [online] http://www.ecosal.org/index.php?option=com_content&view=article&id=520&catid=43&Itemid=139 (2008).
6. Xu, X. M. & Møller, S. G. Iron-sulfur cluster biogenesis systems and their crosstalk. *Chembiochem*. **9**, 2355–2362 (2008).
7. Lill, R. & Mühlenhoff, U. Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. *Annu. Rev. Biochem.* **77**, 669–700 (2008).
8. Rouault, T. A. & Tong, W. H. Iron-sulfur cluster biogenesis and human disease. *Trends Genet.* **24**, 398–407 (2008).
9. Kessler, D. & Papenbrock, J. Iron-sulfur cluster biosynthesis in photosynthetic organisms. *Photosynth. Res.* **86**, 391–407 (2005).
10. Balk, J. & Lobréaux, S. Biogenesis of iron-sulfur proteins in plants. *Trends Plant. Sci.* **10**, 324–331 (2005).
11. Imlay, J. A. Iron-sulphur clusters and the problem with oxygen. *Mol. Microbiol.* **59**, 1073–1082 (2006). **A perspective on the use of Fe–S proteins in relation to the evolution of Earth's atmosphere.**
12. Kuo, C. F., Mashino, T. & Fridovich, I. α,β-Dihydroxyisovalerate dehydratase. A superoxide-sensitive enzyme. *J. Biol. Chem.* **262**, 4724–4727 (1987). **A seminal paper on the relationship between oxidative stress and Fe–S proteins.**
13. Flint, D. H., Tuminello, J. F. & Emptage, M. H. The inactivation of Fe-S cluster containing hydrolyases by superoxide. *J. Biol. Chem.* **268**, 22369–22376 (1993).
14. Keyer, K. & Imlay, J. A. Inactivation of dehydratase [4Fe-4S] clusters and disruption of iron homeostasis upon cell exposure to peroxynitrite. *J. Biol. Chem.* **272**, 27652–27659 (1997).
15. Jang, S. & Imlay, J. A. Micromolar intracellular hydrogen peroxide disrupts metabolism by damaging iron-sulfur enzymes. *J. Biol. Chem.* **282**, 929–937 (2007).
16. Imlay, J. A. Pathways of oxidative damage. *Annu. Rev. Microbiol.* **57**, 395–418 (2003).
17. Mello Filho, A. C., Hoffmann, M. E. & Meneghini, R. Cell killing and DNA damage by hydrogen peroxide are mediated by intracellular iron. *Biochem. J.* **218**, 273–275 (1984).
18. Imlay, J. A., Chin, S. M. & Linn, S. Toxic DNA damage by hydrogen peroxide through the Fenton reaction *in vivo* and *in vitro*. *Science* **240**, 640–642 (1988).
19. Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A. & Collins, J. J. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**, 797–810 (2007).
20. Jacobson, M. R. *et al.* Biochemical and genetic analysis of the *nifUSVWZM* cluster from *Azotobacter vinelandii*. *Mol. Gen. Genet.* **219**, 49–57 (1989). **A landmark article signalling the start of the research into bacterial Fe–S cluster biogenesis.**
21. Zheng, L., Cash, V. L., Flint, D. H. & Dean, D. R. Assembly of iron-sulfur clusters. Identification of an *iscSUA-hscBA-fdx* gene cluster from *Azotobacter vinelandii*. *J. Biol. Chem.* **273**, 13264–13272 (1998). **This paper reports the identification of the Isc system.**
22. Patzer, S. I. & Hantke, K. SufS is a NifS-like protein, and SufD is necessary for stability of the [2Fe-2S] FhuF protein in *Escherichia coli*. *J. Bacteriol.* **181**, 3307–3309 (1999).
23. Nachin, L., El Hassouni, M., Loiseau, L., Expert, D. & Barras, F. SoxR-dependent response to oxidative stress and virulence of *Erwinia chrysanthemi*: the key role of SufC, an orphan ABC ATPase. *Mol. Microbiol.* **39**, 960–972 (2001).
24. Takahashi, Y. & Tokumoto, U. A third bacterial system for the assembly of iron-sulfur clusters with homologs in archaea and plastids. *J. Biol. Chem.* **277**, 28380–28383 (2002). **This paper reports the identification of the Suf system.**
25. Outten, F. W., Djaman, O. & Storz, G. A *suf* operon requirement for Fe-S cluster assembly during iron starvation in *Escherichia coli*. *Mol. Microbiol.* **52**, 861–872 (2004).
26. Johnson, D. C., Unciuleac, M. C. & Dean, D. R. Controlled expression and functional analysis of iron-sulfur cluster biosynthetic components within *Azotobacter vinelandii*. *J. Bacteriol.* **188**, 7551–7561 (2006).
27. Olson, J. W., Agar, J. N., Johnson, M. K. & Maier, R. J. Characterization of the NifU and NifS Fe-S cluster formation proteins essential for viability in *Helicobacter pylori*. *Biochemistry* **39**, 16213–16219 (2000).
28. Takahashi, Y. & Nakamura, M. Functional assignment of the *ORF2-iscS-iscU-iscA-hscB-hscA-fdx-ORF3* gene cluster involved in the assembly of Fe-S clusters in *Escherichia coli*. *J. Biochem.* **126**, 917–926 (1999).
29. Tokumoto, U. & Takahashi, Y. Genetic analysis of the *isc* operon in *Escherichia coli* involved in the biogenesis of cellular iron-sulfur proteins. *J. Biochem.* **130**, 63–71 (2001).
30. Rincon-Enriquez, G., Crêté, P., Barras, F. & Py, B. Biogenesis of Fe/S proteins and pathogenicity: IscR plays a key role in allowing *Erwinia chrysanthemi* to adapt to hostile conditions. *Mol. Microbiol.* **67**, 1257–1273 (2008).
31. Huet, G., Daffé, M. & Saves, I. Identification of the *Mycobacterium tuberculosis* SUF machinery as the exclusive mycobacterial system of [Fe-S] cluster assembly: evidence for its implication in the pathogen's survival. *J. Bacteriol.* **187**, 6137–6146 (2005).
32. Mihara, H. & Esaki, N. Bacterial cysteine desulfurases: their function and mechanisms. *Appl. Microbiol. Biotechnol.* **60**, 12–23 (2002).
33. Zheng, L., White, R. H., Cash, V. L., Jack, R. F. & Dean, D. R. Cysteine desulfurase activity indicates a role for NIFS in metallocluster biosynthesis. *Proc. Natl Acad. Sci. USA* **90**, 2754–2758 (1993). **The first identification of the source and the mechanism of sulphur mobilization for Fe–S cluster formation.**
34. Flint, D. H. *Escherichia coli* contains a protein that is homologous in function and N-terminal sequence to the protein encoded by the *nifS* gene of *Azotobacter vinelandii* and that can participate in the synthesis of the Fe-S cluster of dihydroxy-acid dehydratase. *J. Biol. Chem.* **271**, 16068–16074 (1996).
35. Mihara, H., Kurihara, T., Yoshimura, T. & Esaki, N. Kinetic and mutational studies of three NifS homologs from *Escherichia coli*: mechanistic difference between L-cysteine desulfurase and L-selenocysteine lyase reactions. *J. Biochem.* **127**, 559–567 (2000).
36. Schwartz, C. J., Djaman, O., Imlay, J. A. & Kiley, P. J. The cysteine desulfurase, IscS, has a major role in *in vivo* Fe-S cluster formation in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **97**, 9009–9014 (2000).
37. Mueller, E. G. Trafficking in persulfides: delivering sulfur in biosynthetic pathways. *Nature Chem. Biol.* **2**, 185–194 (2006).
38. Ikeuchi, Y., Shigi, N., Kato, J., Nishimura, A. & Suzuki, T. Mechanistic insights into sulfur relay by multiple sulfur mediators involved in thiouridine biosynthesis at tRNA wobble positions. *Mol. Cell* **21**, 97–108 (2006).
39. Nilsson, K, Lundgren, H. K., Hagervall, T. G. & Björk, G. R. The cysteine desulfurase IscS is required for synthesis of all five thiolated nucleosides present in tRNA from *Salmonella enterica* serovar typhimurium. *J. Bacteriol.* **184**, 6830–6835 (2002).
40. Lauhon, C. T. Requirement for IscS in biosynthesis of all thionucleosides in *Escherichia coli*. *J. Bacteriol.* **184**, 6820–6829 (2002).
41. Nachin, L., Loiseau, L., Expert, D. & Barras, F. SufC: an unorthodox cytoplasmic ABC/ATPase required for [Fe-S] biogenesis under oxidative stress. *EMBO J.* **22**, 427–437 (2003).
42. Lee, J. H., Yeo, W. S. & Roe, J. H. Induction of the *sufA* operon encoding Fe-S assembly proteins by superoxide generators and hydrogen peroxide: involvement of OxyR, IHF and an unidentified oxidant-responsive factor. *Mol. Microbiol.* **51**, 1745–1755 (2004).
43. Yeo, W. S., Lee, J. H., Lee, K. C. & Roe, J. H. IscR acts as an activator in response to oxidative stress for the *suf* operon encoding Fe-S assembly proteins. *Mol. Microbiol.* **61**, 206–218 (2006).
44. Lee, K. C., Yeo, W. S. & Roe, J. H. Oxidant-responsive induction of the *suf* operon, encoding a Fe-S assembly system, through Fur and IscR in *Escherichia coli*. *J. Bacteriol.* **190**, 8244–8247 (2008).
45. Tokumoto, U., Kitamura, S., Fukuyama, K. & Takahashi, Y. Interchangeability and distinct properties of bacterial Fe-S cluster assembly systems: functional replacement of the *isc* and *suf* operons in *Escherichia coli* with the *nifSU*-like operon from *Helicobacter pylori*. *J. Biochem.* **136**, 199–209 (2004).
46. Loiseau, L. *et. al*. Analysis of the heteromeric CsdA-CsdE cysteine desulfurase, assisting Fe-S cluster biogenesis in *Escherichia coli*. *J. Biol. Chem.* **280**, 26760–26769 (2005).
47. Trotter, V. *et al*. The CsdA cysteine desulphurase promotes Fe/S biogenesis by recruiting Suf components and participates to a new sulphur transfer pathway by recruiting CsdL (ex-YgdL), a ubiquitin-modifying-like protein. *Mol. Microbiol.* **74**, 1527–1542 (2009).
48. Urbina, H. D., Silberg, J. J., Hoff, K. G. & Vickery, L. E. Transfer of sulfur from IscS to IscU during Fe/S cluster assembly. *J. Biol. Chem.* **276**, 44521–44526 (2001).
49. Smith, A. D. *et al*. Sulfur transfer from IscS to IscU: the first step in iron-sulfur cluster biosynthesis. *J. Am. Chem. Soc.* **123**, 11103–11104 (2001).
50. Loiseau, L., Ollagnier de Choudens, S., Nachin, L., Fontecave, M. & Barras, F. Biogenesis of Fe-S cluster by the bacterial Suf system: SufS and SufE form a new type of cysteine desulfurase. *J. Biol. Chem.* **278**, 38352–38359 (2003).
51. Ollagnier de Choudens, S. *et al*. Mechanistic studies of the SufS-SufE cysteine desulfurase: evidence for sulfur transfer from SufS to SufE. *FEBS Lett.* **555**, 263–267 (2003).
52. Outten, F. W., Wood, M. J., Munoz, F. M. & Storz, G. The SufE protein and the SufBCD complex enhance SufS cysteine desulfurase activity as part of a sulfur transfer pathway for Fe-S cluster assembly in *Escherichia coli*. *J. Biol. Chem.* **278**, 45713–45719 (2003).
53. Layer, G. *et al.* SufE transfers sulfur from SufS to SufB for iron-sulfur cluster assembly. *J. Biol. Chem.* **282**, 13342–13350 (2007). **The first evidence for the identification of SufBCD as the scaffold in the Suf system.**
54. Sendra, M., Ollagnier de Choudens, S., Lascoux, D., Sanakis, Y. & Fontecave, M. The SUF iron-sulfur cluster biosynthetic machinery: sulfur transfer from the SUFS-SUFE complex to SUFA. *FEBS Lett.* **581**, 1362–1368 (2007).
55. Layer, G., Ollagnier de Choudens, S., Sanakis, Y. & Fontecave, M. Iron-sulfur cluster biosynthesis: characterization of *Escherichia coli* CyaY as an iron donor for the assembly of [2Fe-2S] clusters in the scaffold IscU. *J. Biol. Chem.* **281**, 16256–16263 (2006).
56. Adinolfi, S. *et al.* Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. *Nature Struct. Mol. Biol.* **16**, 390–396 (2009).

57. Shi, R. *et al.* Structural basis for Fe–S cluster assembly and tRNA thiolation mediated by IscS protein–protein interactions. *PLoS Biol.* **8**, e1000354 (2010).
58. Pandolfo, M. & Pastore, A. The pathogenesis of *Friedreich ataxia* and the structure and function of frataxin. *J. Neurol.* **256**, 9–17 (2009).
59. Bou-Abdallah, F., Adinolfi, S., Pastore, A., Laue, T. M. & Chasteen, D. N. Iron binding and oxidation kinetics in frataxin CyaY of *Escherichia coli*. *J. Mol. Biol.* **341**, 605–615 (2004).
60. Pastore, C., Franzese, M., Sica, F., Temussi, P. & Pastore, A. Understanding the binding properties of an unusual metal-binding protein-a study of bacterial frataxin. *FEBS J.* **274**, 4199–4210 (2007).
61. Nair, M. *et al.* Solution structure of the bacterial frataxin ortholog, CyaY: mapping the iron binding sites. *Structure* **12**, 2037–2048 (2004).
62. Li, D. S., Ohshima, K., Jiralerspong, S., Bojanowski, M. W. & Pandolfo, M. Knock-out of the *cyaY* gene in *Escherichia coli* does not affect cellular iron content and sensitivity to oxidants. *FEBS Lett.* **456**, 13–16 (1999).
63. Vivas, E., Skovran, E. & Downs, D. M. *Salmonella enterica* strains lacking the frataxin homolog CyaY show defects in Fe-S cluster metabolism *in vivo*. *J. Bacteriol.* **188**, 1175–1179 (2006).
64. Thorgersen, M. P. & Downs, D. M. Oxidative stress and disruption of labile iron generate specific auxotrophic requirements in *Salmonella enterica*. *Microbiology* **155**, 295–304 (2009).
65. Ding, H. & Clark, R. J. Characterization of iron binding in IscA, an ancient iron-sulphur cluster assembly protein. *Biochem. J.* **379**, 433–440 (2004).
66. Ding, H., Harrison, K. & Lu, J. Thioredoxin reductase system mediates iron binding in IscA and iron delivery for the iron-sulfur cluster assembly in IscU. *J. Biol. Chem.* **280**, 30432–30437 (2005).
67. Yang, J., Bitoun, J. P. & Ding, H. Interplay of IscA and IscU in biogenesis of iron-sulfur clusters. *J. Biol. Chem.* **281**, 27956–27963 (2006).
68. Lu, J., Yang, J., Tan, G. & Ding, H. Complementary roles of SufA and IscA in the biogenesis of iron-sulfur clusters in *Escherichia coli*. *Biochem. J.* **409**, 535–543 (2008).
69. Djaman, O., Outten, F. W. & Imlay, J. A. Repair of oxidized iron-sulfur clusters in *Escherichia coli*. *J. Biol. Chem.* **279**, 44590–44599 (2004).
A rigorous testing of the hypothesis that an Fe–S cluster repair system exists.
70. Bitoun, J. P., Wu, G. & Ding, H. *Escherichia coli* FtnA acts as an iron buffer for re-assembly of iron-sulfur clusters in response to hydrogen peroxide stress. *Biometals* **21**, 693–703 (2008).
71. Velayudhan, J., Castor, M., Richardson, A., Main-Hester, K. L. & Fang, F. C. The role of ferritins in the physiology of *Salmonella enterica* sv. Typhimurium: a unique role for ferritin B in iron-sulphur cluster repair and virulence. *Mol. Microbiol.* **63**, 1495–1507 (2007).
72. Expert, D., Boughammoura, A. & Franza, T. Siderophore-controlled iron assimilation in the enterobacterium *Erwinia chrysanthemi*: evidence for the involvement of bacterioferritin and the Suf iron-sulfur cluster assembly machinery. *J. Biol. Chem.* **283**, 36564–36572 (2008).
73. Dean, D. R. & Brigle, K. E. *Azotobacter vinelandii nifD*- and *nifE*-encoded polypeptides share structural homology. *Proc. Natl Acad. Sci. USA* **82**, 5720–5723 (1985).
74. Goodwin, P. J. *et al.* The *Azotobacter vinelandii* NifEN complex contains two identical [4Fe-4S] clusters. *Biochemistry* **37**, 10420–10428 (1998).
75. Bandyopadhyay, S., Chandramouli, K. & Johnson, M. K. Iron-sulfur cluster biosynthesis. *Biochem. Soc. Trans.* **36**, 1112–1119 (2008).
76. Raulfs, E. C., O'Carroll, I. P., Dos Santos, P. C., Unciuleac, M. C. & Dean, D. R. *In vivo* iron-sulfur cluster formation. *Proc. Natl Acad. Sci. USA* **105**, 8591–8596 (2008).
A genetic analysis supporting the existence of the scaffold.
77. Agar, J. N. *et al.* IscU as a scaffold for iron-sulfur cluster biosynthesis: sequential assembly of [2Fe-2S] and [4Fe-4S] clusters in IscU. *Biochemistry* **39**, 7856–7862 (2000).
Spectroscopy-based evidence for the occurrence of multiple forms of IscU.
78. Chandramouli, K. *et al.* Formation and properties of [4Fe-4S] clusters on the IscU scaffold protein. *Biochemistry* **46**, 6804–6811 (2007).
79. Kato, *S. et al.* Cys-328 of IscS and Cys-63 of IscU are the sites of disulfide bridge formation in a covalently bound IscS/IscU complex: implications for the mechanism of iron-sulfur cluster assembly. *Proc. Natl Acad. Sci. USA* **99**, 5948–5952 (2002).
80. Unciuleac, M. C. *et al. In vitro* activation of apo-aconitase using a [4Fe-4S] cluster-loaded form of the IscU [Fe-S] cluster scaffolding protein. *Biochemistry* **46**, 6812–6821 (2007).
81. Shimomura, Y., Wada, K., Fukuyama, K. & Takahashi, Y. The asymmetric trimeric architecture of [2Fe-2S] IscU: implications for its scaffolding during iron-sulfur cluster biosynthesis. *J. Mol. Biol.* **383**, 133–143 (2008).
82. Vickery, L. E. & Cupp-Vickery, J. R. Molecular chaperones HscA/Ssq1 and HscB/Jac1 and their roles in iron-sulfur protein maturation. *Crit. Rev. Biochem. Mol. Biol.* **42**, 95–111 (2007).
An account of the studies of co-chaperones in Fe–S cluster biogenesis by prominent contributors in the field.
83. Chandramouli, K. & Johnson, M. K. HscA and HscB stimulate [2Fe-2S] cluster transfer from IscU to apoferredoxin in an ATP-dependent reaction. *Biochemistry* **45**, 11087–11095 (2006).
84. Bonomi, F., Iametti, *S.,* Morleo, A., Ta, D. & Vickery, L. E. Studies on the mechanism of catalysis of iron-sulfur cluster transfer from IscU[2Fe2S] by HscA/HscB chaperones. *Biochemistry* **47**, 12795–12801 (2008).
85. Petrovic, A. *et al.* Hydrodynamic characterization of the SufBC and SufCD complexes and their interaction with fluorescent adenosine nucleotides. *Protein Sci.* **17**, 1264–1274 (2008).
86. Wada, K. *et al.* Molecular dynamism of Fe-S cluster biosynthesis implicated by the structure of the $SufC_2$-$SufD_2$ complex. *J. Mol. Biol.* **387**, 245–258 (2009).
87. Chahal, H. K, Dai, Y., Saini, A., Ayala-Castro, C. & Outten, F. W. The SufBCD Fe-S scaffold complex interacts with SufA for Fe-S cluster transfer. *Biochemistry* **48**, 10644–10653 (2009).
88. Eccleston, J. F., Petrovic, A., Davis, C. T., Rangachari, K. & Wilson, R. J. The kinetic mechanism of the SufC ATPase: the cleavage step is accelerated by SufB. *J. Biol. Chem.* **281**, 8371–8378 (2006).
89. Vinella, D., Brochier-Armanet, C., Loiseau, L., Talla, E. & Barras, F. Iron-sulfur (Fe/S) protein biogenesis: phylogenomic and genetic studies of A-type carriers. *PLoS Genet.* **5**, e1000497 (2009).
An *in vivo* analysis of the specificities and redundancies of A-type proteins.
90. Krebs, C. *et al.* IscA, an alternate scaffold for Fe-S cluster biosynthesis. *Biochemistry* **40**, 14069–14680 (2001).
91. Ollagnier de Choudens, S. *et al.* SufA from *Erwinia chrysanthemi*. Characterization of a scaffold protein required for iron-sulfur cluster assembly. *J. Biol. Chem.* **278**, 17993–18001 (2003).
92. Loiseau, L. *et al.* ErpA, an iron sulfur (Fe S) protein of the A-type essential for respiratory metabolism in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **104**, 13626–13631 (2007).
This paper reports the first case of *in vivo* specificity between an A-type protein and a target apoprotein.
93. Gupta, V. *et al.* Native *Escherichia coli* SufA, coexpressed with SufBCDSE, purifies as a [2Fe-2S] protein and acts as an Fe-S transporter to Fe-S target enzymes. *J. Am. Chem. Soc.* **131**, 6149–6153 (2009).
A demonstration that, *in vivo*, IscA binds to Fe–S clusters.
94. Zeng, J. *et al.* The IscA from *Acidithiobacillus ferrooxidans* is an iron-sulfur protein, which assembles the [4Fe-4S] cluster with intracellular iron and sulfur. *Arch. Biochem. Biophys.* **463**, 237–244 (2007).
95. Ollagnier de Choudens, S., Sanakis, Y. & Fontecave, M. SufA/IscA: reactivity studies of a class of scaffold proteins involved in [Fe-S] cluster assembly. *J. Biol. Inorg. Chem.* **9**, 828–838 (2004).
96. Mettert, E. L., Outten, F. W., Wanta, B. & Kiley, P. J. The impact of O_2 on the Fe-S. cluster biogenesis requirements of *Escherichia coli* FNR. *J. Mol. Biol.* **384**, 798–811 (2008).
97. Tan, G., Lu, J., Bitoun, J. P., Huang, H. & Ding, H. IscA/SufA paralogues are required for the [4Fe-4S] cluster assembly in enzymes of multiple physiological pathways in *Escherichia coli* under aerobic growth conditions. *Biochem. J.* **420**, 463–472 (2009).
98. Angelini, S. *et al.* NfuA, a new factor required for maturing Fe/S proteins in *Escherichia coli* under oxidative stress and iron starvation conditions. *J. Biol. Chem.* **283**, 14084–14091 (2008).
99. Bandyopadhyay, S. *et al.* A proposed role for the *Azotobacter vinelandii* NfuA protein as an intermediate iron-sulfur cluster carrier. *J. Biol. Chem.* **283**, 14092–14099 (2008).
100. Balasubramanian, R., Shen, G., Bryant, D. A. & Golbeck, J. H. Regulatory roles for IscA and SufA in iron homeostasis and redox stress responses in the cyanobacterium *Synechococcus* sp. strain PCC 7002. *J. Bacteriol.* **188**, 3182–3191 (2006).
101. Jin, Z. *et al.* Biogenesis of iron-sulfur clusters in photosystem I: holo-NfuA from the cyanobacterium *Synechococcus* sp. PCC 7002 rapidly and efficiently transfers [4Fe-4S] clusters to apo-PsaC *in vitro*. *J. Biol. Chem.* **283**, 28426–28435 (2008).
102. Agar, J. N. *et al.* Modular organization and identification of a mononuclear iron-binding site within the NifU protein. *J. Biol. Inorg. Chem.* **5**, 167–177 (2000).
103. Boyd, J. M., Sondelski, J. L. & Downs, D. M. Bacterial ApbC protein has two biochemical activities that are required for *in vivo* function. *J. Biol. Chem.* **284**, 110–118 (2009).
An important contribution to the understanding of the role of one of the most conserved proteins in all living organisms.
104. Boyd, J. M., Pierik, A. J., Netz, D. J., Lill, R. & Downs, D. M. Bacterial ApbC can bind and effectively transfer iron-sulfur clusters. *Biochemistry* **47**, 8195–81202 (2008).
105. Boyd, J. M., Lewis, J. A., Escalante-Semerena, J. C. & Downs, D. M. *Salmonella enterica* requires ApbC function for growth on tricarballylate: evidence of functional redundancy between ApbC and IscU. *J. Bacteriol.* **190**, 4596–4602 (2008).
106. Bych, K. *et al.* The iron-sulphur protein Ind1 is required for effective complex I assembly. *EMBO J.* **27**, 1736–1746 (2008).
An example of the use of *in vivo* labelling to elucidate the position of an Fe–S cluster biogenesis factor in a pathway.
107. Justino, M. C., Baptista, J. M. & Saraiva, L. M. Di-iron proteins of the Ric family are involved in iron-sulfur cluster repair. *Biometals* **22**, 99–108 (2009).
108. Overton, T. W. *et al.* Widespread distribution in pathogenic bacteria of di-iron proteins that repair oxidative and nitrosative damage to iron-sulfur centers. *J. Bacteriol.* **190**, 2004–2013 (2008).
109. Harrington, J. C. *et al.* Resistance of *Haemophilus influenzae* to reactive nitrogen donors and gamma interferon-stimulated macrophages requires the formate-dependent nitrite reductase regulator-activated *ytfE* gene. *Infect. Immun.* **77**, 1945–1958 (2009).
110. Vine, C. E., Justino, M. C., Saraiva, L. M. & Cole, J. Detection by whole genome microarrays of a spontaneous 126-gene deletion during construction of a *ytfE* mutant: confirmation that a *ytfE* mutation results in loss of repair of iron-sulfur centres in proteins damaged by oxidative or nitrosative stress. *J. Microbiol. Methods.* **81**, 77–79 (2010)
111. Justino, M. C., Vicente, J. B., Teixeira, M. & Saraiva, L. M. New genes implicated in the protection of anaerobically grown *Escherichia coli* against nitric oxide. *J. Biol. Chem.* **280**, 2636–2643 (2005).
112. Justino, M. C., Almeida, C. C., Gonçalves, V. L., Teixeira, M. & Saraiva, L. M. *Escherichia coli* YtfE is a di-iron protein with an important function in assembly of iron-sulphur clusters. *FEMS Microbiol. Lett.* **257**, 278–284 (2006).
113. Todorovic, S. *et al.* Iron-sulfur repair YtfE protein from *Escherichia coli*: structural characterization of the di-iron center. *J. Biol. Inorg. Chem.* **13**, 765–770 (2008).
114. Gralnick, J. & Downs, D. Protection from superoxide damage associated with an increased level of the YggX protein in *Salmonella enterica*. *Proc. Natl Acad. Sci. USA* **98**, 8030–8035 (2001).
115. Tucker, N. P. *et al.* The transcriptional repressor protein NsrR senses nitric oxide directly via a [2Fe-2S] cluster. *PLoS ONE* **3**, e3623 (2008).
116. Schwartz, C. J. *et al.* IscR, an Fe-S cluster-containing transcription factor, represses expression of *Escherichia coli* genes encoding Fe-S cluster assembly proteins. *Proc. Natl Acad. Sci. USA* **98**, 14895–14900 (2001).
The proposal that an autoregulatory system allows bacterial cells to sense Fe–S cluster homeostasis.
117. Giel, J. L., Rodionov, D., Liu, M., Blattner, F. R. & Kiley, P. J. IscR-dependent gene expression links iron-sulphur cluster assembly to the control of O_2-regulated genes in *Escherichia coli*. *Mol. Microbiol.* **60**, 1058–1075 (2006).
This paper shows that IscR regulates a broad range of genes.

118. Wu, Y. & Outten, F. W. IscR controls iron-dependent biofilm formation in *Escherichia coli* by regulating type I fimbria expression. *J. Bacteriol.* **191**, 1248–1257 (2009).
119. Nesbit, A. D., Giel, J. L., Rose, J. C. & Kiley, P. J. Sequence-specific binding to a subset of IscR-regulated promoters does not require IscR Fe-S cluster ligation. *J. Mol. Biol.* **387**, 28–41 (2009).
120. Shen, G. *et al.* SufR coordinates two $[4Fe\text{-}4S]^{2+,1+}$ clusters and functions as a transcriptional repressor of the *sufBCDS* operon and an autoregulator of *sufR* in cyanobacteria. *J. Biol. Chem.* **282**, 31909–31919 (2007).
A description of the Fe–S cluster biogenesis strategy in cyanobacteria.
121. Butland, G. *et al.* Interaction network containing conserved and essential protein complexes in *Escherichia coli*. *Nature* **433**, 531–537 (2005).
122. Arifuzzaman, M. *et al.* Large-scale identification of protein-protein interaction of *Escherichia coli* K-12. *Genome Res.* **16**, 686–691 (2006).
123. Butland, G. *et al.* eSGA: *E. coli* synthetic genetic array analysis. *Nature Methods* **5**, 789–795 (2008).
124. Bandyopadhyay, S. *et al.* Chloroplast monothiol glutaredoxins as scaffold proteins for the assembly and delivery of [2Fe-2S] clusters. *EMBO J.* **27**, 1122–1133 (2008).
125. Mandrand-Berthelot, M. A., Couchoux-Luthaud, G., Santini, C. L. & Giordano, G. Mutants of *Escherichia coli* specifically deficient in respiratory formate dehydrogenase activity. *J. Gen. Microbiol.* **134**, 3129–3139 (1988).
126. Ote, T. *et al.* Involvement of the *Escherichia coli* folate-binding protein YgfZ in RNA modification and regulation of chromosomal replication initiation. *Mol. Microbiol.* **59**, 265–275 (2006).
127. Seaton, B. L. & Vickery, L. E. A gene encoding a DnaK/hsp70 homolog in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **91**, 2066–2070 (1994).
128. Lelivelt, M. J. & Kawula, T. H. Hsc66, an Hsp70 homolog in *Escherichia coli*, is induced by cold shock but not by heat shock. *J. Bacteriol.* **177**, 4900–4907 (1995).
129. Desnoyers, G., Morissette, A., Prévost, K. & Massé, E. Small RNA-induced differential degradation of the polycistronic mRNA *iscRSUA*. *EMBO J.* **28**, 1551–1561 (2009).
The first example of a role for small-RNA-mediated regulation in Fe–S cluster homeostasis.

Acknowledgements
The authors apologize to all colleagues whose studies could not be referred to owing to space constraints. The authors acknowledge past and present colleagues who contributed to Fe–S cluster studies in the F.B. group: S. Angélini, L. Loiseau, L. Nachin, V. Trotter and D. Vinella. Thanks are due to M. Ansaldi for careful reading of the manuscript and to M. Fontecave and S. Ollagnier de Choudens for a warm and efficient collaboration. Financial support is provided by the Centre National de la Recherche Scientifique, the Université de la Méditerranée and the Agence Nationale de la Recherche (ANR-07-BLAN-0101-02).

Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeprj/
Arabidopsis thaliana | *Azotobacter vinelandii* | *Bacillus subtilis* | *Erwinia chrysanthemi* | *Escherichia coli* | *Haemophilus influenzae* | *Helicobacter pylori* | *Mycobacterium tuberculosis* | *Saccharomyces cerevisiae* | *Salmonella enterica* | *Synechococcus* sp. PCC7002 | *Synechocystis* sp. PCC 6803
UniProtKB: http://www.uniprot.org
CsdA | CyaY | ErpA | Fdx | HscA | HscB | IscA | IscR | IscS | IscU | Mrp | NfuA | NifS | NsrR | SoxR | SufA | SufB | SufC | SufD | SufE | SufS | YtfE

FURTHER INFORMATION
Frédéric Barras's homepage: http://lcb.cnrs-mrs.fr

SUPPLEMENTARY INFORMATION
See online article: S1 (table) S2 (box)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF