Iron-sulphur clusters as inhibitors and catalysts of viral replication

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Abstract

A virus hijacks host cellular machineries and metabolites to reproduce. In response, the innate immune system activates different processes to fight back. While many aspects of these processes have been well investigated, the key roles played by iron-sulphur [FeS] clusters, which are among the oldest classes of bio-inorganic cofactors, have barely been considered. Here, we discuss how several [FeS] cluster-containing proteins activate, support, and modulate the innate immune response to restrict viral infections; and how some of these proteins simultaneously support the replication of viruses. We also propose models of function of some proteins in the innate immune response and argue that [FeS] clusters in many of these proteins act as biological ‘fuses’ to control the response. We hope this overview helps to inspire future research in the emerging field of bioinorganic virology/immunology and that such studies may reveal new molecular insight into the links between viral infections and diseases like cancer and neurodegeneration.

Introduction

Iron-sulphur [FeS] clusters constitute one of the most versatile and ancient classes of bioinorganic cofactors. In these clusters the Fe and S atoms exist in different stoichiometries and the coordinating ligands to the irons of the cluster can deviate in multiple ways from the archetypal all-cysteine (Cys) pattern (Fig. 1a).[1] They have diverse functional roles (Fig. 1b-f). (i) They form a relay for long-range electron transfer[2] in enzymes, whose function is fundamental to life on earth, like hydrogenases (enabling many micro-organism to make or use H_2 and conserve energy in the form of ATP),[3] photosystem I (the integral membrane protein complex using light to generate electrons for the reduction of $NADP^+$),[4] or NADH:ubiquinone oxidoreductase (Fig. 1b) (respiratory complex I in mitochondria, a giant, membrane-bound enzyme that plays an integral role in generating the proton-motive force driving ATP synthesis).[5] In these enzymes, each cluster is characterised by a normally negative redox potential and a low activation barrier to electron transfer; the clusters are usually not solvent accessible. (ii) They perform catalysis. A well-studied example is the [4Fe-4S](Cys)$_3$ cluster of the radical S-adenosylmethionine (SAM) enzymes, which in most cases reductively cleaves SAM to generate the 5´-deoxyadenosyl radical (5´-dA• radical) intermediate (Fig. 1c).[6,7] Members of the radical-SAM superfamily of enzymes play various biological functions from modification of RNA or DNA to biosynthesis of cofactors and peptides.[6,7] Another example is the [4Fe-4S](Cys)$_3$ cluster in aconitase. This cluster acts as a Lewis acid (Fig. 1c) to facilitate isomerisation of citrate to iso-citrate in the tricarboxylic acid (TCA) cycle.[8] (iii) The clusters provide structural stability to a protein or a multiprotein complex, e.g. the [4Fe-4S](Cys)$_4$ cluster in the catalytic subunit of DNA polymerase δ (DNA Pol- δ, a multi-subunit protein complex involved in the replication and repair of DNA in eukaryotes).[9] The solvent-exposed cluster is located at the interface of the catalytic and regulatory subunits (Fig. 1d) and insures structural integrity and activity of the protein complex.[10] (iv) The clusters act as sensors of the cellular environments, responding to particular levels of O_2, nitric oxide (NO), or hydrogen peroxide (H_2O_2): by altering the structure of the cluster, these agents produce conformational changes to the respective proteins regulating their functions. Sensory clusters include the [4Fe-4S](Cys)$_4$ cluster in the O_2-sensing fumarate and nitrate reductase regulatory protein (FNR)[11], the [4Fe-4S](Cys)$_3$X

(where X is unknown) rhizobial iron regulator (RirA)[12], and the [4Fe-4S](Cys)$_3$(Asp) cluster of nitrite-responsive repressor (NsrR).[13] In these proteins the reaction of solvent-exposed [FeS] clusters with O_2 or NO induces or blocks the interaction of proteins with DNA, thereby regulating the expression of multiple genes/proteins. Reaction of NO with the [4Fe-4S](Cys)$_3$(Asp) cluster of NsrR results in nitrosylation, degradation of the cluster and formation of a mixture of protein-bound iron nitrosyl species and apo-protein (Fig. 1e).[14] The reaction of the cluster with O_2 in FNR converts the solvent-exposed [4Fe-4S](Cys)$_4$ cluster to a [2Fe-2S](Cys)$_4$ cluster. This conversion induces conformational changes in the protein scaffold and switches the function (Fig. 1e).[15] (v) The cluster acts as a source of sulphur, e.g. the auxiliary [4Fe-4S](Cys)$_3$(Ser) cluster in lipoyl synthase (LipA) supplies two sulphur atoms for the conversion of the protein-bound octanoyl chain to lipoyl cofactor (Fig. 1f).[16] Synthesis and delivery of the [FeS] clusters to the target proteins is a complex process, which has been reviewed in detail elsewhere.[17–20] Briefly, the mitochondrial iron-sulphur cluster (ISC) assembly machinery is involved in the maturation of all cellular iron-sulphur proteins present in mitochondria, cytosol, and the nucleus. Indeed, the ISC machinery presumably provides a [FeS] compound to the cytosolic iron-sulphur protein assembly (CIA) machinery,[21] which carries out the synthesis of [4Fe-4S] clusters in the cytosol.[17–20] In the cytosol, the assembled [4Fe-4S] clusters are then delivered to cytosolic and nuclear target proteins by iron-only hydrogenase-like protein 1 (IOP1) and accessory proteins including one or more components of the CIA targeting complex and the CIA2A protein.[17–20]

Viral infections are a global challenge and cause huge social and economic cost worldwide. The life cycle of all viruses can be divided into four stages (Fig. 2),[22] the details of which vary depending on the virus: (i) host-cell entry, (ii) replication, (iii) assembly of viral particles, and (iv) egress. Some viral proteins contain [FeS] clusters.[23–25] In viral proteins, [FeS] clusters play a structural and/or catalytic role.[25] For example, the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 is shown to have two [4Fe-4S](Cys)$_3$(His) clusters. The clusters are suggested to support catalytic activity of the enzyme and supply structural stability to the RdRp complex.[25] Beside viral factors (proteins, nucleic acids) involved in each step, cellular metabolites and proteins

are hijacked by viruses to complete their life cycle. It is anticipated that the host [FeS] biosynthesis apparatus is also hijacked for the maturation of viral [FeS] proteins.[25]

In response to viral infection, the innate immune system recognises components of the virus inducing expression of proinflammatory cytokines including type I and II interferons (IFNs).[26,27] Next, type I and II IFNs induce expression of interferon stimulated genes (ISGs) to restrict replication of viruses (Fig. 2).[28–30] Expression of ISG proteins and proinflammatory cytokines leads to inflammation, the hallmarks of which are a rise in the production of reactive oxygen and nitrogen species like H_2O_2 and NO,[31,32] and insufficient levels of dioxygen (hypoxia) (Fig. 2).[33] In this review article, we will focus exclusively on the host cell [FeS] proteins involved in the immune response and viral replication process (Table 1). We discuss how [FeS] clusters in different proteins control and modulate the innate immune response to a virus (Fig. 2) from the early steps of viral entry to reprograming metabolism and modulating mitophagy (the process by which damaged mitochondria are selectively degraded). We give examples of the cellular [FeS] proteins involved in inhibiting or catalysing viral replication process and discuss their structural features, the function of [FeS] clusters, and the cellular requirement for [FeS] cluster insertion into these proteins, while highlighting common features. We propose that [FeS] clusters present in some proteins act as a sensor of cellular levels of reactive oxygen species and O_2 enabling feedforward mechanisms to regulate processes like iron homeostasis and mitophagy, while in many other proteins they act as biological 'fuses' enabling a feedback mechanism to prevent an uncontrolled immune response and excessive production of reactive oxygen and nitrogen species (Fig. 2). Our analysis highlights the importance of future studies to elucidate the role of [FeS] clusters in the viral replication process and the innate immune response.

1. Activating the innate immune response

The first step in activation of the innate immune response to viral infections is recognition of the invading virus to initiate a series of cellular processes and restrict viral replication. In this section, we discuss how several [FeS] proteins play central roles in recognising viral components to activate the innate immune response. We demonstrate that these [FeS] proteins recognise viral

components like nucleic acids and proteins and activate the expression of type I and II IFNs and potentially other proinflammatory cytokines and ISGs, while at the same time some of them support expression of viral proteins.

Initiating transcription of IFNs and supporting translation. Many RNA and DNA processing proteins contain [FeS] clusters[34] including the catalytic subunit of DNA polymerase α (DNA Pol-α),[9,10,35] RNA Pol-III,[36,37] and the elongator complex (a multi-subunit protein complex highly conserved in eukaryotes with roles in the activity of RNA Pol-II and in the modification of transfer RNA (tRNA)).[38–40] The [FeS] clusters of POLA1, RNA Pol-III, and the elongator complex support their functions in sensing viral components, initiating translation of IFNs, and supporting translation (Fig. 3a). DNA Pol-α has four subunits: POLA1, a regulatory (POLA2) and two primase (PRIM1 and PRIM2) subunits. DNA Pol-α synthesises the RNA:DNA primers required for DNA replication on both leading and lagging strands of DNA.[41] The C-terminus of POLA1 has a [4Fe-4S](Cys)$_4$ cluster, which is essential for formation of a DNA Pol-α protein complex and its enzymatic activity.[10] In the nucleus, RNA Pol-III transcribes genes encoding 5S ribosomal RNA, tRNA, and other small RNAs.[42] Structural studies of RNA Pol-III in the presence and absence of DNA substrate revealed that the subunit 6 (RPC6) of RNA Pol-III has a [4Fe-4S](Cys)$_4$ cluster,[36,37] which stabilises the C-terminus of the protein (Fig. 3b). The [FeS] cluster domain of RPC6 forms a knot to tie the RPC7-RPC6-RPC3 heterotrimer to the core complex.[36,37] In the absence of DNA, the cluster is solvent exposed via a shallow funnel and upon binding of RNA Pol-III to DNA the funnel is closed by the C-terminal tail of RPC7 subunit (Fig. 3b).

The multiprotein complex RNA Pol-II requires activity of the elongator complex.[43] The catalytic subunit of elongator complex is Elp3, which harbours a [4Fe-4S](Cys)$_3$ cluster (Fig. 3c).[44] Elp3 has two catalytic domains: a C-terminus lysine acetyl–coenzyme A (acetyl-CoA) transferase (KAT) domain and an N-terminus radical-SAM (rSAM) domain, which binds a [4Fe-4S](Cys)$_3$ cluster.[45] Unlike most radical-SAM enzymes, the three cysteine residues coordinating the [4Fe-4S] cluster are not part of a CX_3CX_2C motif but are coordinated by three cysteine residues in a CX_9CX_2C

primary sequence (Fig. 3c). The cluster is accessible from solvent via two channels (Fig. 3c) and it provides structural integrity to the elongator complex,[46] analogous to the role of the [4Fe-4S](Cys)$_4$ cluster in stabilising RNA Pol-III and DNA Pols. When the elongator complex is in the nucleus, Elp3 catalyses acetylation of histones.[47,48] When in the cytoplasm, Elp3 catalyses addition of carboxyl-methyl (cm) to the tRNA third anticodon nucleotide (wobble) uridine at C_5,[49] this is referred to as 'cm^5' modification. The mechanism of DNA acetylation by Elp3 is sparsely characterised, but the mechanism of tRNA modification has been studied in detail.[38,50,51] Amino-acid sequence analysis and biochemical studies using electron paramagnetic resonance (EPR) spectroscopy confirmed that SAM binds to the [4Fe-4S] cluster of Elp3.[44] High resolution cryogenic electron microscopy (Cryo-EM) structures of Elp3 in the catalytic unit of the elongator complex,[38] consisting of Elp1, 2, and 3 (Elp123), showed that Elp3 is sandwiched between Elp1 and Elp2 (Fig. 3c). KAT and rSAM domains have a unique arrangement with a large interface, where tRNA can bind (Fig. 3c).[38,50,51] While the conformation of Elp3 and its catalytic pocket involving the [4Fe-4S] cluster and SAM appears not to change upon tRNA binding, the conformation of tRNA changes, allowing entry of the wobble uridine directly into the channel linked to the active site.[38] Like most radical-SAM enzymes the reduced [4Fe-4S]$^{1+}$ cluster reductively cleaves SAM to generate the 5´-dA• radical intermediate (Fig. 3d), which abstracts a hydrogen atom from the methyl group of acetyl-CoA.[49] The resulting radical attacks the C_5 position of uridine forming a C-C bond. This step is followed by rearrangements, loss of an electron and a proton, and formation of tRNA-acetyl-CoA, which is hydrolysed to generate CoA and cm^{5}U-tRNA (Fig. 3d).

POLA1, RNA Pol-III, and the elongator complex play vital roles in recognising viral components and activating the innate immune response. POLA1 has similarity to DNA polymerases of a number of DNA viruses[52] and interacts with viral DNA in the cytosol[53] or proteins like the non-structural protein (NSP1) of SARS-Co-V2.[54,55] While the function of these interactions are not understood, they can act as a recognition mechanism to activate a type-I IFN response. Specifically, interaction of viral DNAs or proteins with POLA1 may inhibit the activity of DNA Pol-α in synthesis of RNA:DNA primers affecting their cytosolic levels, which modulates IFNs production.[56] Unlike POLA1, the role of RNA Pol-III in recognising viral DNA is clearer. Mutations in

the RNA Pol-III gene are associated with defects in viral DNA recognition and immune response.[57] In the cytoplasm, RNA Pol-III recognises viral DNAs (Fig. 3a) via an unknown mechanism and catalyses the synthesis of double-stranded RNA (dsRNA) with a 5´-triphosphate moiety to stimulate interferon production (Fig. 3a).[58–60] In addition, in the nucleus RNA Pol-III modulates the IFN and antiviral immune response,[61,62] possibly by catalysing transcription of tRNAs and regulating their cellular pool (Fig. 3a).[63] After recognising viral components by cytosolic RNA Pol-III and other cellular mechanisms,[26,27] complex signalling cascades are activated to initiate transcription of mRNA of IFNs and other proinflammatory cytokines in the nucleus.[26,27] The RNA Pol-II complex is recruited to interferon promoters to transcribe IFN mRNAs (Fig. 3a).[64] Recruitment of RNA Pol-II is modulated by acetylation of histones including those of H3 and H4, which are targets of Elp3 (Fig. 3a).[47,48] Viral infection causes hyperacetylation of H3 and H4 at the IFN-β promoter,[65] and acetylation at H3Lys14 promotes hyperacetylation by inducing resistance to deacetylation and demethylation,[66] both of which could repress transcription of pro-inflammatory cytokines like type-1 IFNs.[67] Thus, acetylation of histones H3 and H4 by Elp3 of the elongator complex[47,48] could prime the innate immune response recruiting RNA Pol-II. Consistently, the Elp3 subunit of the elongator complex plays a vital role in epigenetics and regulation of the immune response;[68] and in *Drosophila melanogaster*,[69] plants,[70] and humans[71,72] Elp3 positively regulates the innate immune response. Additionally, in the cytoplasm the radical-SAM activity of Elp3 should play a role in priming the innate and trained immunity. The radical-SAM activity of Elp3 induces cm^5 modification of the tRNA wobble position[49] to modulate the cellular pool of tRNA (Fig. 3a). Changes in the cellular pool of tRNA, which is observed upon interferon stimulation and viral infection,[73] could regulate translation of type I and II IFNs and potentially other proinflammatory cytokines, ISGs, and viral proteins.

While the [4Fe-4S] clusters of RNA Pol-III and Elp3 have both structural and/or catalytic roles to support the function of proteins in activating the innate immune response, the solvent-exposed clusters may also act as sensors of the rising cellular levels of NO or H_2O_2 during inflammation (Fig. 3a), which is caused by the innate immune response (Fig. 2). The mechanism by which the solvent-exposed [4Fe-4S] clusters in RNA Pol-III and Elp3 may sense NO or H_2O_2 is not clear, but

it can be similar to that observed for the solvent exposed [FeS] clusters of NO and O_2 sensory proteins (Fig. 1e). Specifically, NO or H_2O_2 react with the clusters and degrade them, thereby altering and destabilising the structure of RNA Pol-III and the elongator complex. These structural changes block the ability of these proteins to continuously induce expression of IFNs and other pro-inflammatory cytokines, serving as a feedback mechanism to prevent excessive expression of ISG proteins and formation of cytotoxic levels of NO or H_2O_2 (Fig. 3a). Reactivation of the proteins requires the CIA targeting complex to restore the [FeS] clusters and in the case of Elp3 an unknown reducing partner to supply electrons for catalysis (Fig. 3a).

Enabling translation of cytokines, ISGs, and viral proteins. After transcription of proinflammatory cytokines, ISGs, and viral mRNAs, ribosomes translate the mRNAs to proteins (Fig. 2). The eukaryotic translation process consists of four steps: initiation, elongation, termination, and recycling. Two [FeS] proteins whose clusters regulate activity of the translation machinery (Fig. 4a) are Dph2 homodimer in archaea (Dph1-Dph2 heterodimer in eukaryotes)[74] and ABCE1, originally known as an inhibitor of RNase L (RLI1).[75] Dph1-Dph2 is part of a network of genes (Dph1-Dph7) and catalyses the first step in biosynthesis of diphthamide, a post-translational modification of histidine 715 residue (mammals) in the translation elongation factor 2 (EF2).[74] Although the exact function of diphthamide modification has remained elusive, it is shown to be essential for the function of EF2 in translocation of peptidyl-tRNA from the A-site of ribosome to the P-site (peptidyl-tRNA site) and the fidelity of the translation process.[76] While a specific role of diphthamide in the innate immune response is unknown, its loss pre-activates nuclear factor kapa B (NF-kB),[77] a transcription factor and a regulator of the innate immunity. The heterodimeric Dph1-Dph2 has two [4Fe-4S](Cys)$_3$ clusters similar to that present in members of the radical-SAM enzymes. The structure of Dph2 shows that the clusters are solvent exposed (Fig. 4b);[78] however, unlike most radical-SAM enzymes each cluster is coordinated to three distant cysteines in the primary sequence, $CX_{105}CX_{125}C$ (Fig. 4b). Thus, the cluster should confer structural stability and integrity besides its role in catalysis. Biochemical and spectroscopic studies using eukaryotic Dph1-Dph2 heterodimer revealed that the cluster in Dph1 is essential for catalysis and the cluster

in Dph2 is involved in electron transfer to the cluster in Dph1.[79] Unlike most radical-SAM enzymes, the $[4Fe\text{-}4S]^{1+}$ cluster of Dph1-Dph2 reductively cleaves SAM at the γ carbon of the methionine to generate a 3-amino-3-carboxypropyl (ACP)-$[4Fe\text{-}4S]^{3+}$ organometallic intermediate and 5′-methylthioadenosine (MTA) (Fig. 4c).[78,80] Subsequently, the organometallic intermediate attacks the imidazole ring of the histidine residue of EF2 generating an organic radical intermediate, which upon losing an electron and proton generates ACP-modified histidine (Fig. 4c).[78,80]

After the elongation step, the highly conserved ABCE1 protein orchestrates ribosome recycling. This protein is present in all archaea and eukaryotes and its function is vital both for cells[81] and for translation of viral mRNAs.[82] The protein interacts with several translation initiation factors and with 40S ribosomal subunit.[83–85] Biochemical and biophysical experiments using electron paramagnetic resonance (EPR) and Mössbauer spectroscopy confirmed that the protein has two diamagnetic $[4Fe\text{-}4S]^{2+}$ clusters, which cannot be reduced.[86] Structural studies of the ABCE1 from *Pyrococcus abyssi* revealed that the protein has an N-terminal [FeS] cluster-containing domain with two $[4Fe\text{-}4S](Cys)_4$ clusters (Fig. 4d).[87] Each cluster is coordinated by three nearby cysteines and one that is distant in the primary sequence (Fig. 4d), $[4Fe\text{-}4S]_A$: $CX_4CX_4CX_{39}C$, and $[4Fe\text{-}4S]_B$: $CX_{25}CX_2CX_2C$, implying the importance of the [4Fe-4S] clusters for structural stability of the protein. One cysteine residue of each cluster is solvent-exposed (Fig. 4d) and the clusters are prone to degradation by reactive oxygen species (ROS).[88] Extensive structural and biochemical studies have yielded molecular insight into the role of the [FeS] cluster domain in ribosome recycling.[89–96] Briefly, upon ATP binding to both sites in ABCE1, the protein undergoes a conformational switch from an open to a closed ATP-occluded state, which drives ribosome dissociation (Fig. 4e).[95,96] In the closed state, the [FeS] cluster domain of ABCE1 rotates 150° and repositions itself into a binding pocket on the 40S subunit. This movement suggests a collision with A-site factors splitting the ribosomal subunits (Fig. 4e).[93] ATP hydrolysis is required for ABCE1 release from the 40S subunit to re-enter a new cycle.

The activities of [FeS] proteins Dph1-2Dph2 and ABCE1 together orchestrate translation of the mRNAs (Fig. 4a). Their [4Fe-4S] clusters give structural stability to the proteins and enable their function (Fig. 4). The clusters are solvent exposed and can sense the cellular levels of NO or H_2O_2,

which degrade the clusters and inhibit their activity in translation. Hence, like the [FeS] clusters of RNA Pol-III and Elp3, the [FeS] clusters of Dph1-Dph2 and ABCE1 enable a feedback mechanism to respond to the increasing cellular levels of NO and H_2O_2 caused by the innate immune response. This feedback mechanism can serve as a safeguard to prevent excessive production of proinflammatory cytokines and ISG proteins and thus, formation of cytotoxic levels of NO and H_2O_2. After degradation of the clusters, formation of active holo-ABCE1 requires the function of the CIA targeting complex and two accessory proteins, namely YAE1D1 (Yae1 domain containing 1) and OROAV1 (the oral cancer overexpressed 1) proteins, to deliver two [4Fe-4S] clusters (Fig. 4a).[97,98] The specific cellular requirements for formation of holo-Dph1-Dph2 are unknown and it is proposed that iron-binding protein Dph3 can provide electrons to Dph1-Dph2 (Fig. 4a).[99]

2. Reprograming metabolism

A consequence of the induction of expression of proinflammatory cytokines like type I and II IFNs is the induction of expression of ISG proteins.[28–30] One of the ISG proteins is the [FeS] protein RSAD2.[100,101] This protein and other ISG proteins collectively restrict replication of a wide range of viruses and induce a metabolic shift in the cell. Many metabolic proteins also contain [FeS] clusters including respiratory complexes I-III, mitochondrial LipA, and PPAT, which is the first enzyme in the de novo biosynthesis of purine nucleotides. In this section, we discuss how the [FeS] clusters in RSAD2 and PPAT regulate immunometabolism (Fig. 5a).

Modulating central carbon metabolism. The ISG protein RSAD2 was originally identified as an antiviral enzyme,[100,101] which localises at the cytosolic face of the endoplasmic reticulum[102] and lipid droplets.[103] Thus, the enzyme was termed viperin (virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible).[104] This name, however, does not precisely describe the function of the enzyme since its expression can be induced by lipopolysaccharides (LPS),[105] its activity can enhance infectivity of human cytomegalovirus (HCMV),[106] and growing evidence supports a role of the protein in maturation of dendritic cells,[107] promoting adaptive behaviour of natural killer (NK) cells,[108] optimal T helper 2 (Th2) cell cytokine production,[109] and thermogenesis

in adipose tissue.[110] Spectroscopic studies of human and fungal RSAD2[111,112] and structural analysis of vertebrate RSAD2[113] showed that the protein has a [4Fe-4S](Cys)$_3$ cluster (Fig. 5b). The cluster is solvent exposed via a shallow funnel (Fig. 5b) like the [FeS] clusters of many proteins involved in activating the innate immune response. The radical-SAM activity of human RSAD2 catalyses transformation of cytidine triphosphate (CTP) to the nucleotide analogue 3´-deoxy-3´,4´-didehydro CTP (ddhCTP).[114] Structural analysis in the presence of CTP substrate revealed that H4´ of ribose in CTP is positioned toward the C5´ of ribose in S-adenosylhomocysteine (SAH)[115] (Fig. 5b) suggesting that H4´ is abstracted by the 5´-dA• radical, consistent with isotope labelling experiments.[114] The resulting substrate radical intermediate undergoes re-arrangement reactions and one-electron reduction to generate the ddhCTP product (Fig. 5c). The highly conserved tyrosine (Tyr302), which is approximately 5.5 Å away from the ribose of CTP, could act as an electron donor for one-electron reduction of the substrate radical intermediate via a proton-coupled electron transfer step (Fig. 5c).[116] The nucleotide analogue ddhCTP is proposed to be a chain terminator of RNA-dependent RNA polymerases (RdRps) of a number of flaviviruses with IC_{50} values (the inhibitor concentration at which the activity of an enzyme drops to 50% of its maximum value) in the range of 20 - 3,000 mM (Fig. 5a).[114] These IC_{50} values appear to be not physiologically relevant given that the cellular concentration of ddhCTP is approximately 0.1-0.3 mM.[114] Furthermore, the role of ddhCTP as a chain terminator of viral RdRps does not explain many reports describing the effect of RSAD2 enzymatic activity on cellular metabolism and behaviour, e.g. glucose homeostasis,[117] inhibition of mitochondrial trifunctional enzyme complex,[118] thermogenesis in adipose tissue,[110] interference with metabolism of amino acids,[119] maturation of dendritic cells,[107] or promotion of the adaptive behaviour of NK cells.[108] These activities together support an immunometabolic function of the product of the radical-SAM activity of RSAD2.[101] A role of ddhCTP in regulation of immunometabolism was proposed through metabolomics studies of authentic macrophages derived from human induced pluripotent stem cells (iPSCs).[120] Metabolomics studies suggested that the radical-SAM activity of RSAD2 inhibits the NAD^+-dependent activity of different enzymes including the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Fig. 5a).[120] Biochemical studies confirmed that ddhCTP can inhibit NAD^+-dependent activity of different enzymes including that of GAPDH with an

approximate IC_{50} value of 0.05 mM.[120] Inhibition of the NAD^+-dependent activity of GAPDH increases the cellular level of glyceraldehyde 3-phosphate (G3P or GAP)[120] and potentially NAD^+. The concomitant increase in the levels of G3P and NAD^+ can support synthesis of triacylglycerols (TAGs) and phosphatidylcholine (PC), and stimulate synthesis of cyclic ADP-ribose (cADPr) and protein ADP-ribosylation (Fig. 5a).[121]

Together, TAGs, PC, cADPr, and protein ADP-ribosylation can stimulate the immune response in various ways (Fig. 5a):[121] (i) supporting the synthesis of eicosanoids, which are signalling lipids, (ii) supporting antigen cross-presentation via major histocompatibility complex class-I (MHC-I), (iii) stimulating the activity of transcription factors NFAT (nuclear factor of activated T-cells) and NF-kB, and (iv) inducing synthesis of nitric oxide (NO). Besides the function of the [4Fe-4S](Cys)$_3$ cluster in the radical-SAM mediated synthesis of ddhCTP, the cluster provides structural stability to the enzyme,[122] analogous to the [4Fe-4S] cluster in proteins activating the innate immune response (Figs. 3-4). The solvent exposed [4Fe-4S] cluster of RSAD2 can act as a sensor of NO and H_2O_2, which degrade the cluster, induce conformational change in the protein structure, and abolish activity of the enzyme. The degradation of the cluster can modulate the interactions of RSAD2 with other proposed partner proteins.[100] Thus, the [FeS] cluster of RSAD2 could enable a feedback mechanism to regulate the innate immune response and production of toxic levels of ddhCTP, which can cause cell death.[119,123] While the cellular requirement for the reduction of the cluster is not known, insertion of the [4Fe-4S] cluster to form holo-enzyme is proposed to require CIAO1 protein of the CIA targeting complex (Fig. 5a).[124]

Modulating purine metabolism. Nucleotide metabolism is another metabolic process important to the innate immune response and viral infection. While the innate immune response requires nucleotides for transcription and synthesis of proinflammatory cytokines and ISGs mRNAs, and tRNAs, viruses require access to the cellular pool of nucleotides to replicate their genomic material (Fig. 5a). *De novo* biosynthesis of purines requires activation of the pentose phosphate pathway (PPP) to supply 5-phosphoribosyl-1-pyrophosphate (PRPP) (Fig. 5d). Consistently, activation of PPP is a signature of pro-inflammatory macrophages (M1 macrophages)[125] and cells infected with

viruses like influenza activate the *de novo* pathway of purine metabolism.[126,127] The radical-SAM activity of the ISG protein RSAD2 increases the cellular levels of metabolites of PPP including ribose 5-phosphate (R5P),[120] which is the substrate for synthesis of PRPP. Subsequently, the [FeS] enzyme PPAT catalyses the first step in *de novo* biosynthesis of purines by converting PRPP into 5-phosphoribosyl-1-amine (PRA) using the side-chain amine group of a glutamine (Fig. 5a). Crystallographic studies of PPAT from *B. subtilis*,[128] *E. coli*,[129] and *A. thaliana*,[130] in combination with sequence alignments, show that the enzyme from most archaeal, bacterial, and eukaryotic species, including human, is a homotetramer with one [4Fe-4S](Cys)$_4$ cluster per subunit (Fig. 5d). The cluster in the *B. subtilis* enzyme does not differ significantly in geometric[128] or electronic and magnetic structure[131,132] from the [4Fe-4S]$^{2+}$ clusters in electron-transfer proteins, however, it is not reduced under physiological conditions (reduction requires a potential < -600 mV).[133] The cluster is thought not to be directly involved in catalysis since it is located too far away from both the site at which glutamine is hydrolysed and a phosphoribosyl transferase domain where the amine group is added to PRPP.[128,134] Thus, the cluster is a structural element comparable to that of the Zn(II)(Cys)$_4$ site in, e.g., zinc fingers, as it connects distant cysteine residues (Fig. 5d), e.g., in *B. subtilis* PPAT $CX_{145}CX_{76}CX_2C$. Oxidation of the cluster leads to its disintegration, and loss of enzyme activity.[133] One of the inorganic sulphides of the cluster is solvent-exposed via a shallow funnel of 7 Å length and 7 Å entry diameter (Fig. 5d).[128] Therefore, similar to the [4Fe-4S] cluster of RSAD2, the [4Fe-4S] cluster in PPAT can act as lockable switch to turn activity off as a function of the concentrations of NO and H_2O_2 (Fig. 5a). Formation of holo-PPAT and regeneration of the activity of oxidatively damaged enzyme requires the CIA targeting complex.[135]

3. Redox sensing

A universal outcome of the innate immune response to viral infections is inflammation, a hallmark of which is excessive production of reactive oxygen or nitrogen species like H_2O_2 and NO,[31,32] and hypoxia (Fig. 2).[33] These changes are sensed by [FeS] proteins modulating iron homeostasis and mitophagy (Fig. 6a). Iron homeostasis is at the crossroad of viral infection and the immune response,[136–138] and mitophagy is a positive regulator of the innate immune response.[139] The [FeS]

proteins involved in these processes are IRP1,[140,141] FBXL5,[142] and mitoNEET (Fig. 6a).[143] Firstly, IRP1 exists in two forms: holo-protein containing a solvent-exposed [4Fe-4S](Cys)$_3$ cluster and apo-protein lacking the cluster (Fig. 6b). While holo-IRP1 is in a closed state, apo-IRP1 adopts an open state (Fig. 6b) and interacts with specific nucleotide sequences, commonly known as iron-responsive elements (IREs), in the untranslated regions of the mRNAs of ferritin and transferrin receptor (TfR).[140,141] Secondly, FBXL5 harbours an [2Fe-2S](Cys)$_4$ cluster with a solvent-exposed cysteine residue (Fig. 6c).[142] Under normal O_2 levels, the cluster is in the oxidised state, [2Fe-2S]$^{2+}$, and is able to organise the C-terminal loop of FBXL5 that is responsible for recruiting IRP2 (iron-responsive element-binding protein 2) (Fig. 6c).[142] This protein is not an iron-sulphur binding protein, but like IRP1 can bind to IREs. Finally, mitoNEET is a homodimer and each monomer contains a redox active [2Fe-2S](Cys)$_3$(His) cluster (Fig. 6d).[143] The histidine and a cysteine residue coordinating the [2Fe-2S] cluster are exposed to the solvent (Fig. 6d).[144,145] The [2Fe-2S] cluster of mitoNEET has a midpoint redox potential of approximately 0 mV (pH 7.0),[146] which is well above the cytosolic redox potential, i.e. approximately -325 mV (pH 7.0).[147] Thus, under normal physiological conditions mitoNEET is reduced. The cluster is stable in the presence of NO[148] and is reversibly oxidised by H_2O_2.[149]

The [FeS] clusters of IRP1 and FBXL5 modulate iron homeostasis during inflammation (Fig. 6a). The cellular level of IRP1 is affected by inflammation and synthesis of NO,[150] which degrades the [4Fe-4S] cluster presumably via a mechanism similar to that proposed for NO sensory protein NsrR (Fig. 1e).[13] At the same time, the hypoxic conditions caused by inflammation result in a reduced [2Fe-2S]$^{1+}$ cluster in FBXL5 blocking its ability to recruit and mark IRP2 for degradation (Fig. 6a).[142] Consequently, apo-IRP1 and IRP2 bind to the IRE at the 5´-untranslated region of ferritin mRNA, blocking its translation (Fig. 6a), and bind to the 3´-untranslated regions of TfR mRNA, stabilising the mRNA and inducing translation. Overexpression of TfR and a reduction in cellular level of ferritin modulates the immune response to viral infections in two general ways (Fig. 6a). Firstly, it causes an increase in the intracellular level of available iron (i.e. not stored in ferritin). Iron is a critical component of many cofactors, e.g. heme, mononuclear/dinuclear iron (di-iron) sites, and [FeS] clusters. The proteins carrying these bioinorganic cofactors perform many cellular

functions essential for the immune response and viral replication, e.g. [FeS] proteins like RNA Pol-III, Elp3, ABCE1, and RSAD2 and di-iron ribonucleotide reductase of humans and some viruses like human cytomegalovirus (HCMV).[151] Secondly, an increase in expression of TfR can support entry of some viruses to host cells (Fig. 6a).[138,152]

On the other hand, available data suggest that the [FeS] cluster of mitoNEET senses the cellular level of H_2O_2 to regulate mitophagy and the innate immune response (Fig. 6a). Elevated cellular level of H_2O_2 during inflammation, caused by activity of enzymes like NADPH oxidases (NOXs) and/or superoxide dismutase (SOD),[153] leads to oxidation of the [2Fe-2S] cluster of mitoNEET. Oxidised mitoNEET interacts with VDACs (voltage dependent anion channels) and abolishes its redox-gating function in cross talk between the mitochondria and cytosol.[154] Consequently, mitochondrial respiration rate is suppressed (Fig. 6a). This is consistent with previous reports showing that loss of mitoNEET may increase the mitochondrial respiration rate.[155] A decrease in mitochondrial respiration rate induced by the interaction of oxidised mitoNEET with VDACs will lead to a lowering of the mitochondrial membrane potential ($\Delta\Psi_m$). A reduction in the mitochondrial respiration and membrane potential activates the tensin homolog (PTEN)-induced putative kinase 1 (PINK1) (Fig. 6a), which phosphorylates and recruits the E3 ubiquitin protein ligase (PARKIN). The latter induces ubiquitination of target proteins and activates mitophagy.[156,157] Recent studies also support a function of mitoNEET in linking the innate immune response and cellular levels of H_2O_2 to the function of the CIA machinery. Specifically, the complex of anamorsin and NADPH-dependent diflavin oxidoreductase 1 (Ndor1) protein, which acts as an electron donor in the CIA machinery,[158] interacts with mitoNEET and reduces its [2Fe-2S] cluster,[159] and mitoNEET can transfer its [2Fe-2S] clusters to anamorsin.[160] These activities will prevent mitoNEET-induced mitophagy.

Concluding remarks and perspective

We have discussed the essential roles of [FeS] clusters in all steps of the innate immune response (Fig. 2), namely recognising viral components, activating expression of proinflammatory cytokines

and ISG proteins, functioning of the ISG protein RSAD2 and metabolic enzymes like PPAT, and sensing cellular levels of NO, H_2O_2, and O_2. We showed that some of the proteins involved like ABCE1 and PPAT also support viral reproduction. We highlighted that there are two common features associated with the [FeS] clusters in the proteins involved: (i) the clusters confer structural stability on the proteins or protein complexes and (ii) they are solvent-exposed and thus, accessible by NO, H_2O_2, or O_2. We discussed that these features in [FeS] proteins like mitoNEET, IRP1, and FBXL5 enable a feedforward mechanism to modulate the innate immune response, while in other proteins like RNA Pol-III, Elp3, ABCE1, Dph1-Dph2, RSAD2, and PPAT provide a feedback loop to block excessive immune response. In the feedback mechanism, the increasing levels of NO and H_2O_2, which are caused by the innate immune response, are sensed by the solvent-exposed [FeS] clusters. Consequently, the clusters are degraded inactivating the proteins and preventing excessive immune response and production of toxic levels of NO and H_2O_2 or metabolites like ddhCTP, which may have cytotoxicity.[119,123] This halt continues until the cellular levels of NO and H_2O_2 are decreased to a low level and the [FeS] biogenesis machinery restores the activity of the proteins. Recent studies support the proposed feedback mechanism and the action of reactive nitrogen or oxygen species like H_2O_2 in preventing an excessive innate immune response.[161,162] The function of [FeS] clusters as biological ‘fuses’ in enzymes like ABCE1 and PPAT, whose activities not only enable the innate immune system to restrict viral replication but also support reproduction of viral particles, will also limit viral replication process.

We discussed examples of some host cell [FeS] proteins as inhibitors and catalysts of the viral replication process. The role of many [FeS] proteins like Dph1-Dph2, Elp3, ABCE1, mitoNEET, RSAD2, and PPAT in the innate immune response is still not well understood, and [FeS] clusters are emerging as essential components of some viral proteins including RdRp of SARS-CoV-2.[25] Many questions need to be answered, e.g.: How is the [FeS] biogenesis machinery regulated in response to viral infection and during activation of the immune system? What are the critical levels of reactive oxygen and nitrogen species at which [FeS] clusters in different proteins become sensitive? What are the kinetics laws governing the reaction of [FeS] clusters with NO and H_2O_2 and those of [FeS] insertion into target proteins, and how do the different steps combine to regulate

the innate immune response? What is the cellular protein supplying electrons for activating enzymes like Elp3 and RSAD2? How exactly do viral proteins gain their [FeS] clusters? Is the ability of SARS-CoV-2 RdRps to catalyse replication of viral RNA either with [FeS] clusters or Zn(II)[25] an adaptation mechanism to cope with the increasing cellular levels of NO and H_2O_2 caused by the innate immune response? Future studies should help to address these questions revealing fundamental insight into the function of [FeS] clusters in the innate immune system and viral replication processes. These studies will find new drug-targets to fight a wide range of RNA and DNA viruses. Additionally, as the links between the antiviral innate immune response and cancer or neurodegeneration become clearer,[163,164] and because mutations in proteins involved in the [FeS] biogenesis machinery such as frataxin[165] can lead to neurodegenerative diseases like Friedreich's ataxia and oxidative stress,[166] we expect that a better understanding of the roles of [FeS] clusters in the innate immune response will help in understanding the molecular details connecting viral infections and inflammation to cancer and neurodegenerative diseases.

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Acknowledgements

We dedicate this review article to the memory of Andrew Thomson; a true pioneer of iron-sulphur cluster biochemistry who inspired us all in different ways. All authors acknowledge the support from the European COST action FeSBioNET. K.H.E. is grateful for support from the European Molecular Biology Organization (EMBO) (ALTF 157-2015), Edward Penley Abraham Research Fund (RF259), and Cancer Research UK (CRUK) Oxford (CRUKDF-0221-KHE). Y.N. thanks the Commissariat à l'Energie Atomique (CEA) for institutional support. N.L.B. thanks the Biotechnology and Biological Sciences Research Council (BBSRC, grant BB/S001018/1). S.C.B. acknowledges support from the Fondazione Cassa di Risparmio di Firenze (grant no. CRF2018.0920).

Contributions

K.H.E. conceived the idea of the review. K.H.E. wrote the manuscript with contributions from S.C.B., P.L.H., Y.N., N.L.B., W.R.H., and F.A.A.

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Competing interests

The authors declare no competing interests.

Fig. 1. The diversity of [FeS] cluster structures and functions. (a) Six common forms of [FeS] clusters. These clusters have various functional roles in different proteins: (b) Electron transfer, e.g. a relay of [FeS] clusters in the respiratory complex I enables long-range electron transfer (PDB Code:6YJ4); (c) Catalysis, e.g. the $[4Fe\text{-}4S]^{1+}(Cys)_3$ cluster in most members of the radical-SAM enzymes reductively cleaves SAM to generate the 5´-dA• radical intermediate, or the $[4Fe\text{-}4S](Cys)_3$ cluster in aconitase participates in Lewis-acid catalysis; (d) Structural stability, e.g. the solvent-exposed $[4Fe\text{-}4S](Cys)_4$ cluster at the interface of catalytic and regulatory subunits of DNA Pol-δ gives structural integrity to the complex (PDB Code: 6TNY); (e) Sensor, e.g. the solvent-exposed $[4Fe\text{-}4S](Cys)_3(Asp)$ cluster in NsrR (PDB Codes: 5N07 & 5N08) reacts with NO leading to the degradation of the cluster and formation of apo-NsrR and NsrR-bound iron-nitrosyl species. The solvent-exposed $[4Fe\text{-}4S](Cys)_4$ cluster in FNR (PDB Code: 5E44) reacts with O_2 leading to formation of a FNR-bound $[2Fe\text{-}2S](Cys)_4$ cluster inducing conformational change in the protein (PDB Code: 5CVR); (f) Sulphur donor, e.g. the $[4Fe\text{-}4S](Cys)_3(Ser)$ auxiliary cluster in LipA (PDB Code: 5EXJ) supplies two sulphur atoms in the last step of biosynthesis of lipoyl cofactor. 5´-dAH: 5´-deoxyadenosine.

Fig. 2. The activity of all the steps of the innate immune response is regulated by [FeS] clusters. At the top is a summary of the viral life cycle. The steps leading to viral replication are shown by orange arrows. After entry of a virus to a cell, viral factors like nucleic acids and proteins are recognised by [FeS]-containing proteins (Recognition), beside other mechanisms. This signal is translated by [FeS] proteins to the overexpression of proinflammatory cytokines including IFNs and then expression of ISGs and activation of proinflammatory signalling (Transcription & Translation). At the same time, some viral [FeS] proteins (not shown) support replication of viral genome and some host [FeS] proteins support translation of viral proteins (Translation). Some ISG proteins require [FeS] clusters to restrict replication of a virus. Expression of ISG proteins and activation of proinflammatory signalling pathways by cytokines cause inflammation inducing metabolic changes and a burst in production (red arrow) of NO and H_2O_2 and insufficient levels (grey arrow) of O_2 (hypoxia). The steps of the innate immune response leading to the formation of NO and H_2O_2 and to hypoxia are shown by black arrows. The rising cellular levels of NO and H_2O_2 and hypoxia are sensed by [FeS] clusters modulating iron metabolism and mitophagy (feedforward mechanism, purple arrow). At the same time, NO and H_2O_2 can degrade the solvent-exposed [FeS] clusters of many proteins involved in all the steps of the innate immune response halting an excessive production of NO and H_2O_2 (feedback mechanism, blue arrow).

Fig. 3. The [4Fe-4S] clusters in RNA Pol-III and elongator complex activate the innate immune response. (a) A summary of the roles of [4Fe-4S] clusters of RNA Pol-III and Elp3 in activating and regulating the IFNs response due to viral infection (see text for details). While the solvent-exposed clusters are essential in order to recognise viral components, activate the expression of IFNs and modify cellular pool of tRNAs, they may sense the increasing cellular levels of NO or H_2O_2 caused by the innate immune response. Consequently, the clusters are degraded inducing conformational changes in the protein/protein complexes and halting their activity. Delivery of the clusters by the CIA targeting complex will reactivate the proteins. In the case of Elp3, an unknown reducing partner should provide electrons for catalysis. IRF: interferon regulatory factor; Ac: acetyl group. (b) Structure of the RNA Pol-III and its [FeS] binding domain. Comparison of the structure of RNA Pol-III in the presence of DNA (PDB Code: 7AEA) with that in the absence of DNA (PDB Code: 7A6H) shows that binding of DNA allows the long tail of RPC7 to close the shallow funnel exposing the [4Fe-4S] cluster to solvent (dashed arrow). (c) Structure of Elp3 in complex with Elp1 and Elp2 (PDB Code: 6QK7). The KAT (light green) and rSAM (dark green) domains of Elp3 have a large interface forming a pocket, where the wobble position of tRNA can enter (blue arrow). Elp3 has a $[4Fe\text{-}4S](Cys)_3$ cluster, which is exposed to solvent via two

channels (dashed arrows). (d) Mechanism of modification of tRNA by Elp3. In the cytoplasm, the cluster reductively cleaves SAM to initiate the radical-SAM catalysis and addition of carboxyl-methyl (cm) to the tRNA wobble uridine at C_5 (cm^5) (see text for the details).

Fig. 4. Translation of cytokines, ISGs, and viral mRNAs is enabled by [FeS] clusters. (a) The [FeS] cluster-containing proteins Dph1-Dph2 and ABCE1 orchestrate the translation machinery to express proinflammatory cytokines, ISG proteins, and viral proteins. The solvent-exposed clusters sense the increasing cellular level of reactive oxygen or nitrogen species to modulate translation and prevent excessive expression of proinflammatory cytokines and ISG proteins. (b) Structure of Dph2 homodimer (PDB Code: 3LZD) showing the solvent-exposed [4Fe-4S](Cys)$_3$ cluster (dashed arrow). (c) The proposed mechanism by which Dph1-Dph2 is involved in the first step of biosynthesis of diphthamide (see text for detail). MTA: 5′-methylthioadenosine. (d) Structure of ABCE1 showing two [4Fe-4S] clusters in the [FeS] cluster binding domain and two ADP in the twin ATP-binding domain (PDB code: 3BK7). (e) The proposed molecular mechanism of the function of ABCE1 and its [FeS] cluster-binding domain in the ribosome recycling and translation (see text for details). After splitting of ribosomal subunits other factors (not shown, to retain figure clarity)[94] are needed for formation of the initiation complex.

Fig. 5. Immunometabolism is modulated by the action of [FeS] clusters. (a) A summary of the role of the [FeS] clusters of RSAD2 and PPAT in immunometabolism. The nucleotide analogue ddhCTP is proposed to act as a chain terminator of viral RdRps or to inhibit the glycolytic enzyme GAPDH and regulate metabolism (see text for details). Inhibition of GAPDH by ddhCTP increases intermediates of PPP, which support de novo synthesis of purines. The first enzyme in purine biosynthesis is the [FeS] protein PPAT. This enzyme catalyses the first step in the biosynthesis of inosine monophosphate (IMP) to supply ATP and GTP for activity of [FeS] proteins RNA Pol-II and RNA Pol-III supporting transcription of proinflammatory cytokines and ISGs. At the same time, it supplies ATP and GTP for viral genome synthesis. F6P: fructose 6-phosphate. (b) Structure of vertebrate RSAD2 (viperin) showing the solvent-exposed [4Fe-4S](Cys)$_3$ cluster (PDB code: 5VSL). Structural analysis of vertebrate RSAD2 in the presence of S-adenosylhomocysteine (SAH) and CTP (PDB Code: 6Q2P) shows that H4′ faces toward the C5′of the ribose in SAH, suggesting that this hydrogen atom is abstracted by the 5′-dA• radical. The highly conserved Tyr302, which could play a role in catalysis, is shown. (c) The proposed mechanism of the catalytic conversion of CTP to ddhCTP by RSAD2. (d) Structure of *B. subtilis* PPAT (PDB Code: 1AO0). The enzyme is a homotetramer, and each subunit has a solvent-exposed [4Fe-4S](Cys)$_4$ cluster.

Fig. 6. Cellular redox state is sensed by [FeS] clusters to modulate mitophagy and iron metabolism. (a) A summary of the roles of the [FeS] clusters of IRP1, FBXL5 and mitoNEET in the regulation of iron homeostasis and mitophagy in response to the cellular levels of NO, H_2O_2, and O_2 (feedforward). (b) The [4Fe-4S](Cys)$_3$ cluster of IRP1 acts as a conformational switch: holo-IRP1 (PDB Code: 2B3X) has a close conformation (top) and cannot bind to IRE, while apo-IRP1 has an open conformation and binds to IRE (PDB Code: 3SN2). (c) FBXL5 has a [2Fe-2S](Cys)$_4$ cluster, and one of the cysteine residues is solvent-exposed (dashed arrow) (PDB Code: 6VCD). When the [2Fe-2S](Cys)$_4$ cluster of FBXL5 is oxidised, the protein binds to IRP2. (d) Each monomer of the homodimeric mitoNEET has a redox-active [2Fe-2S](Cys)$_3$(His) cluster (PDB Code: 3EW0). The histidine and a cysteine residue coordinating to the cluster are solvent-exposed (dashed arrow). Ub: Ubiquitin.

Table 1. A glossary of the key [FeS] proteins discussed in the review, the type of their clusters, the roles of their clusters in immune response, and the steps of the innate immune response they are involved in.

Protein	Cluster	Role of cluster	Step involved
POLA1 (Catalytic subunit of DNA Pol-α)	[4Fe-4S](Cys)$_4$	Biological fuse & structural stability	Interaction with and sensing of viral DNA and proteins
RNA Pol-III (RNA polymerase III)	[4Fe-4S](Cys)$_4$	Biological fuse & structural stability	Interaction with and sensing of viral RNA
Elp3 (Elongator protein 3 of the elongator complex)	[4Fe-4S](Cys)$_3$	Catalysis, Biological fuse & structural stability	Recruitment of RNA Pol-II and transcription of type-1 IFNs; modification of tRNA to modulate translation
Dph1-Dph2 (Diphthamide biosynthesis protein subunits 1-2)	[4Fe-4S](Cys)$_3$	Catalysis, Biological fuse & structural stability	Modification of tRNA to modulate translation
ABCE1 (ATP-binding cassette sub-family E member 1)	[4Fe-4S](Cys)$_4$	Biological fuse & structural stability	Ribosome recycling and translation of mRNA
RSAD2 (Radical S-adenosylmethionine domain-containing protein 2)	[4Fe-4S](Cys)$_3$	Catalysis, Biological fuse & structural stability	Modulation of immunometabolism and restriction of viral replication
PPAT (Phosphoribosyl pyrophosphate amidotransferase)	[4Fe-4S](Cys)$_4$	Biological fuse & structural stability	Modulation of biosynthesis of purines
IRP1 (The iron-responsive element-binding proteins 1)	[4Fe-4S](Cys)$_3$	Sensor of NO, & conformational change	Regulation of iron metabolism
FBXL5 (An E3 ubiquitin ligase)	[2Fe-2S](Cys)$_4$	Sensor of O_2 & conformational change	Regulation of iron metabolism
MitoNEET (Outer membrane mitochondrial-associated protein containing the amino acid sequence Asn-Glu-Glu-Thr)	[2Fe-2S](Cys)$_3$(His)	Sensor of H_2O_2 & conformational change	Regulation of mitophagy

Table of Content summary

We show that iron-sulphur [FeS] clusters in several proteins activate the innate immune system to catalyse or inhibit viral replication. We propose that in many of these proteins the clusters act as biological ‘fuses’ in reponse to toxic levels of reactive oxygen and nitrogen species further regulating the immune response.