Review

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Guardians of the Genome: Iron-Sulfur Proteins in the Nucleus

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Abstract: Iron-sulfur (Fe-S) clusters are essential cofactors found in many proteins in the mitochondria, cytosol, and nucleus of the cell. These versatile cofactors may undergo reversible oxidation-reduction reactions to enable electron transfers; they may be structural and confer stability to a folded protein; they may be regulatory and transduce an iron signal that alters the function or stability of a recipient protein. Of the nearly 70 proteins described in mammalian cells that bind Fe-S clusters, about half localize exclusively or partially to the nucleus, where they are required for DNA replication and repair, telomere maintenance, transcription, mitosis, and cell cycle control. Most nuclear Fe-S cluster proteins interact with DNA, including DNA polymerases, primase, helicases, and glycosylases. However, the specific roles of the clusters in the enzymatic activities of these proteins and their interplay with DNA remain a matter of debate. Defects in the metallation of nuclear Fe-S proteins cause genome instability and alter the regulation of cell division and proliferation, which are hallmarks of various genetic diseases and cancers. Here, we provide an inventory of the nuclear Fe-S cluster binding proteins and discuss cluster types, binding sites, the process of cluster acquisition, and potential roles of the cluster in the function of the proteins. However, many questions remain unresolved. We highlight critical gaps in our understanding of cluster delivery to nuclear client proteins, the potential for cluster repair, and the mechanistic roles that clusters play in these enzymes. Taken together, this review brings the focus to the nucleus of the human cell as a hotspot for Fe-S cluster proteins and aims to inspire new research in the roles of iron in DNA metabolism and the maintenance of genome integrity.

Keywords: Iron-sulfur cluster; nucleus; iron trafficking; DNA replication; DNA repair.

1. Introduction

Iron-sulfur (Fe-S) clusters are essential cofactors found in a wide variety of proteins across all domains of life. These clusters consist of iron and sulfur ions with different compositions, structures, redox states, and coordination ligands in proteins. The most frequently found clusters are the rhombic [2Fe-2S] and the cubic or cubane [4Fe-4S], but [3Fe-4S] and [4Fe-3S] clusters have also been described. The chemistry of these clusters makes them ideal for sensing redox signals, as they can accommodate different electronic configurations by accepting or donating unpaired electrons. Consequently, these clusters serve a wide spectrum of functions within proteins, including electron transfer, enzymatic catalysis, protein structural stability, cofactor transfer, oxygen and iron sensing, as well as redox stress [1]. These functions render Fe-S proteins crucial roles in multiple cellular processes, including energy production, metabolite transformation, DNA maintenance, gene expression regulation, protein synthesis, mitosis, and antiviral defense [2].

In the early stages of identifying and characterizing Fe-S clusters in the human proteome, the proteins discovered were primarily associated with mitochondrial and cytosolic functions, such as ferredoxins, aconitases, and respiratory complexes [3]. When the first Fe-S cluster bound to a helicase was reported in 2006, the authors stated "*Fe-S clusters are rare in nuclear proteins*" [4]. Nearly two decades later, we see that this perspective has completely changed, with the nucleus now known to contain about 35 proteins that bind Fe-S clusters, turning the nuclear compartment into a hot spot for

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Fe-S-related processes. The inventory of nuclear Fe-S cluster binding proteins described to date is listed in Table 1.

The link between iron metabolism and nucleic acid metabolism is becoming increasingly clear. This is evident in processes related to genome integrity, with Fe-S clusters found in many proteins involved in DNA replication and repair, as well as in transcription, with Fe-S clusters present in RNA polymerase, transcription factors, and RNA modification proteins. Although the precise role of these Fe-S clusters often remains elusive, their prevalence in proteins associated to nucleic acid transactions, and the fact that they have not been replaced by less redox-active metals throughout evolution, suggest that they play a crucial role in nucleic acid metabolism.

In this review, we discuss the current knowledge of nuclear Fe-S cluster proteins, examine accumulating evidence suggesting that Fe-S clusters may have additional and unexpected roles in cells, and highlight areas of research that may have practical significance but for which no definitive evidence has yet emerged.

Table 1. Inventory of Fe-S cluster binding proteins that localize to the nucleus of mammalian cells. The localization of the proteins reported here is based on consolidating information from references (when available) and from three different databases: The Human Protein Atlas [5] (https://www.proteinatlas.org), The UniProt Knowledgebase [6] (https://www.uniprot.org), and COMPARTMENTS [7] (https://compartments.jensenlab.org). An extended version of the table, including the residues that coordinate the clusters, is available as Supplementary Information. The asterisks (*) denote that Fe-S cluster ligands are shared between oligomeric partners from the CIA or ISC pathways. Question marks indicate unknown or debatable cluster binding residues, cluster types, or stoichiometries. SAM: S-adenosyl-L-methionine.

Participates in	Name	Consolidated Localization	Fe-S cluster type
DNA replication DNA repair	PRIM2	Nucleus	[4Fe-4S] $(Cys)_4$ [8,9]
	POLA1	Nucleus, Cytosol [10]	[4Fe-4S] $(Cys)_4$ [11]
	POLD1	Nucleus, Cytosol [12]	[4Fe-4S] $(Cys)_4$ [11]
	POLE	Nucleus	[4Fe-4S] $(Cys)_4$ [11,13]
	REV3L	Nucleus, Cytosol [14]	[4Fe-4S] $(Cys)_4$ [11]
	FANCJ	Nucleus, Cytosol [15,16]	[4Fe-4S] $(Cys)_4$ [4,17]
	DDX11	Nucleus [18]	[4Fe-4S] $(Cys)_4$ [19]
	RTEL1	Nucleus [16]	[4Fe-4S] $(Cys)_4$ [20]
	XPD	Nucleus, Cytosol [16,21]	[4Fe-4S] $(Cys)_4$ [4]
	DNA2	Nucleus, Mitochondria [22]	[4Fe-4S] $(Cys)_4$ [23,24]
	EXO5	Nucleus, Cytosol [25]	[4Fe-4S] $(Cys)_4$ [25]
	MUTYH	Nucleus, Mitochondria [26]	[4Fe-4S] $(Cys)_4$ [27,28]
	NTHL1	Nucleus, Mitochondria [29]	[4Fe-4S] $(Cys)_4$ [30,31]
RNA transactions	ELP3	Nucleus, Cytosol [32]	[4Fe-4S] $(Cys)_3$ SAM [33,34]
	RPC6	Nucleus [35]	[4Fe-4S] $(Cys)_4$ [36,37]
	CPSF4	Nucleus [38]	[2Fe-2S] $(Cys)_3$ (His)? [39,40]
	TYW1	Nucleus, Cytosol	[4Fe-4S] $(Cys)_3$ SAM [34,41]
	TYW1B	Nucleus	[4Fe-4S] $(Cys)_3$ SAM [34,41]
	CDK5RAP1	Nucleus, Mitochondria, Cytosol [42]	[4Fe-4S] $(Cys)_4$, [4Fe-4S] $(Cys)_3$ SAM [34,43]
Mitosis	KIF4A	Nucleus [44]	[4Fe-4S] $(Cys)_4$? [45]
	KIF4B	Nucleus [44]	[4Fe-4S] $(Cys)_4$? [45]
Iron metabolism	NCOA4	Nucleus, Cytosol [46]	[3Fe-4S] $(Cys)_4$? [47,48]

 doi:10.20944/preprints202410.2446.v1

	FBXL5	Nucleus, Perinuclear region [49]	[2Fe-2S] $(Cys)_4$ [50]
Post-translational modifications	DPH1	Nucleus, Cytosol [51]	[4Fe-4S] $(Cys)_3$ SAM [52]
	DPH2	Nucleus, Cytosol	[4Fe-4S] $(Cys)_3$ SAM [52]
	ATE1	Nucleus, Cytosol [53]	[4Fe-4S] $(Cys)_4$ [54]
Respiration	SDHB	Mitochondria, Nucleus [55]	[2Fe-2S] $(Cys)_4$, [3Fe-4S] $(Cys)_3$, [4Fe-4S] $(Cys)_4$ [55]
Unknown	RFESD	Nucleus	[2Fe-2S] $(Cys)_2$ $(His)_2$
CIA	CIAPIN1	Nucleus, Mitochondria, Cytosol [56]	[2Fe-2S] $(Cys)_4$, [4Fe-4S] $(Cys)_4$? [57,58]
	BOLA2	Nucleus, Cytosol [59]	[2Fe-2S]* Ligands shared with GLRX3 [60,61]
	GLRX3	Nucleus, Cytosol [62]	[2Fe-2S]* Ligands shared with BOLA2 [60,61]
	NUBP2	Nucleus, Cytosol [63]	[4Fe-4S]* Ligands shared with NUBP1 [64,65]
ISC	NFU1	Nucleus, Mitochondria, Cytosol [66]	[4Fe-4S]* Ligands shared between dimers [66,67]
	NFS1	Nucleus, Mitochondria, Cytosol [68]	[2Fe-2S]* Ligands shared with ISCU [69,70]
	GLRX2	Nucleus, Mitochondria [71]	[2Fe-2S]* Ligands shared between dimers [72]

2. Metallation of Nuclear Fe-S Cluster Proteins

The assembly of Fe-S clusters in mammalian cells involves a complex interplay between two machineries: the mitochondrial Iron-Sulfur Cluster (ISC) pathway and the Cytosolic Iron-Sulfur Assembly (CIA) pathway. This organized, multistep process relies on the functions of multiple proteins located inside and outside the mitochondria and consists of common underlying mechanisms of cluster synthesis, trafficking, and insertion into the final recipient proteins. Cofactor maturation of the nuclear [4Fe-4S] proteins depends on the function of both the ISC and the CIA pathways, as evidenced by the defects in the metallation of nuclear Fe-S proteins when depleting either ISC or CIA components [11,65,73–79]. Excellent recent reviews have focused on the known aspects of the *de novo* assembly of clusters inside and outside the mitochondria [2,80,81]. Although numerous studies in yeast and mammalian cells indicate that mitochondria provide the raw materials for the *de novo* assembly of clusters, studies in mammalian cells suggest that *de novo* assembly of Fe-S clusters also occurs in the cytosol via a dual localization of some ISC components in the mitochondria and the cytosol [66,68,82–84]. However, the relative contribution of each pathway to the assembly of clusters in mammalian cells remains unclear.

Similarly, the source of the iron used for the assembly of clusters has not been determined. In the cytosol, the iron chaperone poly C-binding protein 1 (PCBP1) binds iron from the kinetically labile, exchangeable iron pool (called the labile iron pool) and delivers it to ferritin and non-heme iron enzymes [85–88]. The co-chaperone complex comprised of PCBP1 and BOLA2, formed via a bridging Fe ligand, serves as an intermediate for the assembly of [2Fe-2S] clusters on the chaperone complex formed by BOLA2 and the monothiol glutaredoxin GLRX3 [89]. In mitochondria, however, the source of iron for cluster assembly is still an open question due to the lack of known iron chaperones residing in the mitochondrial compartment. Although some early studies suggested that frataxin could act as the mitochondrial iron donor for the ISC [90–93], mounting evidence does not support an iron chaperone function for frataxin, but instead indicates a role as an allosteric activator of the persulfide transfer process during the initial steps of Fe-S cluster assembly [81,94,95]. Consequently, the source of iron for mitochondrial ISC-mediated cluster synthesis remains unknown.

A fully assembled Fe-S cluster may be transferred to a recipient apoprotein that requires the cluster for function. This associative transfer occurs via protein-protein interactions, which protect the labile cofactor from chemical attack during delivery and prevent the cofactor from engaging in harmful redox chemistry. More than one system may be involved in the transfer of clusters to recipient apoproteins. The insertion of Fe-S clusters into apoproteins is facilitated by chaperone

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proteins such as HSC20 [82,84] or co-chaperone systems of the CIA-targeting complex (CTC), composed of three proteins: CIAO1, MMS19, and FAM96B/CIAO2B [74,77,78,96,97]. To assist the incorporation of Fe-S clusters into target proteins, HSC20 recognizes target proteins containing a short peptide (LYR) motif [82]. In contrast, the CTC guides the Fe-S clusters from the CIA to client apoproteins containing a conserved [LIM]-[DES]-[WF] C-terminal tripeptide motif, called the CTC recognition motif, which facilitates the identification of about a quarter of the known CTC clients or their adaptors [98]. However, this C-terminal signature is only present in a handful of the known Fe-S cluster-binding proteins (Table 1). It is still unclear how the apo-client recognition occurs for the remaining 75% of the Fe-S cluster proteins that do not contain the described CTC recognition motif. Alternative recognition motifs and new adaptor proteins mediating cluster transfer from CTC to apoproteins are likely yet to be discovered.

It is broadly assumed that Fe-S clusters are inserted into client apoproteins during protein synthesis and folding in the cytosol and remain bound during the lifetime of the Fe-S cluster protein. This seems necessary for proteins in which the cluster is buried deeply within the tertiary structure of the protein. For example, xanthine oxidase (XDH) contains 2 Fe-S clusters buried in the interior of the protein. The free exchange of these clusters seems unlikely due to steric hindrance inherent in the structure of the folded protein (Figure 1A). However, evidence is lacking that shows Fe-S metallation steps must occur exclusively during folding in the cytosol and before the nuclear protein is translocated to the nucleus. An additional hypothesis points to the possibility of cluster transfer after full translation and folding of the apoproteins. Two interesting observations may shed light in this regard: the evidence of Fe-S cluster transfer via adaptor-mediated protein-protein interactions [98], and the presence of multiple CIA components in the nucleus of the cell.

First, emerging evidence suggests that a subset of Fe-S proteins require an adaptor protein for their recognition by the CTC [98,99]. This is the case for the DNA primase subunit 2 (PRIM2) with its adaptor PRIM1, the elongator subunit ELP3 with its adaptor ELP4, and the ribosome recycling factor ABCE1 with its adaptor LTO1 [98,99]. These three [4Fe-4S] cluster proteins receive clusters from the CTC but do not contain the C-terminal [LIM]-[DES]-[WF] CTC recognition motif in their sequences. Instead, the CTC recognition motif is present in the C-terminus of the corresponding adaptor proteins [98]. This suggests that the transfer of the metalloclusters through adaptor-mediated protein-protein interactions should occur once the proteins have been synthesized and folded, and the client-adaptor complexes have been formed. Supporting this idea, the PRIM2/PRIM1 complex appears to form prior to Fe-S cluster incorporation into PRIM2 [100]. Both PRIM2 and PRIM1, while in complex, directly interact with the CIA targeting complex for cluster transfer [101]. This may only be possible for proteins harboring readily accessible, solvent-exposed cluster binding sites. Figure 1B-D shows that the three proteins, ABCE1, PRIM2, and ELP3, that use an adaptor protein for CTC recruitment have, in fact, solvent-exposed clusters. Thus, it is probable that the CTC donates cofactors to fully- or partially folded proteins with accessible cluster binding sites, while proteins with buried clusters may utilize a co-translational system that delivers clusters during protein folding. Further studies are needed to clarify how this parallel process occurs.

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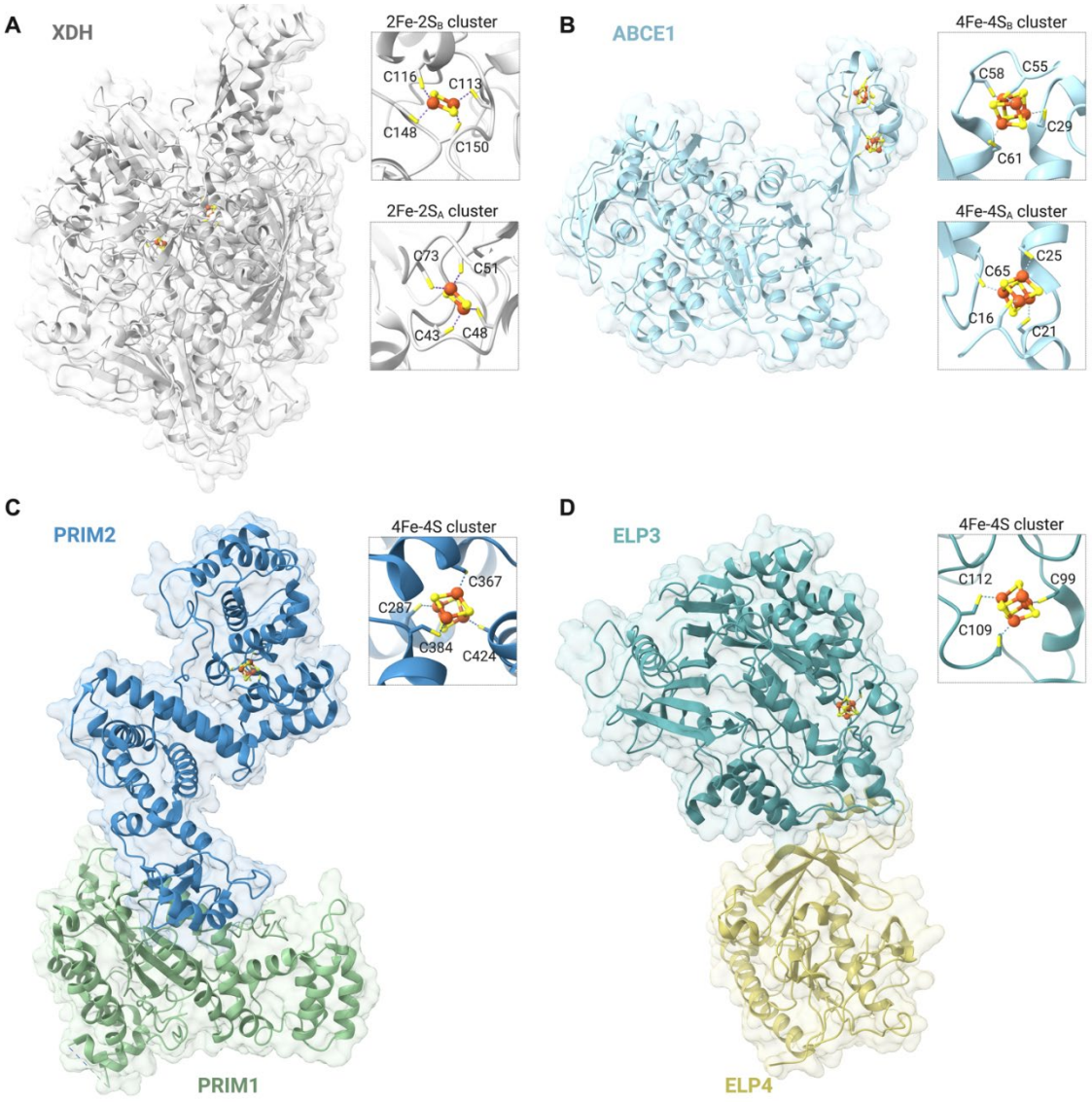

Figure 1. Comparison of solvent-exposed and buried Fe-S clusters in proteins. Structures (left) and close-ups of the Fe-S binding domains (square, right). **A**. X-ray structure of human xanthine oxidase (XDH) showing the two 2Fe-2S clusters deeply buried within the protein structure (PDB 2CKJ). **B.** Cryo-EM structure of the human ribosome recycling factor ABCE1 showing the two 4Fe-4S clusters situated in solvent-exposed loops at the N-terminal domain (PDB 7A09). The clusters in ABCE1 are prone to degradation [102]. Each cluster in ABCE1 is coordinated by three nearby Cys residues and one distant Cys residue in the primary sequence, suggesting a structural role for these clusters [103]. **C.** Cryo-EM structure of human DNA primase subunits, PRIM1 (green) and PRIM2 (blue) (PDB 8QI7). The 4Fe-4S cluster bound to PRIM2 locates at the junction of the N-terminal and C-terminal domains close to the protein surface. PRIM1 is proposed to serve as an adaptor for the recognition of PRIM2 by the CTC to facilitate its metallation. **D.** Cryo-EM structure of the yeast elongator complex proteins ELP3 (green) and ELP4 (yellow) (PDB 8ASV). The radical-SAM enzyme ELP3 has a [4Fe-4S](Cys)$_3$ cluster, which is exposed to solvent via two channels [103]. ELP4 is proposed to serve as an adaptor for the recognition of ELP3 by the CTC to facilitate its metallation.

Although solvent-exposed clusters are inherently more susceptible to oxidative damage [104–107] and may require replacement or repair to maintain their function, proteins with exposed clusters may also exchange cofactors more readily. This lability could confer to these proteins the capacity to sense and respond to environmental cues. For example, the variable metallation status of the iron-responsive protein 1 (IRP1/ACO1) [108], the nuclear receptor coactivator 4 (NCOA4) [109], or the F-

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box and leucine-rich repeat protein 5 (FBXL5) [50], determine the biological activity of these three proteins. They are central to the regulation of intracellular iron metabolism and Fe-S cluster binding allows for coordination of the intracellular iron supply with their cellular activities. These three proteins also harbor solvent-exposed clusters. However, it remains to be demonstrated whether other Fe-S proteins, including nuclear Fe-S enzymes with solvent-exposed cluster binding sites, may exist as a pool of fully folded proteins that incorporate the cluster only when required to respond to cellular needs. Interestingly, although most of the proteins involved in the assembly and delivery of clusters are present in the cytosol, the nucleus of the cell also houses many of them including, MMS19 [21,110], CIAPIN1 [56], CIAO1 [110], CIAO2B [21], GLRX3 [62], NFU1 [66], NFS1 [68], NUBP2 [63], GLRX2 [71], and PCBP1 [5]. Whether these proteins assist in the *in-situ* transfer of iron or Fe-S clusters to client proteins in the nucleus remains elusive.

3. Fe-S Clusters Proteins Involved in Nuclear DNA Transactions

Defects in Fe-S cluster biogenesis result in DNA damage and genome instability. This is due to the requirement of Fe-S clusters for the function of multiple enzymes involved in DNA transactions [76–78,111–114]. In fact, the fastest-growing category of Fe-S binding proteins consists of enzymes that participate in nucleic acid metabolism, including DNA polymerases, primases, glycosylases, helicases, nucleases, transcription factors, RNA polymerases, and RNA-modifying enzymes.

3.1. Fe-S Clusters and DNA Replication

DNA replication in eukaryotic cells relies entirely on several Fe-S cluster-binding proteins, whose function and cluster binding properties are summarized below. Fe-S clusters may link the initiation of DNA replication to intracellular iron availability through licensing factors that ensure replication occurs only when the cell's iron levels are sufficient to meet the high demand for this essential cofactor during DNA synthesis.

3.1.1. DNA Polymerases

The human genome encodes at least 14 DNA-dependent DNA polymerases [115]. Among these, DNA polymerases Pol α, δ, and ε, all located in the nucleus, are considered the replicative DNA polymerases. These multisubunit complexes are classified as class B DNA polymerases and play crucial roles in faithful nuclear DNA duplication [115]. Pol α initiates DNA replication at origins by synthesizing an RNA-DNA hybrid primer of 20-30 nucleotides. This primer is further extended by the highly processive Pol δ and ε on the lagging and leading strands of DNA, respectively [115] (Figure 2A). The catalytic subunits of all eukaryotic replicative DNA polymerases (POLA1, POLD1, and POLE) harbor a [4Fe-4S] cluster that is essential for replisome stability [11,116,117]. The fourth and final member of the class B DNA polymerase family, the error-prone DNA polymerase Pol ζ (zeta) [115], also binds a [4Fe-4S] cluster using a Cys-rich motif at the C-terminal domain of its catalytic subunit, REV3L [11]. Mutations that prevent cluster binding to Pol ζ abrogate its activity in UV mutagenesis repair [118]. Fe-S cluster binding has not been described for other classes of DNA polymerases [11]. Interestingly, although all class B DNA polymerases bind the cubane [4Fe-4S] cluster via a classical 4 Cys arrangement, the site of cluster binding varies. The cysteine motifs CysA and CysB, located at the C-terminus, are conserved among the four members of the family. CysB serves as the cluster binding site for Pol δ and Pol ζ [118]. In contrast, the catalytic domain of Pol ε binds the [4Fe-4S] cluster at an additional Cys-rich motif in the N-terminus, known as CysX, which is unique to Pol ε (C651, C654, C663 and C747) [13,116]. This positions the cluster in an exposed area, away from the DNA template, at the base of the processivity domain (P-domain), near the junction of the P and Palm domains [11,13,116,117]. Mutation of the cluster binding site in Pol ε abolishes DNA binding and proofreading exonuclease activities [119].

The metalation of all class B DNA polymerases depends on the function of the CIA pathway. Mutations in the CIA machinery lead to the loss of clusters in DNA polymerases, resulting in reduced protein stability and activity that causes genome instability [11,74,77,78]. Although the precise roles

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of Fe-S clusters in these proteins remain enigmatic, increasing evidence suggests that they may function as redox switches controlling the speed of DNA synthesis. Faster synthesis is associated with the reduced form of the [4Fe-4S] cluster present in Pol δ. This may provide a rapid and reversible mechanism to respond to replication stress by slowing down the DNA polymerase through cluster oxidation, allowing the accumulation and activation of DNA repair factors at lesion sites. Once DNA is repaired, reduction of the polymerase cluster may enable replication to resume [120]. This model needs further validation.

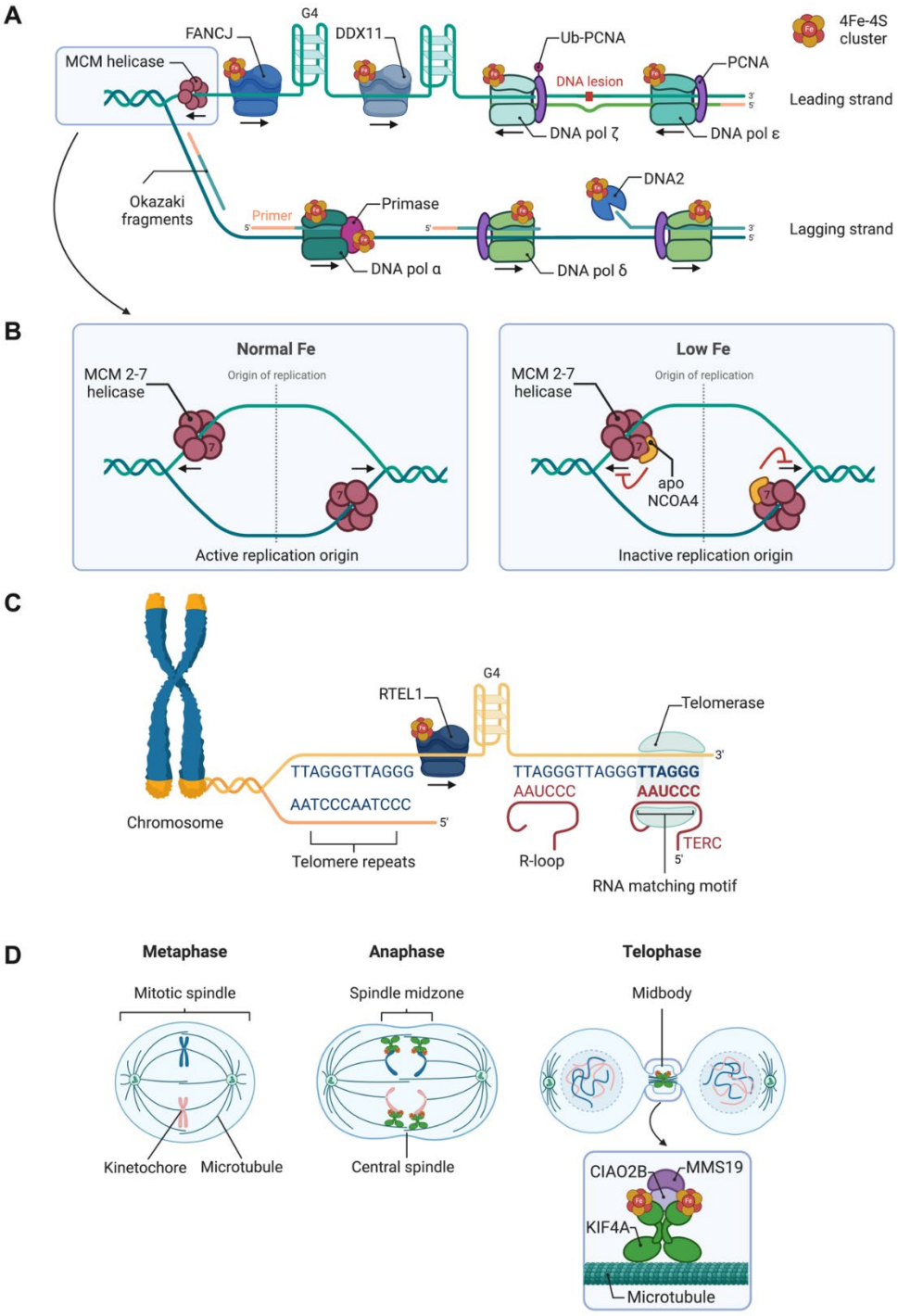

Figure 2. Essential roles of nuclear Fe-S cluster-binding proteins in DNA replication, telomere maintenance, and mitosis. **A.** Representation of a eukaryotic DNA replication fork. The mini-chromosome maintenance (MCM2-7) helicase complex unwinds DNA at replication origins, separating the double helix to generate the leading and lagging strands. All replicative DNA

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polymerases, Pol α, δ, and ε, bind a 4Fe-4S cluster. DNA replication initiates with an RNA primer (orange) synthesized by DNA primase, with the regulatory subunit PRIM2 also binding a 4Fe-4S cluster. Pol ε further extends the primer on the leading strand, while Pol δ does so on the lagging strand. The proliferating cell nuclear antigen sliding clamp PCNA enhances the processivity of DNA polymerases and facilitates DNA repair. The 4Fe-4S cluster DNA helicases FANCJ and DDX11 unwind G4s to prevent stalling of the replisome. DNA lesions obstruct replication, triggering monoubiquitylation of PCNA, which recruits the DNA damage bypass DNA polymerase Pol ζ. The catalytic subunit of Pol ζ also binds a 4Fe-4S cluster. Finally, the 4Fe-4S cluster nuclease-helicase DNA2 cleaves exposed single stranded DNA ends from Okazaki fragments and stalled replication forks. **B.** Under Fe-sufficient conditions, the MCM helicase moves in opposite directions from the activated origin of replication. This results in the formation of two functional replication forks, enabling DNA synthesis. Under iron-depleted conditions, apo NCOA4 accumulates in the nucleus and binds to the helicase subunit MCM7 hindering MCM helicase activity, and inactivating replication origins. This ensures that DNA synthesis only occurs when there is a sufficient pool of metallated Fe-S enzymes, thereby maintaining genomic integrity and preventing replication under suboptimal conditions. **C.** The 4Fe-4S cluster helicase RTEL1 unwinds G4s and R-loops at the telomeric repeats, facilitating telomere replication by telomerase and preventing telomere shortening. **D.** During the late stages of mitosis (metaphase, anaphase, and telophase), the nuclear 4Fe-4S cluster mitotic factor KIF4A binds the arms of condensed chromosomes. As a kinesin, KIF4A moves along microtubules to mobilize cargoes. KIF4A localizes at the central spindle, accumulating at the spindle midzone and midbody. The close-up image on the bottom illustrates the co-localization of the CIA targeting factors MMS19 and CIAO2B with KIF4A during mitosis, suggesting an *in situ* metallation of KIF4A during function.

The presence of the cluster in Pol α is somewhat controversial because structural data shows Zn $[2]^{+}$ bound at the position of the Fe-S cluster, which suggests that the presence of a cluster in Pol α might prevent its interaction with the corresponding B subunits [118,121–125]. Although no structures of Pol α showing a bound [4Fe-4S] cluster are currently available in the Protein Data Bank (PDB), it is not uncommon to find zinc replacing *bona fide* Fe-S clusters in protein structures due to the intrinsic sensitivity of these clusters to oxidative damage under aerobic conditions [116,126–131]. Resolving these discrepancies is essential to bridging a critical gap in our understanding of the role of Fe-S cluster in DNA polymerases.

3.1.2. DNA Primase

DNA replication begins with the synthesis of a short RNA primer of 8–12 nucleotides catalyzed by DNA primase. The primer is then extended by the catalytic subunit of Pol α [115] (Figure 2A). The primosome consists of four subunits: the catalytic subunit POLA1, the accessory subunit POLA2, and two primase subunits, the small catalytic subunit PRIM1 and the large regulatory subunit PRIM2. Each is located in the nucleoplasm, [5] where DNA replication occurs. In addition to the cluster in POLA1, the primase complex contains an additional [4Fe-4S] cluster bound to the C-terminal domain of PRIM2, which is essential for primer synthesis [8,9,132]. None of the smaller non-catalytic subunits of Pol δ or Pol ε harbor Fe-S clusters [11]. PRIM2, which is present only in archaeal and eukaryotic primases [132–134], bridges the two catalytic domains of the primosome–POLA1 and PRIM1, thereby facilitating the transition from RNA primer synthesis to DNA elongation [132].

The metalation of PRIM2 relies on the CIA machinery [16,77,97,132]. It has been proposed that PRIM1 may act as an adaptor protein between PRIM2 and the CIA targeting complex (Figure 1C), aiding in the client protein recognition necessary for cluster transfer [98,101]. The [4Fe-4S] cluster in PRIM2 is bound via a canonical four-Cys motif (C287, C367, C384, C424), located at the junction of the N-terminal and C-terminal domains, with each domain contributing two binding Cys residues [8,9,121,133–135] (Figure 1C). This arrangement suggests that the Fe-S cluster serves a structural rather than a redox-active role in the protein. However, one might question why such a complex cofactor would be used solely to preserve the structural integrity of a small domain. Recent evidence indicates that the [4Fe-4S] cluster in DNA primase functions as a redox switch, utilizing DNA charge

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transport to regulate the protein's DNA binding activity [136,137]. Briefly, oxidized [4Fe-4S] $[3]^{+}$ PRIM2 binds more tightly to DNA than its reduced counterpart [136,137]. Electrostatic analyses revealed that the oxidation/reduction of the cluster, along with the accumulation of negative charges on the newly synthesized primers, induces structural changes at the DNA-protein interface [138]. The redox switch model proposes that the redox state of the [4Fe-4S] cluster in DNA primase acts as a reversible switch for DNA binding. The oxidized cluster activates the primase, enhancing its binding to DNA to initiate primer synthesis. As the primase extends the RNA primer, Pol α interacts with the RNA/DNA primer-template, and its [4Fe-4S] facilitates DNA-mediated redox signaling for primer truncation. This promotes PRIM2 dissociation by reducing its cluster, which allows the handoff of the primed template to Pol α [136,137,139]. This is a model and further research is needed to determine whether a DNA-mediated electron transfer relay coordinates the association, transfer, and dissociation steps of replication.

3.1.3. Fe-S Clusters and DNA Replication Origins

Nuclear receptor coactivator 4 (NCOA4), is the protein responsible for targeting ferritin for degradation during iron starvation, reintroducing iron into the labile iron pool. Under iron-replete conditions, iron-bound NCOA4 is targeted for proteasomal degradation by the ubiquitin ligase HERC2, which maintains ferritinophagy at basal levels [140,141]. It was recently shown that NCOA4 binds a 3Fe-4S cluster [48]. The insertion of the Fe-S cluster in NCOA4 promotes its recognition by HERC2, triggering its degradation and inhibiting ferritinophagy [47]. How NCOA4 acquires the Fe-S cluster is unknown. In addition to its role in ferritinophagy, NCOA4 is a multifunctional protein also implicated in coordination between cellular iron availability and the DNA replication machinery [109]. Federico et al. demonstrated that under low-iron conditions, apo-NCOA4 binds to DNA replication origins, inhibiting their activation and preventing replication stress [109]. NCOA4 specifically interacts with MCM7, a subunit of the MCM2-7 helicase complex that unwinds DNA during replication. Binding of NCOA4 to the MCM complex hinders its helicase activity [46] (Figure 2B). This mechanism may allow mammalian cells to respond to low iron by delaying initiation of DNA replication, an adaptive response when the pool of metallated Fe-S enzymes is limited. By finely tuning DNA replication to fluctuations in intracellular iron levels, this mechanism may help to safeguard genome stability during periods of diminished bioavailable iron and replicative stress [109].

3.1.4. Fe-S Clusters in Helicase Activity

Helicases are motor proteins that move along linear nucleic acid tracks using the energy from the hydrolysis of a nucleoside triphosphate. They function by destabilizing the hydrogen bonds between the complementary base pairs of unwinding DNA or RNA strands to generate the single-stranded intermediates required for processes like replication, repair, recombination, transcription, and splicing. Helicases are classified into six superfamilies, SF1-6 [142]. From the about 24 DNA helicases present in human cells, only five are known to bind Fe-S clusters. Those belong to the two largest superfamilies of DNA helicases (SF1 and SF2) [143]. Yet not all members of those families bind a cluster. Four nuclear DNA helicases from the SF2 family (Rad3/XPD group) contain Fe–S clusters in mammalian cells: the Fanconi Anemia (FA) protein J FANCJ, the Warsaw Breakage Syndrome protein ChlR1/DDX11, the telomere maintenance helicase RTEL1, and the component of the TFIIH complex involved in transcription and nucleotide excision repair XPD/ERCC2 [4,19,20,77,78,143]. XPD, the founding member of the Fe-S helicase family, is a central component of the general transcription factor TFIIH which plays major roles in transcription and nucleotide excision repair (NER), discussed below. FANCJ participates in the homologous recombination pathway of DNA double strand break repair, and it is part of the FA pathway proposed to function downstream of FANCD2. FANCJ also interacts with the breast cancer type 1 susceptibility protein BRCA1 and contributes to its DNA repair function. DDX11 has roles in replication of DNA, establishment of sister chromatid cohesion, and maintenance of chromosome architecture. RTEL1 is essential for preserving telomere length as discussed below. The SF1 helicase/nuclease DNA2 is the fifth helicase known to

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bind a Fe-S cluster in mammalian cells [23,143,144]. Although initially reported to be exclusively in the mitochondria [145], DNA2 also localizes to the nucleus [22]. DNA2 participates in the resolution of stalled replication forks during DNA synthesis [24] (Figure 2A). All these Fe-S helicases have been linked to multiple cancers and hereditary disorders characterized by genome instability [4,21,146–150] and mutations resulting in loss of the cluster are associated with the onset of disease. For example, mutations adjacent to the Fe-S cluster-binding Cys residues (M299I and A349P) in FANCJ are linked to breast cancer and Fanconi anemia, respectively [15,151–153].

The four SF2 helicases bind a single cubane [4Fe-4S] cluster using a canonical 4Cys arrangement (Table 1) located within the helicase core at the N-terminus of the proteins [4,17]. The cluster binding to DNA2 is unusual, as one of the four Cys residues coordinating the cubane [4Fe-4S] is located a few hundred amino acids upstream of the other three (C136, C393, C396, C402) [24]. Structurally speaking, there are two different types of helicases: those that form ring-like hexameric structures and those that do not. None of the known Fe-S cluster binding helicases form hexameric ring-like structures.

All known eukaryotic Fe-S cluster-containing helicases have a 5'-3' polarity and unwind DNA substrates. Although the clusters are essential for the DNA unwinding activity of the SF2 helicases [17,146,153], they do not affect the DNA binding and ATP hydrolysis capacities [153]. In contrast, loss of the cluster in DNA2 not only impairs DNA binding, but also impairs the nuclease and ATPase activities [24]. The Fe-S helicases exhibit an apparent specialization for the resolution of non-canonical DNA structures, including G4-quadruplexes (G4s), D-loops, and R-loops [154]. However, not all G4-resolving helicases have been reported to bind an Fe-S cluster, such as the helicases BLM, and WRN [146]. Non-canonical DNA structures play roles in genome organization, gene regulation, replication initiation and progression, recombination, and telomere metabolism [155,156]. However, unscheduled accumulation of these secondary DNA structures stall replication forks, causing genome instability. FANCJ efficiently resolves G4 DNA structures to enable smooth DNA synthesis during replication [157–159] (Figure 2A). RTEL1 resolves R-loops and G4s [160–162] and binds to the rG4 formed by the Telomeric Repeat-Containing RNA (TERRA) for to the maintenance of *TERRA*-containing R-loops, enhancing telomere stability [163]. DDX11 also resolves G4 DNA structures [164] to support sister chromatid cohesion [165]. And finally, G4 unwinding activity is also sustained by the archaeal XPD helicase [166]. Although it is clear, at least for FANCJ, that the presence of the Fe-S cluster is essential for this G4 unwinding activity [153], the mechanism by which the Fe-S cluster facilitates the unwinding of non-B DNA structures catalyzed by these helicases is still poorly understood.

The metallation of the helicases likely depends on the CIA machinery, as suggested by the interaction of MMS19 with XPD, FANCJ, DDX11, RTEL1, and DNA2 [16,21,77,78,100,110,167,168] and the decreased protein levels of these helicases when MMS19 is depleted [16,77,78]. The interaction of HSC20 with XPD, and DDX11 has been also confirmed [84]. However, several questions remain regarding the cluster insertion on those enzymes. For example, when the interaction of these helicases with other components of the CIA targeting complex was examined, XPD, DNA2, and RTEL1 were found to interact with CIAO1 and CIAO2B [97], however FANCJ did not interact with CIAO1 [16]. CIAO1 is the CTC component that offers the docking site for CIA client proteins to interact with the CIA delivery system. In general, it is not known how these Fe-S proteins are recognized for cluster delivery as they all lack the C-terminal CTC recognition motif proposed to facilitate the recognition of CIA clients. Further investigation is required to identify the factors that mediate the delivery of cluster to all the nuclear DNA helicases.

Surprisingly, Fe-S cluster binding in RNA helicases has not been described in mammalian cells. The only example of an RNA helicase binding an Fe-S cluster is the viral 5′-3′ SF1 helicase nsp13, the metalation of which depends on the host Fe-S cluster assembly machinery [127]. However, helicases that are currently annotated as Zn-binding proteins may in fact bind Fe-S clusters.

3.1.5. Fe-S Clusters and Telomere Maintenance

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Telomeres, the protective caps at the ends of chromosomes that prevent degradation and fusion of chromosomes, also rely on functional Fe-S cluster enzymes for their maintenance. Replication of the telomeres is mechanistically challenging due to their repetitive nature and the presence of non-canonical DNA structures, such as G4s. Ultimately this results in the shortening of the telomeres during cell division. Key among the proteins involved in telomere maintenance is the ATP-dependent DNA helicase RTEL1, that unwinds G-quadruplex structures at telomeric DNA to facilitate telomere replication, preventing telomere loss [169] (Figure 2C). RTEL1 is located in the nucleoplasm and in nuclear speckles [5] and binds a redox-active cubane [4Fe-4S] cluster that is essential for its helicase activity. The cluster is bound at the N-terminus via a four-Cys arrangement (C145, C163, C172, C207) [20]. Although the role of the cluster in RTEL1 activity has not been determined, the physical interaction of RTEL1 with MMS19 [77,78] and the altered telomere length observed in MMS19 mutants [170] confirm that cluster insertion in RTEL1 depends on the CIA machinery.

3.2. Fe-S Clusters and DNA Repair

An estimate of 50,000 DNA-damaging events occur in our cells per day [171]. These lesions block replication-fork progression, cause cell cycle arrest, and constitute a major source for genome instability. At least six major DNA repair systems are required to remove the distinct types of DNA damage: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), non-homologous end joining (NHEJ), and translesion DNA synthesis (TLS) [172]. Fe-S clusters are integral components of the molecular machinery that repair DNA to ensure the faithful replication and distribution of genetic material during cell division (Figure 3). In addition to the Fe-S DNA polymerases and helicases discussed above, which are central to all DNA repair mechanisms [172,173], two Fe-S cluster DNA glycosylases, MUTYH and NTHL1, play central roles in the repair of oxidative DNA damage [174–176]. These enzymes cleave the glycosidic bond between bases and sugars to remove damaged or mispaired bases from the DNA duplex and act as initiators of BER [177]. MUTYH is specific for removing mismatched adenines and 8-dihydro-8-oxodeoxyguanine (8-oxoG) [178] and NTHL1 removes oxidatively-damaged pyrimidines in DNA [179]. They bind a redox-active cubane [4Fe-4S] cluster in the catalytic domain using a four-cysteinyl motif located in a solvent-exposed loop, referred to as the Fe-S cluster loop (FCL) motif (Table 1) [28,180–183]. Although originally assumed to play purely structural roles, the Fe-S clusters in DNA glycosylases are required for DNA binding and affect the detection and removal of the damaged bases [183–185]. Electrochemical studies using purified proteins indicate that cluster oxidation in the bacterial DNA glycosylases EndoIII and MutY promotes an increase in binding affinity [186,187]. The hypothesis is that the cluster binding motif is positioned for interaction with the DNA backbone [27,182,188] and the redox potential of the Fe-S clusters may aid glycosylases in scanning the DNA duplex for damaged bases [184]. Finally, although the metallation of these nuclear DNA glycosylases relies on the CIA machinery [110], they lack the C-terminal motif believed to guide the CIA targeting complex to client apo-proteins.

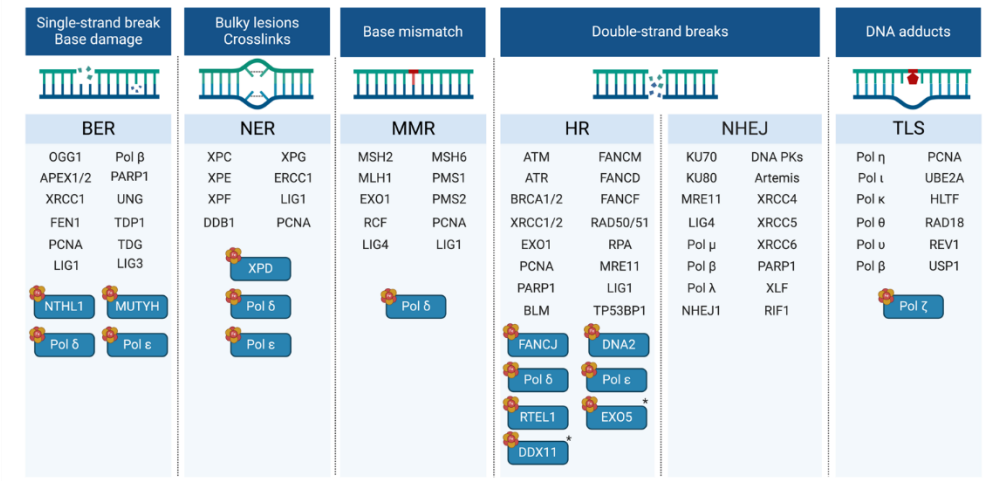

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Figure 3. Fe-S cluster proteins within the six major DNA repair pathways: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), non-homologous end joining (NHEJ), and translesion DNA synthesis (TLS). Schematic representation of DNA lesions and the corresponding repair pathways. Key proteins involved in each pathways are organized according Knijnenburg et al. [172]. DNA repair proteins that bind 4Fe-4S clusters are shown in a blue square at the bottom. * While DDX11 and EXO5 are not formally categorized under HR, their functions align with this repair mechanism.

3.2.1. Fe-S Clusters, Transcription, and Nuclear RNA Transactions

Fe-S proteins are also involved in RNA processing pathways by modulating the activity of transcription factors, RNA polymerases, and RNA modifying enzymes. These proteins can be found as subunits of the RNA polymerase complex III, the transcription factor IIH, the elongator complex, and the pre-mRNA processing complex in mammalian cells. Although Fe-S binding transcriptional regulators that sense intracellular iron levels have not been described in mammalian cells, examples from yeast will be reviewed below to illustrate the transfer of Fe-S clusters between regulators in the nucleus of eukaryotic cells.

3.2.2. Fe-S Cluster Binding Fe-Dependent Transcriptional Regulators

Fe-dependent transcriptional regulators that bind Fe-S clusters and thereby sense intracellular iron levels have been described in yeast species but are not yet found in mammalian cells. In this discussion, we focus on fungal transcriptional regulators that sense intracellular iron levels, as their metalation appears to occur in the nucleus, suggesting the presence of dynamic Fe-S trafficking within the nuclear space. Although the iron regulatory mechanisms in the nonpathogenic fungi *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are fundamentally distinct, both rely on Fe-S clusters as signals to regulate iron acquisition, storage, and utilization within the cell. In *S. cerevisiae*, the Fe-S cluster-binding transcriptional activators Aft1 and Aft2 regulate the expression of genes involved in iron uptake and use. Under iron-sufficient conditions, a heterodimeric [2Fe-2S] chaperone complex comprised of a monothiol glutaredoxin (Grx3/4) and a BolA-like protein (Bol2) binds and delivers the cluster to the DNA-bound transcriptional activators, altering their oligomeric state and leading to their dissociation from DNA, thereby suppressing the transcription of iron uptake genes [189–191]. In *S. pombe*, the transcriptional repressors Fep1 and Php4 regulate the expression of iron uptake and iron utilization genes, respectively. Under iron-depleted conditions, Php4 accumulates in the nucleus and associates with the DNA-binding complex Php2/3/5 to suppress the expression of iron utilization genes. As iron levels increase, a [2Fe-2S] cluster-dependent interaction between Php4 and Grx4 promotes the dissociation of Php4 from the Php2/3/5 complex, leading to the de-repression of iron-usage gene expression [192–194]. In contrast, the transcriptional repressor Fep1 binds a [2Fe-2S] cluster under iron-sufficient conditions, which facilitates its binding to DNA and halts the expression of iron uptake genes [195,196]. Under iron-depleted conditions, the cluster is unidirectionally transferred from Fep1 to Grx4-Fra2, causing the apo form of Fep1 to detach from DNA, allowing the expression of iron uptake genes [195]. The mechanism by which Fep1 acquires the cluster remains unclear. Notably, Fep1 localizes to the nucleus under both iron-replete and iron-deficient conditions [197], and remains bound to DNA when iron is sufficient. This suggests that the transfer of the cluster between Fep1 and the Grx4-Fra2 chaperone system occurs within the nucleus.

The [2Fe-2S]-coordinating complex formed by the glutaredoxin protein Glrx3 and the BolA-like protein BolA2 is also present in the cytosol of mammalian cells, where it participates in the maturation of cytosolic Fe-S cluster proteins [61]. Although mammals, unlike fungi, do not have iron-responsive transcriptional regulators such as Aft1/2, Fep1, or Php4, it is tempting to infer from the fungal evidence that the Glrx3-[2Fe-2S]-BolA2 system may constitute a pool of bioavailable, exchangeable clusters that are transferred to client partners for signaling purposes. However, these proposed roles remain to be experimentally tested.

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3.2.3. RNA Polymerase III

Pol III is a DNA-dependent RNA polymerase specialized for the transcription of tRNAs and other short, essential RNAs. It is responsible for transcribing approximately 15% of total cellular RNA, including tRNA, 5S ribosomal RNA, U6 spliceosomal RNA, and other small ubiquitous RNAs. Misregulation of human Pol III is associated with tumor transformation, neurodegenerative and developmental disorders, and increased susceptibility to viral infections [198,199]. Pol III is a multi-subunit complex composed of 17 subunits, with a ten-subunit catalytic core and a peripheral heterodimeric stalk [35,200]. RPC6 (POLR3F), the subunit that binds a cubane [4Fe-4S] cluster, is part of the peripheral heterotrimeric complex RPC3/RPC6/RPC7 that modulates Pol III dynamics during transcription initiation [36,37,201]. The Fe-S cluster binds to the C-terminus of RPC6 via the thiol groups of four Cys residues (C287, C290, C296, C307). The Fe-S cluster is sandwiched between RPC3, RPC7, and the RPC1 clamp, tethering the interactions between the catalytic core and the RPC3-RPC6-RPC7 heterotrimer, thereby stabilizing Pol III [37]. Interestingly, although the Fe-S binding residues of RPC6 are widely conserved in many organisms, they are missing in *Saccharomyces cerevisiae* [199]. Distinct from other RNA polymerases, Pol III exhibits functional activities in both the nucleus and the cytoplasm. The canonical function of Pol III involves transcribing tRNAs, 5S rRNAs, and other small RNAs within the nucleus. Interestingly, cytoplasmic Pol III plays a vital role as a viral DNA sensor, synthesizing RNA from AT-rich viral DNA templates and triggering innate immune responses [202,203]. The current model suggests that Pol III is assembled in the cytosol, with some of it translocated into the nucleus and some remaining in the cytosol to participate in viral DNA sensing and response [203]. However, the actual factors facilitating Fe-S cluster insertion in RPC6 remain to be elucidated.

3.2.4. Transcription Factor IIH

The general transcription factor TFIIH participates in transcription initiation and nucleotide excision repair [204]**.** It is required for promoter opening and the transition from the pre-initiation complex to active transcription. TFIIH is a protein complex composed of ten subunits, divided into two subcomplexes: the core (XPB, p62, p52, p44, p34, p8) and the CDK-activating kinase (CDK7, cyclin H, MAT1) [205]. These subcomplexes are bridged by the helicase XPD, which connects p44 and MAT1. XPD (ERCC2) is a mostly nuclear helicase [5] that binds a [4Fe-4S] cluster via 4 Cys (C116, C134, C155, C190) in its N-terminal domain [4]. Mutations in XPD affect the interaction between XPD and p44, resulting in dissociation of XPD from the TFIIH complex. This disruption results in defects in the phosphorylation of various transcriptional activators, causing aberrant transcriptional activation [206–208].

3.2.5. RNA Modifying Proteins

In addition to capping and splicing, pre-mRNA undergoes 3' end maturation before protein translation. The cleavage and polyadenylation specific factor complex (CPSF) plays a crucial role in this pre-mRNA processing step by adding a poly-A tail to the mRNA. CPSF consists of six different proteins that recognize the highly conserved polyadenylation signal in the pre-mRNA, cleave the 3′ end, and recruit additional polyadenylation factors. CPSF4, one of the subunits of the complex, binds one [2Fe-2S] cluster and four Zn $[2]^{+}$ ions as cofactors. *In vitro* analyses have shown that both metal cofactors are necessary to recognizing the polyadenylation signal in the pre-mRNA [129]. CPSF4 contains 5 CCCH domains, which are known to bind Zn and Fe-S clusters. The binding site for the [2Fe-2S] cluster in CPSF4 exhibits flexibility due to redundant iron-binding sites, allowing the cluster to be bound to an alternative site when the preferred site is mutated [129,209]. It is currently unknown which CCCH domain of CPSF4 is the preferred binding site for the [2Fe-2S] cluster *in vivo*. It is of interest to determine whether such flexibility in cluster binding sites have a meaningful functional effect on the biological role of the protein. Notably, CPSF4 is the only protein, exclusively localized in the nucleus known to bind a [2Fe-2S] cluster. Although a physical interaction between CPSF4 and

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the Fe-S chaperone HSC20 has been described [84], the mechanism by which CPSF4 acquires its cluster is not well established.

The multi-functional Elongator complex facilitates transcription by RNA Pol II [32], but is also responsible for the post-transcriptional modification of tRNAs at the wobble uridine (U_{34}), a crucial modification that enhances translation efficiency [34]. It is composed of two copies of each of its six individual protein subunits, ELP1-ELP6, that form two discrete subcomplexes known as ELP123 and ELP456 [210]. ELP3 is the catalytic subunit of the complex with tRNA acetyltransferase activity, which is necessary for multiple tRNA modifications, including formation of 5-methoxycarbonylmethyl uridine (mcm5U) and its derivates [34]. ELP3 harbors a cubane [4Fe-4S] cluster bound to its N-terminus via 3 cysteines (C99, C109, C112) and an exchangeable S-adenosyl-L-methionine (SAM) (Figure 1D) [33,210–212]. Interestingly, ELP3 is the only Elongator subunit that has orthologs present in eukaryotes, archaea, bacteria, and viruses [211]. ELP3 interacts with MMS19 [78,110], and deficiency in ELP3, and other Fe-S proteins, was observed in cell lines derived from patients with biallelic loss of function in *CIAO1* [213], confirming that ELP3 acquires its cluster from the CIA complex. As discussed above, ELP4 may serve as an adaptor protein between CTC and ELP3 (Figure 1D), exposing the C-terminal tryptophan motif recognized by the CIA targeting complex for the delivery of [4Fe-4S] clusters to client proteins [98]. However, demonstration of the involvement of other CIA factors and ELP4 in facilitating the metallation of ELP3 is still lacking.

Humans have three other tRNA-modifying enzymes in the nucleus that bind Fe-S clusters. The SAM-dependent tRNA 4-demethylwyosine synthases, TYW1 and TYW1B, are involved in the wybutosine biosynthesis pathway and are responsible for forming imidazoline rings. This RNA modification is important for stabilizing the codon-anticodon interaction, maintaining the reading frame and preventing errors during translation [34,214]. Information reported in databases suggest that TYW1 localizes to the nucleus (reason for the inclusion in Table 1). However, a recent publication shows that TYW1 mainly localizes in the cytosol in human cells [215], which is the compartment where the type of tRNA modification catalyzed by this enzyme is assumed to occur [216]. The third protein, CDK5RAP1, is a methylthiotransferase responsible for modifying four mitochondrial DNA-encoded tRNAs to optimize mitochondrial translation [217]. CDK5RAP1 is primarily located in the mitochondria but can also be found in the nucleoplasm and cytosol. Its RNA modification activity is not limited to mitochondrial tRNA, as there is evidence that it also introduces modification into nuclear RNA species [42]. These three proteins bind cubane [4Fe-4S] clusters via 3 cysteines and an exchangeable SAM (Table 1).

4. Fe-S Cluster Proteins in Mitosis

Once DNA is fully replicated, cells enter mitosis following an intricate and highly regulated process that ensures each new cell receives an exact copy of the genetic blueprint. It has long been established that MMS19 and CIA2B localize to the mitotic spindle during mitosis and that their knockdown disrupts the mitotic pathway, leading to poor alignment of chromosomes, improper segregation, accumulation of nuclei with abnormal shapes, and mislocalization of key mitotic factors [21]. However, it was only a few years ago that a mitotic factor was identified as binding an Fe-S cluster. The chromokinesins KIF4A, which is involved in faithful chromosome segregation during mitosis, binds an Fe-S cluster [45] (Figure 2D). Molecular motor kinesins are the primary source of cellular forces and drive many types of intracellular movement along microtubule networks. KIF4A and KIF4B are the two variants of the kinesin family 4 present in humans. They share 98% homology in their DNA sequence with different chromosomal location [218]. This ATP-dependent microtubule-based motor kinesin resides in the nucleoplasm during interphase and on the arms of condensed chromosomes during mitosis. KIF4A accumulates at the central spindle that connects the two daughter cells during the late stages of mitosis, from late anaphase through cytokinesis [44,219,220]. This stabilizes the bipolar mitotic spindle, which is essential for successful midzone formation and cytokinesis. In KIF4-deficient cells, the central spindle becomes disorganized, leading to cytokinesis failure [221,222], chromosome misalignment, spindle defects, and chromosome missegregation. This

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results in shorter and wider chromosomes, lagging chromosomes, aneuploidy, and ultimately tumor formation [220,223].

Unlike other motor kinesins, KIF4A resides in the nucleus during interphase where it performs additional non-mitotic functions. KIF4A has been proposed to play a role in the DNA damage response by modulating the BRCA2/Rad51 pathway. Its C-terminal cargo-binding domain interacts with the conserved C-terminal region of BRCA2, facilitating the translocation of BRCA2 to damaged DNA regions following injury [224]. Depletion of KIF4A results in a significant slowdown of DNA replication and decreased homologous recombination [224]. KIF4 also acts as a modulator of large-scale chromatin architecture. It binds globally to chromatin and its depletion leads to chromatin decondensation and loss of heterochromatin domains [223]. Overall, KIF4 deficiency has been associated with developmental delays and intellectual disabilities [225] and it is abnormally expressed in multiple cancers [218]. KIF4A has been also implicated in the infection process of Hepatitis B and D viruses by regulating the transport of the NTCP receptor from the cytoplasm to the cell surface, where it functions as a receptor for viral entry [218,226].

Structurally speaking, KIF4A features a highly conserved ATPase/motor domain at the N-terminus that binds to microtubules and provides the mechanochemical force for movement. This is followed by a central α-helical stalk domain and a C-terminal cargo-docking domain responsible for capturing cargoes [227,228]. The [4Fe-4S] cluster in KIF4A is bound to a conserved cysteine-rich domain located at the C-terminus [45]. However, it remains unclear which of the nine conserved Cys residues located in that region coordinate the Fe-S cluster. Whether the cluster is redox-active is also unknown.

In mitotic cells, KIF4A colocalizes with the CIA components CIAO2B and MMS19 at the spindle midzone and midbody between separating chromosomes [45] (Figure 2D). KIF4A also co-immunoprecipitates with CIAO2B and MMS19, confirming their physical interaction [45]. This interaction is enhanced after iron chelation, suggesting a more stable association between the CIA complex and the apo client protein [45]. Similar enhancements in interactions between CIA and client proteins under iron deprived conditions has been observed for other Fe-S proteins, including XPD, FANCJ, and Pol δ [78]. The mislocalization of the KIF4A variant lacking the conserved C-terminal cysteine domain that binds the cluster, along with the reduced association of endogenous KIF4A with the midzone/midbody following CIAO3 depletion, suggests that the Fe-S cluster in KIF4A modulates the proper subcellular localization of the protein at the mitotic spindle. This likely influences the protein-protein interactions essential for the mitotic process [45].

Although the cluster in KIF4A is quite labile in [55]Fe radiolabeling experiments, its absence does not appear to affect the stability of the protein [45], as seen for other Fe-S cluster binding proteins. This dynamic nature may be biologically relevant and allow for the assembly and disassembly of the Fe-S cluster in KIF4A to regulate the progression of mitosis [45], which could explain why the late-acting CIA factors colocalize at the midbody with components of the mitotic machinery. This suggests that the cluster is transferred to a folded client protein at the site of function, rather than co-translationally transferred. Further mechanistic studies are needed to elucidate the role of the Fe-S cluster in modulating KIF4A activity and whether the metallation of KIF4A occurs only after the breakdown of the nuclear membrane when cells enter mitosis, or if KIF4A residing in the nucleus during interphase also binds an Fe-S cluster.

KIF4A and KIF4B are currently the only known components of the mitotic machinery that bind an Fe-S cluster. However, the significant mitotic defects observed when silencing the CIA, along with the colocalization of late-acting CIA factors with components of the mitotic machinery, suggest the potential existence of other, yet-to-be-discovered Fe-S cluster proteins that are essential for successful mitosis.

5. Other Fe-S Cluster Proteins in the Nucleus

The inventory of nuclear Fe-S cluster proteins presented in Table 1 includes some unexpected proteins whose functions are typically associated with other cellular compartments. These proteins are involved in post-translational modifications (ATE1, DPH1, and DPH2), nucleotide metabolism

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(XDH), maturation of cytosolic Fe-S proteins (CIAPIN1, GLRX3, and BOLA2), immune modulation and restriction of viral replication (RSAD2), and mitochondrial function (SDHB, GLRX2, NFS1, and NFU1). It remains unclear why these proteins are also localized in the nucleus and whether they retain the function already characterized in other subcellular localizations or have alternative roles while in the nucleus.

6. Conclusions and Open Questions

About half of all known Fe-S binding proteins in human cells localize to the nucleus, with many identified only recently. A few decades ago, the presence of iron cofactors in the nucleus was considered unlikely due to the threat that the reactive iron poses to the genetic material. However, chaperoning and trafficking systems that safely mobilize these cofactors facilitate their incorporation in DNA metabolic enzymes, leveraging their redox capabilities while minimizing risks. The connection between iron cofactors and genome integrity is now well established, with an increasing number of nuclear Fe-S proteins, particularly those involved in nucleic acid metabolism such as DNA replication and repair. The high metabolic expense associated with incorporating clusters into proteins suggest that they play essential roles. However, the mechanistic benefit of incorporating Fe-S clusters into nucleic acid-binding proteins remains unclear. Mismetallation of these nuclear Fe-S cluster enzymes often leads to genome instability, which is linked to various diseases, including breast and ovarian cancer, Fanconi anemia, Xeroderma pigmentosum, and the Cockayne, Warsaw breakage and Hoyeraal-Hreidarsson syndromes.

Most nuclear Fe-S cluster proteins bind cubane [4Fe-4S] clusters using a canonical four-cysteinyl ligand structure. A few exceptions exhibit alternative architectures for cluster binding sites and stoichiometries, including the [2Fe-2S] proteins CPSF4 and FBXL5, the [3Fe-4S] protein NCOA4, and the radical SAM enzymes that bind the [4Fe-4S] cluster using three cysteines and an exchangeable S-adenosyl-L-methionine. In some cases, such as CPSF4 and KIF4A/B, the exact residues responsible for cluster binding have yet to be determined.

There is clear evidence that most nuclear Fe-S proteins depend on the CIA machinery for their metallation. However, a systematic survey of the specific CIA subcomplexes and adaptors required for the metallation of each nuclear Fe-S protein is necessary to fully understand how, when, and where Fe-S proteins acquire their clusters, as well as how this process is regulated. Notably, nuclear Fe-S proteins appear to bind the clusters at solvent-exposed loops. New evidence suggests that cluster transfer can occur to fully folded proteins forming complexes. Investigation of newly obtained structures of Fe-S proteins would help us understand whether solvent accessible clusters are a general characteristic of [4Fe-4S] proteins and if this has functional implications. One can speculate that these accessible positions may allow for sensory roles of the clusters, enabling dynamic cluster disassembly and reassembly in response to intracellular cues like redox status or iron levels, thus allowing cells to modulate Fe-S enzyme activity according to their needs. Do solvent-accessible, redox-active clusters play a role in sensing structural changes in DNA, e. g. DNA damage? [229] The possibility of re-metallation or repair of exchangeable clusters for reactivating *apo* proteins raises important questions about the dynamic trafficking of bioavailable Fe-S clusters and whether an equilibrium exists between the *apo* and *holo* forms of these proteins. Trafficking systems, such as the Glrx3-[2Fe-2S]-BolA2 system that exchanges Fe-S clusters with nuclear iron-responsive transcriptional regulators in fungi, may also traffic a pool of bioavailable Fe-S clusters in the nucleus of mammalian cells. Alternatively, CIA targeting complexes may deliver clusters *in-situ* when and where needed, as observed for the mitotic factor KIF4A, which colocalizes with MMS19 at the mitotic spindle. All these hypotheses remain to be experimentally tested.

The discovery of Fe-S clusters in viral proteins, including the SARS-CoV-2 RNA-dependent RNA polymerase [128] and the helicase nsp13 [127], as well as the hepatitis B virus protein HBx, which localizes to the nucleus of mammalian cells during infection [230,231], indicates a broad role for Fe-S clusters in viral infection. It seems likely that additional nuclear Fe-S binding proteins will be identified, particularly among those automatically annotated in the proteome as Zn-binding proteins. We hope this review encourage further research on the unresolved questions regarding Fe-

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S clusters in human cells, potentially leading to promising avenues for the development of novel therapeutic strategies.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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