The elemental role of iron in DNA synthesis and repair

Sergi Puig[1]*, Lucia Ramos-Alonso[1], Antonia María Romero[1] and María Teresa Martínez-Pastor[2]*

[1]Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de Alimentos (IATA), Consejo Superior de Investigaciones Científicas (CSIC), Ave. Agustín Escardino 7, 46980, Paterna, Valencia, Spain.

[2]Departamento de Bioquímica y Biología Molecular, Universitat de València, Ave. Doctor Moliner 50, 46100, Burjassot, Valencia, Spain.

*Corresponding authors: E-mail: spuig@iata.csic.es; maria.teresa.martinez@uv.es

Running title: Iron in DNA synthesis

Keywords: iron cofactor, iron-sulfur cluster, DNA synthesis, DNA repair, yeast, *Saccharomyces cerevisiae*, iron deficiency, ribonucleotide reductase.

Abstract

Iron is an essential redox element that functions as a cofactor in many metabolic pathways. Critical enzymes in DNA metabolism, including multiple DNA repair enzymes (helicases, nucleases, glycosylases, demethylases) and ribonucleotide reductase, use iron as an indispensable cofactor to function. Recent striking results have revealed that the catalytic subunit of DNA polymerases also contains conserved cysteine-rich motifs that bind iron-sulfur (Fe/S) clusters that are essential for the formation of stable and active complexes. In line with this, mitochondrial and cytoplasmic defects in Fe/S cluster biogenesis and insertion into the nuclear iron-requiring enzymes involved in DNA synthesis and repair lead to DNA damage and genome instability. Recent studies have shown that yeast cells possess multi-layered mechanisms that regulate the ribonucleotide reductase function in response to fluctuations in iron bioavailability to maintain optimal deoxyribonucleotide concentrations. Finally, a fascinating DNA charge transport model indicates how the redox active Fe/S centers present in DNA repair machinery components are critical for detecting and repairing DNA mismatches along the genome by long-range charge transfers through double-stranded DNA. These unexpected connections between iron and DNA replication and repair have to be considered to properly understand cancer, aging and other DNA-related diseases.

Introduction

Iron is an essential micronutrient for all eukaryotes because it participates as a redox cofactor in critical biological processes. Despite its propensity to generate reactive oxygen species (ROS) such as hydroxyl radicals via the Fenton reaction, and its potential harmful effect on DNA integrity and genome stability, iron is now recognized as an essential component of multiple enzymes implicated in fundamental DNA metabolism functions.[1-4] Although the biological reasons for the persistent role of iron in DNA metabolism are unknown, a fascinating model proposes that iron participates in DNA charge transport, which electronically probes DNA integrity.[5, 6] The enzymes required for DNA synthesis and repair that harbor functionally relevant iron cofactors include replicative DNA polymerases and primase, DNA helicases, nucleases, glycosylases and demethylases, as well as ribonucleotide reductases (see Table 1 for a list). Genetic mutations or defects in these proteins can be lethal or give rise to cancer-prone, developmental and aging-related diseases, including xeroderma pigmentosum, Cockayne's syndrome, trichothiodystrophy or Warsaw breakage syndrome. The budding yeast *Saccharomyces cerevisiae* has been used as a eukaryotic model organism to characterize cellular iron metabolism. This review focuses on yeast proteins, and discusses human and other protein homologs only when considered relevant. This paper refers to *S. cerevisiae* simply as yeast throughout.

Iron-sulfur cluster assembly and genomic integrity

Mitochondria are double-membrane organelles present in the cytoplasm of eukaryotic cells that perform critical cellular functions, including energy

production via oxidative phosphorylation, intermediary metabolism, ROS production, programmed cell death and iron metabolism. In the yeast *S. cerevisiae*, mitochondria are essential for viability because of their role in Fe/S cluster biogenesis, whereas respiration or mitochondrial DNA (mtDNA) are dispensable. A pioneering work has uncovered that yeast cells without mtDNA exhibit nuclear genome instability, a hallmark of cancer and aging.[7] By using yeast mutants that increased the inner mitochondrial membrane electrochemical potential and fluorescent reporters, these authors have shown that genomic instability correlates with a low mitochondrial membrane potential.[7] Mutants in the genes required for different mitochondrial electron transport chain steps maintained the integrity of their nuclear genome, which suggests that defects in respiration are not the origin of DNA damage. Instead the above authors have observed that the down-regulation of Nar1, an essential component of the maturation machinery of non mitochondrial Fe/S proteins (see below), is sufficient to alter nuclear genome stability.[7] Consistent with these observations, the cells that lack mitochondrial co-chaperone Zim17, which is required for the biogenesis of Fe/S proteins, show increased mutation frequencies, DNA damage and genomic recombination.[8, 9] Given that many enzymes required for DNA synthesis and repair harbor iron cofactors that are essential for their function (Table 1), these observations suggest that defects in Fe/S biogenesis and distribution might be the origin of genomic instability.

Work by Roland Lill's group and others in budding yeast has been fundamental to elucidate the conserved molecular mechanisms that govern the maturation of (non plant) eukaryotic Fe/S proteins.[4, 10, 11] The biogenesis of Fe/S clusters can be dissected into three fundamental stages (Figure 1): (i) an initial

stage catalyzed by mitochondrial core iron-sulfur cluster (ISC) assembly machinery; (ii) a mitochondrial export step mediated by ATP-binding cassette transporter Atm1; and (iii) a cytosolic ISC protein assembly (CIA) process responsible for the maturation of extra-mitochondrial Fe/S proteins. The core ISC assembly system performs a primary function in the assembly of all Fe/S proteins, including those in the cytoplasm and the nucleus. Various components cooperate in the *de novo* assembly of an initial [2Fe-2S] cluster: (i) mitochondrial inner membrane proteins Mrs3 and Mrs4 import cytosolic iron into mitochondria; (ii) cysteine desulfurase complex Nfs1-Isd11 donates the sulfur released from cysteine; (iii) an electron transfer chain, which consists of ferredoxin reductase and ferredoxin, reduces sulfur to sulfide; (iv) frataxin, which is defective in Friedreich's ataxia, has been proposed to assist in sulfur transfer and iron supply. In this way, the [2Fe–2S] cluster is assembled on scaffold protein Isu1 (and Isu2). Then a dedicated Hsp70 chaperone system (Ssq1-Jac1-Mge1), stabilized by co-chaperone Zim17,[12] and monothiol glutaredoxin Grx5 release the [2Fe–2S] cluster from Isu1 by transferring it toward specific mitochondrial apoproteins in a GSH-dependent manner. In humans, defects in conserved mitochondrial Fe/S biogenesis factors lead to lethality during early embryonic development or to severe neurodegenerative, hematological and metabolic diseases.[12] These core ISC assembly components are sufficient to support Atm1-mediated export and the subsequent assembly of cytosolic and nuclear Fe/S proteins. Specialized Fe/S components assist in the generation and insertion of [4Fe–4S] clusters into specific mitochondrial client apoproteins. A sulfur-containing product (denoted X-S) generated by core ISC machinery is exported from mitochondria by the conserved Atm1 transporter (ABCB7 in humans) within the mitochondrial inner membrane

(Figure 1).[13,14,13, 14] The recent resolution of the crystal structures of yeast Atm1 and a bacterial homolog has shown that glutathione (GSH) associates with the Atm1 substrate-binding pocket, and strongly suggests that GSH forms part of the X-S molecule.[15, 16] The potential nature of this exported compound, its role in iron sensing and the activation of the so-called iron regulon have been recently addressed, but are not the focus of this review.[11, 13, 14] The exported X-S compound is possibly transformed into a [2Fe-2S] cluster coordinated by two GSH molecules and two monothiol glutaredoxins, either Grx3 or Grx4.[17] Then CIA machinery components facilitate the maturation of extra-mitochondrial Fe/S proteins (Figure 1). First the sulfur source X-S, the electron transport chain composed of NADPH–diflavin reductase Tah18, the Fe/S protein Dre2, and possibly the [2Fe-2S]-Grx3/4-GSH complex, assemble a transient bridging [4Fe-4S] cluster on the cytosolic heterotetrameric Cfd1-Nbp35 scaffold complex. Then later-acting CIA targeting factors, including the Fe/S protein Nar1 and dynamic CIA targeting complex Cia1-Cia2-Mms19, distribute and insert the [4Fe-4S] cluster into dedicated cytosolic and nuclear apoproteins.

Two recent innovative studies have used protein-protein interaction approaches in both yeast and human cells to demonstrate that protein Mms19, which is the only CIA member that is not essential for cell viability, interacts *in vivo* with CIA machinery components and with a subset of Fe/S enzymes involved in DNA metabolism (DNA helicase Rad3, DNA polymerase Pol δ, DNA helicase/nuclease Dna2, and DNA glycosylase Ntg2), amino acid biosynthesis (sulfite reductase Met5-Met10 and isopropylmalate isomerase Leu1 required for methionine and leucine synthesis, respectively), and ribosome biogenesis and translation termination factor Rli1 (ABCE1 in humans).[18, 19] These findings suggest

that Mms19 may be implicated in the biogenesis and delivery of Fe/S clusters to specific proteins (Figure 1). A powerful yeast assay, which determines the incorporation of ^{55}Fe into immunoprecipitated proteins, has demonstrated that Mms19 facilitates Fe/S clusters insertion into Rad3 (and human XPD), Ntg2, Leu1, Met5-Met10 and Rli1 proteins, but not into early-acting CIA components Cfd1 and Nbp35. This finding suggests that Mms19 serves as a late-acting member of the CIA machinery.[18, 19] As expected, the reduced iron binding to Leu1 and Met5-Met10 correlates with a drop in isopropylmalate isomerase and sulfite reductase enzymatic activities, respectively.[18] Therefore, Mms19 is a conserved late-acting CIA component implicated in Fe/S cluster insertion into a subset of apoproteins that specialize mostly in DNA metabolism (and amino acid synthesis in yeast). However, very little is known about the mechanisms that the CIA machinery uses to discriminate particular Fe/S clusters recipient proteins from other proteins. Two yeast dedicated adaptors, Lto1 and Yae1, specifically facilitate Fe/S cluster insertion into Rli1, but not the assembly of other Fe/S proteins.[20] Thus the Yae1-Lto1 complex functions as a target-specific adaptor that recruits apo-Rli1 to the CIA targeting complex formed by Cia1-Cia2-Mms19. The function of Yae1 and Lto1 can be bypassed under anaerobic conditions, which highlights the great sensitivity of Fe/S protein biogenesis to oxygen.[20] Interestingly, the interaction between Yae1-Lto1 and CIA components is reinforced when cytosolic Fe/S protein maturation is impaired, which suggests that cells possess mechanisms to select which proteins are the preferred recipients of iron cofactor delivery during iron deficiency. In human cells, the association of CIA1 (yeast Cia1 homolog) and MIP18/CIA2B (yeast Cia2 homolog) with the MMS19 carboxy-terminal region forms a docking site for Fe/S proteins, including DNA Pol δ, primase large subunit

PriL and helicase RTEL1.[21, 22] However, Fe/S cluster delivery to helicase XPD seems to follow a different pathway as it can interact directly with the MMS19 amino-terminus independently of CIA1 and MIP18.[21] Furthermore, MMS19 seems to protect MIP18 from proteasomal degradation.[21] In addition to the CIA1-CIA2B-MMS19 complex, human CIA components can also form CIA1-CIA2A heterodimers, which facilitate iron regulatory protein 1 (IRP1) maturation.[22] Collectively, these data suggest that eukaryotic cells assemble specialized late CIA complexes to specifically target different Fe/S proteins.

An active role for Fe/S clusters in replicative DNA polymerases

In eukaryotic cells, nuclear genome replication is achieved through the coordinated action of three class B family DNA polymerases (Pol): Pol α, Pol δ and Pol ε.[23] These polymerases are multimeric complexes composed of a catalytic subunit A, a regulatory subunit B, and other accessory subunits. Subunits A and B are highly conserved and form a constitutive heterodimer that acts as the functional core complex. In *S. cerevisiae*, Pol α forms part of a four-subunit DNA polymerase-Primase (Pol-Prim) holoenzyme, where catalytic subunit Pol1 interacts with its B subunit Pol12, and also with the primase heterodimer formed by Pri1 and Pri2 (Figure 2). DNA replication initiates when the primase synthesizes an RNA primer of ~10 nucleotides, which is extended by DNA Pol α that incorporates ~20 additional nucleotides of DNA.[23] Then the replication factor C (RFC) complex recognizes the RNA-DNA primer, facilitates DNA Pol-Prim complex dissociation, and promotes the loading of the proliferating cell nuclear antigen (PCNA) complex, a sliding clamp that encircles DNA.[23] PCNA recruits DNA

Pol δ and Pol ε, which increase DNA replication processivity and fidelity due to their 3'-5' exonuclease proofreading activity (Figure 3). DNA Pol δ and Pol ε respectively execute the synthesis of the lagging strand and the leading strand. Pol δ is also a multimeric complex composed of three subunits; Pol3 (the catalytic subunit A), Pol31 and Pol32, while Pol ε is integrated by Pol2, Dpb2, Dpb3 and Dpb4 (A, B, and accessory subunits, respectively) (Figure 2). A fourth specialized B family DNA polymerase exists known as Pol ζ, which is the main enzyme that acts in translesion DNA synthesis (TLS) to prevent replication fork collapse and to assure genomic integrity at the expense of higher mutation rates. Pol ζ was first described to be formed by the catalytic Rev3 subunit and the accessory Rev7 subunit,[24] but several recent reports have shown that Rev3 also interacts with the Pol31 and Pol32 accessory subunits (Figures 2 and 3).[25-27]

The carboxy-terminal domain (CTD) of the catalytic subunits of the four polymerase complexes is quite conserved and contains two cysteine-rich metal-binding motifs, CysA and CysB, which were first believed to be zinc (Zn) finger modules for several reasons. First, CTD shows similarity to other Zn finger motifs. Second, the anti-cancer drug cisplatin, known to inactivate several polymerases through covalent binding to cysteine residues, deactivates the Pol α obtained from prostate PA-3 cells, whereas Zn is released in the process.[28] Third, the structure of a Zn-reconstituted synthetic peptide has been determined for the CysB domain of Pol α.[29] Fourth, a crystal structure of yeast core Pol α, including the Pol1 and Pol12 subunits, obtained after the expression in the bacterium *Escherichia coli,* shows that CTD contains two Zn^{2+} ions bound to each CysA and CysB motif.[30] This structural analysis has also shown that Pol1 CTD is important for the interaction

with the B subunit, which corroborates previous studies where the CTDs of the three replicative polymerases had been implicated in these interactions.[31, 32] However, the fact that the mutant allele *pol3-13* of yeast Pol3, the catalytic subunit of Pol δ, displays synthetic lethality with CIA members Nbp35, Dre2 and Tah18 has led to the identification of an Fe/S cluster in the CTD of Pol δ and, by extension, in other B family polymerases.[33, 34] An analysis of ^{55}Fe incorporation, purification of complexes and spectrometric characterization has provided *in vitro* and *in vivo* evidence that a [4Fe-4S] cluster binds to the CysB motif of the four catalytic subunits of yeast B family polymerases Pol1, Pol2, Pol3 and Rev3, and has also confirmed that CysA is a *bona fide* Zn binding motif.[34] Both yeast two-hybrid experiments and isolation of Pol3 from cell extracts have demonstrated that the Fe/S cluster in the CysB motif of Pol3 is required for the assembly of the Pol δ complex. Specifically, the mutation of two essential cysteines in the Pol3 CysB motif abrogates its interaction with Pol31, and indirectly with Pol32.[34] The mutations of the cysteines in the Pol3 CysA motif do not affect the interaction with Pol31 and Pol32, but are important for the binding of processivity factor PCNA.[34] Both CIA and mitochondrial ISC systems are necessary for Fe/S cluster insertion into the four polymerases, as insertion is impaired when mitochondrial cysteine desulfurase Nfs1 is depleted.[34] Although the precise physiological role of the Fe/S cluster has not yet been determined, in principle it seems to play a structural role. First, Pol3 CTD becomes unstable in the absence of its Fe/S cluster; second, the Fe/S cluster is important for the replication function by apparently mediating interactions with accessory subunits. This role is supported by the fact that the CTD domain is separated from the catalytic domain, and basal activity still remains when the CysB motif is mutated. Presence of an Fe/S cluster has been

independently confirmed for DNA Pol ζ and Pol ε.[25, 27, 35] In humans, the Fe/S cluster has been corroborated in the CTDs of Pol δ and Pol ζ, but not in Pol α and Pol ε, which have been proposed to bind Zn.[27] The Fe/S cluster in Pol α has been also questioned because of a new crystal structure of human Pol α in complex with its B subunit, where Zn ions are bound to the CysA and CysB motifs.[36] However as in the previous case, the purified human protein is heterologously expressed in *E. coli*, which is known to frequently promote the substitution of Fe/S clusters for Zn.[37] The presence of Fe/S clusters, as several *in vivo* experiments performed in yeast have concluded, is highly consistent.[34] Furthermore, the striking similarity between the CysB motif of the four polymerases supports the presence of the Fe/S cluster in all the polymerases. However, we cannot definitely rule out that replacing the Fe/S cluster with Zn would play a physiological role.

Human Pol δ and Pol ζ share the accessory B and C subunits described previously for Pol δ (Figure 2).[27] Subunit sharing between polymerases δ and ζ has been confirmed by the isolation in yeast of a four-subunit Pol ζ complex composed of proteins Rev3, Rev7, Pol31 and Pol32.[25, 26] In the Pol ζ complex, the Rev7 subunit interacts with the Rev3 amino-terminal domain, whereas the integrity of Rev3 CTD, which contains the [4Fe-4S] cluster, is necessary for its association with Pol31, and for the assembly and stabilization of the Pol ζ complex.[25, 26] Subunits Pol31 and Pol32 facilitate the interaction of Pol ζ with PCNA and are essential for the PCNA-dependent function of Pol ζ in TLS.[25, 26] Consistent with this, the Rev3 CysB double cysteine mutants with a disrupted Fe/S cluster are defective for ultraviolet (UV) damage-induced mutagenesis, while CysA mutants show no damage-induced phenotype.[25] Interestingly, a Rev3 mutant that lacks its CTD, and

which cannot interact with subunits Pol31 and Pol32, still maintains some UV-induced mutagenesis activity, which depends on Pol32 and other factors.[38] These results corroborate that the integrity of Pol δ is important for TLS, but also reveal that Pol32 is differentially regulated depending on the polymerase with which it associates.[38] Previous findings obtained with the thermosensitive yeast *pol3-13* mutant strain, which affects the binding of the Fe/S cluster to the CysB motif of Pol δ, have shown DNA damage sensitivity and defects in induced mutagenesis.[39] A recent report has demonstrated that yeast *pol3-13* mutants also exhibit a high spontaneous mutation rate that includes large deletions, transversions and other complex mutations.[40] All these mutations require a functional Pol ζ, which suggests that the Fe/S cluster of Pol δ differentially regulates the role of Pol ζ in spontaneous or induced mutations.[40] Collectively, these results indicate that anchoring the accessory subunits shared by polymerases δ and ζ to the DNA lesion allows Pol δ to be replaced with Pol ζ, which occurs during the replication of damaged DNA while TLS takes place. Briefly, after the stalling of Pol δ and ubiquitylation of PCNA, catalytic Pol δ subunit Pol3 dissociates and Pol ζ subunits Rev3 and Rev7 are recruited by Pol31-Pol32 to the lesion site.[27, 40] The Fe/S cluster in Pol ζ is essential for interactions with accessory subunits, and consequently for the polymerase switch. Polymerase exchange has been proposed to possibly depend on the communication between the Fe/S clusters of Pol δ and Pol ζ.[40]

A recent report has added a new and unexpected Fe/S cluster to the replicative DNA polymerases scene.[35] Pol ε catalytic subunit Pol2 contains a second [4Fe-4S] cluster within its active polymerase domain, as well as the Fe/S cluster present in its CTD.[35] Whereas a Pol2 mutant that lacks its CTD still maintains its

polymerase activity, additional mutations in the cysteines predicted to coordinate the Fe/S cluster in the catalytic domain abrogate DNA synthesis activity, but still maintain exonuclease activity.[35] The more solvent-exposed nature of the Fe/S cluster from the catalytic domain has led to the proposal of a possible regulation by the redox state of the cell.

A structural and redox role for the primase Fe/S cluster

As eukaryotic DNA polymerases are unable to start *de novo* the synthesis of a DNA strand, DNA replication is initiated by primase, a DNA-dependent RNA polymerase.[41] As mentioned above, the coordinated activities of Pol-Prim complex components catalyze the synthesis of short RNA-DNA primers, which allows further replication of lagging and leading DNA strands by more processive Pol δ and Pol ε, respectively.[23] DNA primase is a conserved heterodimeric enzyme formed by a small (yeast Pri1 or human PriS) and a large subunit (yeast Pri2 or human PriL), which are both essential for viability.[42-44] Although the ribonucleotide-binding site resides solely in the small subunit, both subunits participate in the formation of the catalytic site and are necessary for primase activity.[45] Work with yeast and mammalian cells has assigned key roles to the large Pri2/PriL subunit, which include (i) initiating RNA primer synthesis; (ii) stabilizing the small Pri1/PriS subunit; (iii) determining RNA primer size; and (iv) promoting the switch to DNA Pol α activity.[46, 47]

The presence of a conserved Fe/S cluster has been demonstrated for the large primase subunit of human, yeast and archaeal cells, and adds primase to the group of DNA metabolism enzymes that contain iron.[48, 49] As mentioned above,

biochemical data have recently shown that human complex CIA1-MIP18-MMS19 interacts with primase, which strongly suggests that CIA machinery mediates Fe/S cofactor assembly into primase.[21] Both human primase subunits PriS and PriL interact with the CIA complex, which indicates that the primase complex forms before the Fe/S cofactor incorporates into PriL.[21] Apo-primase seems unstable since yeast Pri2 protein levels drop when (i) key Fe/S binding residues are mutated; (ii) cells are grown under iron-deficient conditions; or (iii) Fe/S cluster delivery is impaired by the deletion of the CIA component *TAH18*.[50] Structural data have indicated that four conserved cysteines within the CTD of Pri2/PriL coordinate a [4Fe-4S] cluster.[48, 49] *In vitro* experiments have shown that the disruption of Fe/S cluster incorporation into yeast Pri2 eliminates primase activity, partly as a result of apo-CTD unfolding.[49] These results demonstrate the essentiality of the Fe/S cluster for initiating RNA synthesis, but not for primer elongation.[49] More recently, *in vivo* data have shown that Fe/S binding cysteines are required for: (i) iron incorporation into yeast primase; (ii) growth at high temperatures; (iii) Pol-Prim complex loading into early replication origins; (iv) cell cycle progress through the S phase, which implicates the Fe/S cofactor in DNA replication initiation.[50] The mutation of a single cysteine residue in human PriL suffices to eliminate iron binding and to abrogate the initiation of RNA synthesis activity, which corroborate that the Fe/S cluster is essential for primase function.[48] A second crystallografic analysis of yeast Pri2 CTD has shown that the Fe/S cluster locates at the interface between two largely independent helical folds within CTD.[51]

The exact role played by the Fe/S cluster in primase activity has not yet been demonstrated. Although initial studies have suggested a structural function,

more recent evidence offers new possibilities. Assembly of iron into yeast Pri2 is sensitive to oxidative stress as it is impaired in cells defective in superoxide dismutase activity.[50] This observation suggests that DNA replication might be influenced by the cellular redox state. By *in vitro* DNA-mediated electrochemistry, a recent study by Jacqueline Barton's group has shown that changes in the redox state of the [4Fe-4S] cluster in human DNA primase act as a reversible switch that modulates DNA binding.[52] Thus oxidized $[4Fe\text{-}4S]^{3+}$-PriL would bind tightly to DNA, while PriL would transit to a looser association in the reduced state. This group proposed that the electron transfer, which switches the cluster on and off, would occur through an internal charge transfer pathway in the PriL protein, which would tunnel electrons between the Fe/S cluster and DNA throughout several tyrosine residues. The primase redox switch is essential for initiation in single-stranded DNA (ssDNA), but not for nucleotide polymerization.[52] Additionally, the introduction of mismatches into the primer inhibits DNA charge transport through the DNA-RNA duplex, which impairs the necessary truncation of the primer by primase to proceed to the handoff to Pol α.[52] This work supports an exciting hypothesis about communication between the different polymerases in replication machinery through the DNA-mediated charge transport between Fe/S clusters (further details below).

Multitasking nuclease/helicase Dna2

Dna2 is an essential multitasking enzyme involved in Okazaki fragment processing during replication, DNA double-strand break (DSB) repair, telomere maintenance, processing and restarting reversed replication forks, and activation

of the cell cycle checkpoint response.[53-56] Conserved regions in Dna2 proteins include an Fe/S cluster-containing domain with RecB ssDNA-specific endonuclease activity and a superfamily 1 ATPase/helicase domain.[57] The yeast Dna2 protein contains an additional unstructured amino-terminal region that has been implicated in checkpoint activation and hairpin DNA binding.[58] Yeast Dna2 nuclease activity is essential for cell survival, whereas 5' to 3' helicase activity is dispensable, but leads to growth defects and sensitivity to DNA damage.[59, 60] Similarly to bacterial AddAB nucleases, a $Cx_{248}Cx_2Cx_5C$ Fe/S-coordinating motif spans the Dna2 nuclease domain.[61, 62] Protein-protein interaction data suggest that CIA protein Mms19 participates in the assembly of the Fe/S cofactor into Dna2 in both yeast and human cells.[18, 19] A recent study has also experimentally demonstrated that the yeast Dna2 protein coordinates an Fe/S cluster through conserved cysteine residues.[62] The mutagenesis of the cysteine residues that coordinate the Fe/S cluster does not alter Dna2 affinity for DNA, but diminishes the DNA nuclease function and ssDNA-dependent ATPase/helicase activity. These findings support the notion that both the nuclease and helicase domains of Dna2 are coupled.[62] The Fe/S cluster seems essential for Dna2 function in DNA replication and repair.[62] However, further studies are necessary to decipher the relevance of the iron cofactor and bioavailability in other Dna2 cellular tasks.

DNA helicase Rad3 functions in nucleotide excision repair and transcription initiation

Yeast Rad3 is an essential 5' to 3' ATP-dependent DNA helicase that belongs to the broad and conserved superfamily II of DNA helicases denoted XPD (from

Xeroderma Pigmentosum complementation group D), with members in the three domains of life.[63-66] Mutations in human XPD, the Rad3 ortholog, that affect DNA repair and transcription give rise to three different skin cancer-prone or premature aging genetic diseases: skin xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. Eukaryotic Rad3/XPD proteins function as part of the RNA Pol II initiation factor TFIIH, which is implicated in transcription initiation and nucleotide excision repair (NER). NER is a DNA repair pathway that removes bulky lesions, including photoproducts induced by UV light (Figure 3).[67] Unlike eukaryotic Rad3/XPD, which associate with other TFIIH complex members, archaeal XPD paralogs seem to function as monomers. This characteristic has facilitated the purification and tridimensional structure determination of several archaeal XPD helicases.[68-72] The archaeal XPD architecture comprises four domains, which contain the majority of disease-related mutation sites: two canonical RecA-like helicase motor domains present in all superfamily II helicases with a central interface to which ATP binds and hydrolyses; a third novel fold denoted the Arch domain, which comes into contact with both motor regions; a fourth Fe/S domain, which contains a [4Fe-4S] cluster coordinated by four conserved cysteine ligands. The larger Arch domain present in eukaryotic XPDs may increase the interaction surface with TFIIH components. The recent elucidation of an archaeal XPD structure in a complex with a short DNA fragment and the biochemical analysis of specific mutants have revealed how 5' to 3' translocation polarity is achieved.[69, 73] A wedge-shaped region that flanks the Fe/S cluster constitutes the point of DNA duplex separation.[73] The Arch and Fe/S domains are inserted into the first motor domain and form a positively charged channel through which DNA is led due to the energy of the ATP hydrolysis by

helicase domains, which enables unwinding.[69, 73] Thus DNA interacts with residues within the first motor and Fe/S domains.[69, 73] A recent biochemical and structural work has studied the mechanism of DNA loading onto archaeal XPD by characterizing the conformational changes that occur during this process.[74] Its authors conclude that after ssDNA binding to the second motor domain, the interface between the Arch and Fe/S domains transiently opens to allow access to the first helicase domain, which directs DNA through the pore.[74] Biochemical data with archaeal XPD have also shown that the Fe/S domain is essential for helicase activity, but dispensable for binding to ssDNA and ATP hydrolysis.[68, 70, 72] A missense mutation in a conserved arginine residue within the Fe/S domain of human XPD (R112H) leads to trichothiodystrophy disease. Biochemical analyses, with the corresponding archaeal arginine mutant, have demonstrated that this XPD variant does not contain iron and, subsequently, has completely lost its helicase activity, but still binds ssDNA and possesses ATPase activity.[68] The expression of the equivalent arginine Rad3 mutant in yeast leads to UV sensitivity.[68] The fact that this arginine residue indicates a narrow pocket involved in damage recognition has allowed speculations to be made about its potential role as a DNA damage sensor.[65] Similarly to helicase activity, the Fe/S cluster is not essential for transcription and cell survival. Yeast cells that harbor mutations in the conserved Rad3 residues required for Fe/S cluster binding display sensitivity to UV light. This fact supports a critical role for the iron cofactor, and therefore helicase activity, in NER.[68] Further studies with archaeal XPDs have suggested that the Fe/S cluster plays a structural function by facilitating the folding and stabilization of various XPD domains required for helicase targeting to the DNA junction, and to couple ATP hydrolysis to the unidirectional DNA translocation.

However, a potential redox role in DNA damage sensing has not been ruled out.[65, 70, 72, 73] Thus the archaeal XPD incubated with DNA-modified gold electrodes produces an ATP-dependent redox signal, which is mediated by Fe/S cluster coupling to DNA.[75] Archaeal XPD helicases have been widely used to explore the molecular bases of multiple clinically relevant XPD mutations, including those at the Fe/S domain.[68-73] However, we consider that further *in vivo* studies with yeast Rad3 could considerably contribute to our current understanding of XPD-related diseases and the effect of iron bioavailability on function and regulation.

Data on the mechanism that eukaryotic cells utilize to assemble the [4Fe-4S] cofactor into Rad3/XPD proteins date back to the late 1970s when Prakash and colleagues showed that yeast cells defective in the conserved CIA-component Mms19 were sensitive to UV light, DNA cross-linking agents and alkylating agent methyl methanesulfonate (MMS).[76, 77] Two decades later, they demonstrated that yeast *mms19Δ* cells were defective in two NER modes (transcription-coupled and global genome repair) and exhibited defects in RNA Pol II transcription at high temperatures.[78] Both the transcriptional and NER defects displayed by *mms19Δ* mutants have been complemented *in vitro* by the TFIIH complex, but not by the Mms19 protein itself.[78, 79] Furthermore, biochemical and genetic data have shown that NER defects in yeast *mms19Δ* mutants are rescued by overexpressing Rad3.[79] However, Rad3 overexpression is unable to correct the temperature sensitivity of *mms19Δ* yeast cells.[79] Finally, recent data have demonstrated that the Mms19 protein interacts *in vivo* with CIA machinery components Cia1 and Cia2, and also with Rad3/XPD helicases, which explains most previous NER- and transcription-related phenotypes associated with *mms19Δ* mutants.[18, 19, 80-82] In humans, an

ADP/ATP translocase called ANT2 also associates with a complex that contains MMS19 and XPD proteins, but not to each protein separately, possibly to stabilize interactions.[81, 82] Incorporation of ^{55}Fe into immunoprecipitated Rad3 and XPD has also demonstrated that Mms19 facilitates Fe/S cluster insertion into both proteins.[18, 19] Unlike other Fe/S proteins, human XPD can interact directly with MMS19 independently of CIAO1 (yeast Cia1) and MIP18 (yeast Cia2).[21] Interestingly, yeast *mms19Δ* mutants exhibit low Rad3 protein levels, which suggests that Mms19 is necessary to sustain an adequate TFIIH component concentration.[79] Given that impaired cofactor delivery to Fe/S cluster-containing proteins has been associated with apoprotein degradation, these results indicate that apo-Rad3 might be destabilized compared to holo-Rad3. A more detailed study in human cells has reported that assembly into XPD occurs in a sequential fashion in which MMS19 receives the Fe/S cluster from CIA factors in the cytoplasm, and transfers it to XPD in the nucleus,[83] although the MMS19 simultaneous interaction with XPD and CIA proteins has been reported.[82] In any case, XPD associates with TFIIH and MMS19 in a mutually exclusive manner.[83] Only when the Fe/S cluster is properly inserted can XPD assemble into the TFIIH complex.[83] In agreement with this, XPD association with TFIIH is prevented under iron-deficient conditions upon MMS19 depletion, and in XPD mutants in the residues that are required for Fe/S cluster or CIA binding.[18, 19, 83] This sequential assembly process could constitute a quality control mechanism to prevent immature apo-XPD from being incorporated into TFIIH.

DNA helicase Chl1 links replication to chromosome segregation

In addition to Rad3/XPD, the human superfamily II of Fe/S cluster-dependent DNA helicases includes FANCJ, which is involved in DNA cross-link repair and is mutated in Fanconi anemia patients; RTEL, which contributes to telomere maintenance, and ChlR1 (also called DDX11), which is implicated in chromosome segregation and is responsible for a rare genetic developmental disorder known as Warsaw breakage syndrome.[84] Among these DNA helicases, only ChlR1 possesses a yeast paralog denoted Chl1. To ensure the proper distribution of chromosomes between daughter cells, eukaryotes establish a ring-shaped multisubunit complex called cohesin, which encircles DNA and holds together sister chromatids (Figure 3). Yeast chromosome cohesion assays and mammalian chromosomal spreads analyses have demonstrated that *CHL1*/*ChlR1*-defective cells display defects in the sister chromatid linkage.[85-90] Cohesin assembles onto chromosomes in the late G1 phase and accompanies DNA replication in the S phase. In consistence with this, both yeast Chl1 and human ChlR1 genetically and physically associate with Fen1, an endonuclease required for processing Okazaki fragments during DNA replication. This scenario suggests Chl1/ChlR1 couple lagging strand synthesis to chromatid establishment.[91, 92] Holding sister chromatids together requires the stabilization of cohesin rings on chromosomes. This is achieved in yeast through acetylation by replication fork-associated acetyltransferase Eco1. However, recent data have demonstrated that, in parallel to Eco1, Chl1 is recruited to the DNA replication fork in the S phase by Ctf4, a structural component of the replisome that interacts with DNA polymerase α to stabilize chromosome cohesion.[93, 94]

Human ChlR1 possesses DNA-dependent ATPase and helicase activities that unwind duplex DNA substrates, preferentially with a 5' to 3' directionality.[91, 95] The

mutations present in Warsaw breakage syndrome disease abolish ChlR1 DNA binding and helicase activities, which leads to defects in cohesin function and DNA repair that cause microcephaly, growth retardation and abnormal skin pigmentation.[96-98] Both the yeast *CHL1* and human *ChlR1* mutants that abolish ATP binding inactivate its catalytic function in chromosome segregation.[95, 99] Surprisingly, recent data have indicated that helicase activity is dispensable for the yeast Chl1 function in sister chromatid cohesion, but is essential for recruitment to the replisome and replication fork progress under genotoxic stress conditions.[93] Both Chl1/ChlR1 helicases contain the conserved cysteine residues that coordinate an Fe/S cluster in other superfamily II helicases such as Rad3/XPD. ChlR1 interacts with MMS19 and CIA protein MIP18, probably to assemble the Fe/S cluster.[82] It is noteworthy that novel Warsaw breakage syndrome patients carry a missense mutation in the Fe/S domain of ChlR1, which impairs ATPase and helicase activity.[96, 100] However, the presence of an Fe/S cluster and its potential structural and functional relevance for the role of Chl1 in chromosome segregation, DNA synthesis and repair have not yet been experimentally addressed.

Yeast Fe/S protein glycosylase Ntg2

Base excision repair (BER) is one of the most important mechanisms to remove the oxidative DNA damage produced by ROS during normal metabolism or environmental stresses. BER is initiated by DNA N-glycosylases that recognize and cut the glycosylic bond between a damaged nitrogen base and its sugar in DNA. The resulting abasic site (AP-site) is cleaved by the AP-lyase activity of the glycosidase itself or by another AP-endonuclease. Finally, DNA polymerases and

DNA ligases complete DNA repair. The bacterium *E. coli* possesses [4Fe-4S]-containing DNA glycosylases, such as EndoIII and MutY, which are implicated in the repair of a variety of oxidized pyrimidine bases from duplex DNA. The conserved regions in this family of DNA glycosylases include a helix-hairpin-helix motif involved in the catalytic mechanism and a $Cx_6Cx_2Cx_5C$ cysteine motif within a carboxyl-terminal loop that coordinates an Fe/S cluster.[101-104] The positively charged Fe/S domain is not directly involved in the base excision reaction, but plays a structural role by recognizing and positioning DNA for base cleavage.[105] *In vitro* data suggest that the Fe/S cluster participates in the DNA-mediated charge transfers between the DNA repair enzymes that facilitate DNA damage detection (see below for further details).[106, 107]

S. cerevisiae expresses two homologs of bacterial EndoIII and MutY called Ntg1 and Ntg2, which contain the conserved helix-hairpin-helix motif.[108-111] Both yeast enzymes possess N-glycosylase and AP lyase activities, which preferentially remove oxidized pyrimidines from DNA.[108, 109, 112] The yeast cells that lack either *NTG1* or *NTG2* show higher mutation frequencies than wild-type cells.[109] The rate of mutations is even higher in a double *ntg1Δntg2Δ* mutant, which suggests that both proteins perform separate functions in DNA repair.[109] Therefore, despite both yeast proteins functioning in oxidative DNA damage removal, they exhibit many differences. First, Ntg2 contains the $Cx_6Cx_2Cx_5C$ motif that coordinates the Fe/S cluster in bacterial EndoIII and MutY, whereas Ntg1 does not have any of these cysteine residues, which precludes Fe/S cluster binding.[110] Second, both enzymes have overlapping, but not identical, substrate specificity and reaction modes.[109, 113, 114] Third, while Ntg1 moves to the nucleus or mitochondria depend on the oxidative status of each organelle, Ngt2 seems to be constitutively localized in the

nucleus.[109, 113, 115] Sumoylation targets Ntg1 to the nucleus upon nuclear oxidative damage.[115] However, the binding of an Fe/S cluster to Ntg2 glycosylase and the physiological relevance of the iron cofactor have not yet been experimentally addressed in yeast.

Multiple mechanisms regulate diferric ribonucleotide reductase enzyme

Ribonucleotide reductase (RNR) is an essential oxygen- and iron-dependent enzyme that catalyzes the rate-limiting step in the *de novo* biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides.[116-118] Eukaryotic class Ia RNR comprises a large R1 (α_2) and a small iron-containing R2 (β_2 or $\beta\beta'$) subunit. The yeast R1 subunit consists in an Rnr1 homodimer that houses catalytic and allosteric effector sites. Rnr1 contains two allosteric sites: a specificity S effector site that controls the specificity for each substrate, and therefore regulates the balance between the four deoxyribonucleoside triphosphate (dNTP) pools; an activity A allosteric site that regulates overall enzyme activity by monitoring the adenosine triphosphate/deoxyadenosine triphosphate (dATP/ATP) ratio, with dATP inhibiting and ATP activating RNR activity. The binding of either dATP or ATP to the A site regulates the assembly of various R1 and R2 subunits into an $R1_3R2_n$ quaternary structure, where n is 1 or 3. In response to genotoxic stress, yeast cells activate the expression of a second and less abundant large R1 subunit called Rnr3, which occasionally heterodimerizes with Rnr1. Although the Rnr1-Rnr3 heterodimer has been proposed to enhance catalytic activity, its physiological relevance is currently unknown. Unlike most eukaryotes, including human, in which R2 is composed of an iron-containing homodimer, the yeast R2

subunit is formed by an Rnr2-Rnr4 heterodimer. Whereas Rnr2 harbors the essential diferric $(Fe^{3+})_2$ center that generates and maintains a tyrosyl radical (Y·), which is indispensable to initiate nucleotide reduction at the catalytic site, Rnr4 is structurally homologous to Rnr2, but catalytically inactive because it lacks key iron ligands. Notwithstanding, Rnr4 plays a crucial role in the RNR function by favoring the proper folding and assembly of the Rnr2 cofactor, and by improving the interaction between subunits R1 and R2. After substrate binding to R1, a long-range proton-coupled electron transfer, which involves aromatic residues in both the R1 and R2 subunits, causes ribonucleotide reduction. Reducing equivalents for dNDP synthesis come from two conserved cysteine residues in the R1 subunit that form a disulfide bond after catalysis. These cysteines have to be reduced by thioredoxin and glutaredoxin to reactivate the enzyme.

Diferric tyrosyl $[(Fe^{3+})_2Y\cdot]$ cofactor assembly into RNR is poorly characterized.[118, 119] Yeast cells depleted of monothiol glutaredoxins Grx3 and Grx4, which function in intracellular iron sensing and trafficking, exhibit considerably reduced iron insertion, as well as reduced specific activity of RNR and other di-iron containing proteins.[17] However, it is not clear how direct the role played by monothiol glutaredoxins is in the iron cofactor delivery to these enzymes. Interestingly, the yeast cells depleted of conserved CIA machinery members Dre2 and Tah18 display diminished RNR cofactor assembly and activity, which suggests that the Dre2-Tah18 complex may serve as the electron donor for RNR cofactor synthesis.[120-122] The physical interaction between Dre2 and Rnr2 proteins supports this direct electron transfer.[122] However, a recent report has demonstrated that iron loading into Rnr2 requires Nfs1, Atm1 and Grx3/Grx4, but not Dre2 and other CIA components.[123] These results suggest that the electron

delivery by Dre2 occurs in a second step of $(Fe^{3+})_2Y\cdot$ cofactor assembly that is not necessary for initial iron loading. By measuring RNR activity, these authors show that $(Fe^{3+})_2Y\cdot$ cofactor formation requires the mitochondrial ISC machinery and Dre2, but not CIA components downstream of Dre2.[123] Collectively, these results indicate that there is a separation between the maturation of cytosolic 4Fe-4S clusters pathway and the assembly of the RNR metallocofactor that occurs after the Dre2-Tah18 step.[123] Further *in vitro* and *in vivo* studies will be necessary to unravel additional molecular details of this assembly process.

In addition to allosteric regulation, a wide range of cellular mechanisms tightly controls eukaryotic RNR activity. Defect, excess or imbalances in the total or relative levels of dNTPs cause DNA replication and repair problems by increasing mutation rates and genome instability. Human RNR is an important target in anticancer therapies because its catalytic activity increases in several tumor cell types.[124] Under normal conditions, different mechanisms limit RNR activity in yeast. First, the Sml1 protein binds to the R1 carboxy-terminal tail and inhibits its catalytic activity. Second, the R1 subunit resides in the cytoplasm, whereas the R2 subunit is constantly transported to the nucleus by the Dif1 protein and is anchored there by Wtm1. Third, the expression of RNR genes is low and inhibited by transcriptional repressors, including Crt1. In response to DNA damage and DNA replication stress, the yeast Mec1-Rad53-Dun1 checkpoint kinase cascade activates RNR at multiple levels, which increases the dNTP concentration.[125-127] First, phosphorylation of the R1 inhibitor Sml1 promotes its degradation by the proteasome. Second, the phosphorylation and degradation of Dif1, and the reduced interaction between R2 and nuclear anchor protein Wtm1, promote the redistribution of the small R2 subunit to the cytoplasm, where the catalytic R1

subunit resides. Third, the phosphorylation of the Crt1 transcriptional repressor promotes its release from *RNR2-4* promoters and their transcriptional activation. More recently, Mec1 and Rad53, but not Dun1, have been found to be implicated in the Ixr1-mediated transcriptional activation of *RNR1* by genotoxic stress.[128] The heterodimeric Swi6-Mbp1 transcription complex transiently activates the expression of yeast *RNR1* during the transition between the G1 and S phases of the cell cycle.

Iron deficiency decreases the bioavailability of iron cofactors and, consequently, the catalytic activity of many iron-dependent enzymes, including RNR. However, very little is known about the mechanisms that eukaryotic cells utilize to maintain the RNR function when its iron cofactor is scarce. These regulatory strategies would be especially relevant when iron chelators are used to treat tumor growth. In the last few years, various overlapping mechanisms that activate RNR in response to iron deficiency have been described (Figure 4). Nutritional and genetic iron deficiencies seem to activate the Mec1-Rad53-Dun1 kinase pathway at different stages by an unknown mechanism, probably related to the fundamental role played by iron in DNA synthesis and repair. First, iron scarcity promotes the Dun1-dependent phosphorylation and vacuolar/proteasomal degradation of Sml1, which improves dNTP synthesis.[129, 130] Second, upon iron limitation, Dun1 kinase phosphorylates specific serine residues in the Dif1 protein that facilitate R2 relocalization to the cytoplasm.[131] Dun1 phosphorylates different Dif1 residues in response to genotoxic stress and iron deficiency.[131] In consistence with these results, yeast cells defective in different mitochondrial core ISC assembly machinery steps activate a DNA damage response, which increases RNR activity by promoting Rad53 phosphorylation,

reducing Sml1 protein levels, redistributing R2 to the cytoplasm and activating *RNR3* expression.[18, 122, 130] Collectively, these results support the notion that the Mec1-Rad53-Dun1 checkpoint kinase cascade is differently activated in response to iron deficiency, defects in Fe/S cluster biogenesis and genotoxic stress through various signaling pathway mediators that converge on Dun1 kinase.[122, 129-131]

In addition to the checkpoint kinase cascade, a novel pathway that involves iron-regulated RNA-binding proteins Cth1 and Cth2 (Cth1/2) activates RNR in response to low iron levels. In response to iron deficiency, yeast cells express *CTH1* and *CTH2*, which encode soluble proteins that harbor a conserved tandem-zinc finger that directly binds to the AU-rich elements (AREs) present in the 3' untranslated region (3'UTR) of multiple mRNAs to thus promote their targeted degradation.[132-135] Cth1/2 promote the turnover of many mRNAs, which encode proteins that contain iron as a cofactor, or which participate in iron-dependent pathways.[132, 133, 136] Consequently, Cth1/2 activate a global metabolic reprogramming directed to optimize iron utilization in essential processes, but not in dispensable pathways for yeast survival, such as mitochondrial respiration.[132, 133] Cth1/2 promote the degradation of *WTM1* mRNA, which leads to a drop in the protein levels of Wtm1, the nuclear R2 anchor that facilitates R2 redistribution to the cytoplasm when iron is scarce.[127] The Cth1/2-mediated post-transcriptional regulatory mechanism of RNR increases dNTP production 6-fold during iron deficiency.[127] It is interesting to note that the 3'UTRs of *RNR2* and *RNR4* mRNAs contain functional AREs, which facilitate their slight Cth1/2-dependent degradation and the subsequent subtle drop in the R2 subunit levels during iron deficiency.[127] Although the physiological relevance of R2 down-regulation under low iron conditions is not known, we suggest that it is a cellular strategy to lower

the levels of apo-R2 dimers. Additional mechanisms, uncharacterized at the molecular level, regulate RNR expression in response to iron scarcity. For instance, Rnr1 protein levels increase by iron depletion through a mechanism that is independent of Rad53 and Dun1 kinases.[129] These results in yeast cells suggest that multiple iron-dependent mechanisms may control mammalian RNR activity. Deciphering these regulatory pathways will be crucial for our understanding of how patients' iron status influences their response to RNR-targeted chemotherapies.

Tpa1 functions as iron-dependent DNA demethylase

Fe^{2+}/2-oxoglutarate-dependent dioxygenases (2-OGDOs) comprise a large family of enzymes that catalyze the oxidation of many organic substrates. They play important roles in a highly diverse biological processes, including oxygen and energy sensing, metabolism of iron, fatty acids and collagen, translation and nucleic acids repair.[137, 138] Budding yeast expresses an iron-dependent 2-OGDO called Tpa1 composed of two structurally similar domains with a double-stranded β-helix fold. The smaller amino-terminal domain (NTD) contains the conserved active site and the $H_1X(D/E)...H_2$ iron-coordinating motif, whereas the larger CTD lacks the dioxygenase active site for the binding of Fe^{2+} and 2-oxoglutarate.[139, 140] Tpa1 was originally described to interact with translation release factors and the poly(A)-binding protein, and thus influences translation termination efficiency, as well as mRNA deadenylation and turnover.[141] More recently, simultaneous studies with yeast Tpa1 and its homolog proteins in humans and fruit flies have demonstrated that Tpa1 hydroxylates proline 64 in ribosomal protein Rps23 and

allows proper translation termination.[142-144] Cells defective in Tpa1 alter translation as they lead to enhanced termination or readthrough, depending on the specific stop codon context.[141, 142] Noteworthily, the Tpa1 residues that coordinate iron are required for prolyl hydroxylation and accurate translation termination.[140, 142]

Bacterial 2-OGDO family member AlkB has been implicated in the oxidative reversal of DNA alkylation damage.[145] Specifically, AlkB functions as a demethylase that uses molecular oxygen to oxidize the alkyl groups in a wide variety of alkylated nucleic acid base lesions by releasing an aldehyde and regenerating the undamaged base. Despite yeast cells not containing a sequence homolog of AlkB, a recent study has provided plenty of evidence to support the idea that Tpa1 is the yeast functional ortholog of AlkB, which is implicated in DNA repair.[146] First, the heterologous expression of either the whole Tpa1 protein or its iron-containing NTD functionally complements *E. coli alkb* mutants. Second, different *in vitro* approaches have shown that purified recombinant Tpa1 protein displays DNA demethylase activity and is able to repair MMS-damaged DNA, while the mutation of iron-binding sites abrogates its activity. Third, the genetic interactions between yeast *tpa1Δ* and mutants in other DNA repair enzymes, such as *MAG1*, which encodes for a DNA glycosylase involved in BER, and *REV3*, which encodes for the catalytic subunit of error-prone DNA Pol ζ, provide *in vivo* evidence for the physiological implication of Tpa1 in DNA repair. The mild MMS-sensitive phenotype, displayed by either *tpa1Δ* or *mag1Δ* single mutants, is exacerbated in a *tpa1Δmag1Δ* double mutant, which suggests that both enzymes repair methylated bases.[146] It would be particularly interesting to explore whether the Tpa1 human homolog, which is not included among the nine mammalian AlkB subfamily

members, also exhibits DNA demethylase activity in addition to its well-established role as Rps23 prolyl hydroxylase.

DNA charge transport between iron centers emerges as a universal DNA surveillance mechanism

One decade ago, not many enzymes that acted on DNA were known to contain iron as a prosthetic group. As we cover in this review, an increasing number of DNA processing enzymes have been discovered in recent years to possess Fe/S clusters or other iron cofactors. Now that the essentiality of iron in DNA metabolism enzymes has been established, the question is: why does a redox active metal like iron locate so close to DNA? Iron was initially considered to mainly play a structural role. By using various *in vitro* platforms and, more recently, *in vivo* approaches, Barton's group proposed an enthralling model that assigns a redox active role to Fe/S clusters within DNA metabolism enzymes.[5, 6] This model introduces the concept of DNA as a wire-like structure, where electrons or electron holes rapidly travel long molecular distances along the DNA helix through the π-stacking interactions between the nitrogen bases in a process called DNA charge transport (CT) (Figure 5). *In vitro* studies with DNA modified electrode systems, where DNA monolayers are assembled on gold electrodes and CT is monitored with redox active intercalative probes, have shown that the CT process depends on the integrity of the π-stack of base pairs and is, therefore, extremely sensitive to perturbations such as mismatched base pairs, base modifications or other lesions;[106] e.g., the binding of proteins that bend DNA, such as the TATA-binding protein, or that flip out a base attenuate DNA CT. Therefore,

DNA CT would allow genome-scale monitoring to efficiently detect DNA damage sites.

Electrochemical approaches have permitted the changes in the redox potential of various DNA enzymes when bound to DNA to be measured. Thus DNA glycosylases (*E. coli* EndoIII and MutY) and DNA helicases (*E. coli* DinG and archaeal XPD) shift the redox potential of their Fe/S clusters when bound to DNA, which allows oxidation/reduction reactions to occur under physiological conditions.[75, 106, 107, 147-149] When these enzymes bind to DNA, they oxidize their Fe/S clusters and, therefore, enhance their affinity for DNA. If the intervening DNA region is not damaged, then the electron travels along it to reduce another Fe/S-containing glycosylase, which is then released from DNA (Figure 5). Atomic force microscopy has confirmed that this mechanism allows Fe/S-containing DNA repair proteins to rapidly sort big undamaged regions within the genome, while being redistributed to concentrate in the vicinity of damaged sites.[147, 150, 151] As mentioned before, recent data suggest that human primase also follows a similar strategy.[52] Although a biological role for DNA CT has not yet been definitely established, there is some evidence to support its feasibility *in vivo*; e.g., CT has been detected in nucleosome-bound DNA and in the nuclei isolated from HeLa cells.[152] Once again, atomic force microscopy has provided *in vitro* evidence that similarities among the DNA-bound redox potentials of Fe/S clusters enable interprotein communication between different types of DNA repair proteins, including bacterial EndoIII, MutY and DinG, and archaeal XPD, which cooperate to increase the efficiency of DNA damage detection.[107, 150] Genetic assays have provided *in vivo* support for such DNA-mediated cross-talk among enzymes EndoIII, MutY and DinG.[147, 150] Taken together, these *in vitro* and *in vivo*

observations support the cooperation via DNA CT between different Fe/S cluster machineries to rapidly prove DNA fidelity.[3] The recent discovery of additional DNA metabolism enzymes that contain Fe/S clusters, such as replicative DNA polymerases, further increases the feasibility of DNA CT communication as a universal DNA surveillance mechanism that maintains genome integrity in living organisms.

Perspective

As reviewed here, multiple enzymes required for DNA synthesis and repair contain iron cofactors that are essential for their function. However, many aspects of DNA metabolism need to be further studied, especially under iron deficient conditions. How does iron deficiency affect the fidelity of DNA replication and repair? Do mechanisms exist that control iron cofactor assembly into the prefered enzymes implicated in DNA metabolism? Is human ribonucleotide reductase regulated by iron availability? And, if it were, should we consider the body iron status before using ribonucleotide reductase as a target in anticancer therapies? What is the biological relevance of the DNA-mediated charge transport between Fe/S clusters within DNA polymerases and repair enzymes in monitoring DNA damage sites? The elemental, and stricking, role of iron in different DNA metabolism steps opens up these and many other fascinating questions which need to be answered in the future.

Acknowledgements

Antonia María Romero and Lucía Ramos-Alonso are recipients of predoctoral fellowships from the Spanish Ministry of Economy and Competitiveness. Work in our group is supported by the BIO2014-56298-P grant from the Spanish Ministry of Economy and Competitiveness.

Table 1. List of the iron-containing enzymes dedicated to DNA metabolism in the yeast *S. cerevisiae*. Human homologs and functions are indicated. An asterik (*) indicates that it has not been experimentally demonstrated in yeast. Y·: tyrosyl radical. 2-OG: 2-oxoglutarate.

Yeast	Human	Enzyme (function)	Cofactor
Pol1	POLA	Catalytic subunit of DNA Pol α (DNA replication)	[4Fe-4S]
Pol2	POLE1	Catalytic subunit of DNA Pol ε (DNA replication)	[4Fe-4S]
Pol3	POLD1	Catalytic subunit of DNA Pol δ (DNA replication)	[4Fe-4S]
Rev3	REV3L	Catalytic subunit of DNA Pol ζ (translesion DNA synthesis)	[4Fe-4S]
Pri2	PRIM2	Subunit of DNA primase (DNA synthesis and double-strand break repair)	[4Fe-4S]
Dna2	DNA2	Helicase/nuclease (Okazaki fragment maturation, DNA repair and telomere maintenance)	[4Fe-4S]

Rad3	XPD	Helicase subunit of TFIIH (nucleotide excision repair and transcription)	[4Fe-4S]
Chl1	CHLR1	Helicase (sister chromatid cohesion and DNA replication)	[4Fe-4S]*
Ntg2	NTHL1	DNA glycosylase (base excision repair)	[4Fe-4S]*
Rnr2	RRM2	Small subunit of ribonucleotide reductase (dNDP synthesis)	$[(Fe^{3+})_2Y\cdot]$
Tpa1	OGFOD1	Fe^{2+}/2-oxoglutarate-dependent dioxygenase (translation termination and DNA alkylation repair)	Fe^{2+}/2-OG

Figure legends

Figure 1. Current model for iron cofactor assembly in a subset of *S. cerevisiae* cytosolic and nuclear Fe/S cluster proteins. The core mitochondrial ISC assembly machinery synthesizes a sulfur-containing molecule, denoted X-S, which is exported to the cytoplasm by ATP-binding cassette transporter Atm1. The mitochondrial exported X-S product is transformed into a [2Fe-2S] cluster coordinated by two GSH molecules and Grx3 or Grx4 monothiol glutaredoxins. The CIA machinery facilitates the maturation of extra-mitochondrial Fe/S proteins. Briefly, the electron transport chain composed of NADPH–diflavin reductase Tah18, the Fe/S protein Dre2 and the [2Fe-2S]-Grx3/4-GSH complex use the sulfur source X-S to assemble a bridging [4Fe-4S] cluster on the Cfd1-Nbp35 heterotetrameric scaffold complex. Then the Fe/S protein Nar1 and the CIA targeting complex Cia1-Cia2-Mms19 transfer and insert the [4Fe-4S] cluster into proteins involved in DNA metabolism (Pol3, Pri2, Dna2, Rad3, Ntg2), amino acid synthesis (Met5, Leu1) and translation (Rli1). Two adaptors, Lto1 and Yae1, specifically facilitate Fe/S cluster insertion into yeast Rli1. CIA: cytosolic iron-sulfur cluster protein assembly. ISC: iron-sulfur cluster.

Figure 2. Representation of the multisubunit structure of yeast DNA polymerases. Each holoenzyme is composed of a catalytic subunit A (Pol1, Pol3, Pol2, Rev3), a regulatory subunit B (Pol12, Pol31, Dpb2), and other accessory subunits (Pri1, Pri2, Pol32, Dpb3, Dpb4). The CTD of each Pol catalytic subunit that

contains the [4Fe-4S] cluster and the second [4Fe-4S] cluster in Pol2 are depicted. Pol δ subunits Pol31 and Pol32 also form part of the Pol ζ holoenzyme.

Figure 3. Schematic view of the replicative fork and the main yeast Fe/S cluster enzymes implicated in DNA replication and repair. The Fe/S enzymes involved in DNA replication and repair include the polymerases Pol α–Prim, Pol δ, Pol ε and Pol ζ, the helicases Rad3 and Chl1, the helicase/nuclease Dna2 and the glycosylase Ntg2. DNA replication starts with primase synthesizing an RNA primer that is extended by DNA Pol α. The binding of the RFC complex (not shown) to the RNA-DNA duplex promotes DNA Pol-Prim complex dissociation and PCNA loading. PCNA recruits DNA Pol δ and Pol ε, which increase DNA replication processivity and fidelity of the lagging strand and the leading strand respectively. DNA Pol ζ acts in TLS to prevent replication fork collapse. Helicase/nuclease Dna2 is implicated in Okazaki fragment processing during replication, DNA repair and telomere maintenance. XPD family member DNA helicase Rad3 functions in NER (and transcription initiation) as part of RNA Pol II initiation factor TFIIH. Glycosylase Ntg2 removes oxidative DNA damage through BER. Finally, DNA helicase Chl1 associates with clamp factor PCNA, endonuclease Fen1, Ctf4 (not shown), acetyltransferase Eco1 and cohesin to ensure proper chromosome segregation between daughter cells. Leading and lagging DNA strands are indicated. BER: base excision repair. NER: nucleotide excision repair. PCNA: proliferating cell nuclear antigen. RFC: replication factor C. TLS: translesion DNA synthesis.

Figure 4. **Mechanisms of the activation of *S. cerevisiae* ribonucleotide reductase in response to iron deficiency and Fe/S cluster synthesis defects.** If low iron bioavailability or defects in Fe/S biogenesis occur, the Mec1-Rad53-Dun1 checkpoint kinase cascade and mRNA-binding proteins Cth1 and Cth2 (Cth1/2) optimize ribonucleotide reductase activity by multiple overlapping mechanisms. First, Dun1 kinase phosphorylates Sml1 and Dif1 proteins, which are degraded (indicated by a red "X"), to cause an increase in Rnr1 activity and the relocalization of Rnr2-Rnr4 to the cytoplasm, respectively. Second, defects in mitochondrial ISC synthesis activate *RNR3* expression. Third and finally, in response to iron scarcity, Aft1 and Aft2 (Aft1/2) activate the transcription of *CTH1* and *CTH2* genes. Cth1/2 proteins bind to the AU-rich elements (ARE) within *WTM1* mRNA thus promoting its turnover. Reduced Wtm1 protein abundance (indicated by a red arrow) facilitates Rnr2-Rnr4 redistribution to the cytoplasm.

Figure 5. Barton's model for the DNA charge transfer between Fe/S clusters in DNA repair. When a $[4Fe\text{-}4S]^{2+}$-containing protein binds to DNA, it can oxidize to the $[4Fe\text{-}4S]^{3+}$ state and transfer an electron (e^-) to a nearby $[4Fe\text{-}4S]^{3+}$ protein. This charge transfer promotes the reduction of the recipient protein to the $[4Fe\text{-}4S]^{2+}$ state and its dissociation from DNA. This released protein can scan another genome region. If there is a DNA lesion (indicated by a red "X") between two [4Fe-4S] proteins, the DNA charge transfer is blocked and both proteins remain bound to DNA in their $[4Fe\text{-}4S]^{3+}$ oxidation state. This mechanism facilitates DNA damage localization by redistributing DNA repair proteins from undamaged regions to sites with lesions.

References

1. M. F. White and M. S. Dillingham, Iron-sulphur clusters in nucleic acid processing enzymes, *Curr Opin Struct Biol*, 2012, **22**, 94-100.
2. C. Zhang, Essential functions of iron-requiring proteins in DNA replication, repair and cell cycle control, *Protein Cell*, 2014, **5**, 750-760.
3. J. O. Fuss, C. L. Tsai, J. P. Ishida and J. A. Tainer, Emerging critical roles of Fe-S clusters in DNA replication and repair, *Biochim Biophys Acta*, 2015, **1853**, 1253-1271.
4. V. D. Paul and R. Lill, Biogenesis of cytosolic and nuclear iron-sulfur proteins and their role in genome stability, *Biochim Biophys Acta*, 2015, **1853**, 1528-1539.
5. M. A. Grodick, N. B. Muren and J. K. Barton, DNA charge transport within the cell, *Biochemistry*, 2015, **54**, 962-973.
6. A. R. Arnold, M. A. Grodick and J. K. Barton, DNA Charge Transport: from Chemical Principles to the Cell, *Cell Chem Biol*, 2016, **23**, 183-197.
7. J. R. Veatch, M. A. McMurray, Z. W. Nelson and D. E. Gottschling, Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect, *Cell*, 2009, **137**, 1247-1258.
8. M. C. Diaz de la Loza, M. Gallardo, M. L. Garcia-Rubio, A. Izquierdo, E. Herrero, A. Aguilera and R. E. Wellinger, Zim17/Tim15 links mitochondrial iron-sulfur cluster biosynthesis to nuclear genome stability, *Nucleic Acids Res*, 2011, **39**, 6002-6015.
9. I. Lewrenz, N. Rietzschel, B. Guiard, R. Lill, M. van der Laan and W. Voos, The functional interaction of mitochondrial Hsp70s with the escort protein Zim17 is critical for Fe/S biogenesis and substrate interaction at the inner membrane preprotein translocase, *J Biol Chem*, 2013, **288**, 30931-30943.
10. D. J. Netz, J. Mascarenhas, O. Stehling, A. J. Pierik and R. Lill, Maturation of cytosolic and nuclear iron-sulfur proteins, *Trends Cell Biol*, 2014, **24**, 303-312.
11. R. Lill, R. Dutkiewicz, S. A. Freibert, T. Heidenreich, J. Mascarenhas, D. J. Netz, V. D. Paul, A. J. Pierik, N. Richter, M. Stumpfig, V. Srinivasan, O. Stehling and U. Muhlenhoff, The role of mitochondria and the CIA machinery in the maturation of cytosolic and nuclear iron-sulfur proteins, *Eur J Cell Biol*, 2015, **94**, 280-291.
12. O. Stehling, C. Wilbrecht and R. Lill, Mitochondrial iron-sulfur protein biogenesis and human disease, *Biochimie*, 2014, **100**, 61-77.
13. C. E. Outten and A. N. Albetel, Iron sensing and regulation in Saccharomyces cerevisiae: Ironing out the mechanistic details, *Curr Opin Microbiol*, 2013, **16**, 662-668.
14. M. T. Martinez-Pastor, A. Perea-Garcia and S. Puig, Mechanisms of iron sensing and regulation in the yeast Saccharomyces cerevisiae, *World J Microbiol Biotechnol*, 2017, **33**, 75.
15. J. Y. Lee, J. G. Yang, D. Zhitnitsky, O. Lewinson and D. C. Rees, Structural basis for heavy metal detoxification by an Atm1-type ABC exporter, *Science*, 2014, **343**, 1133-1136.
16. V. Srinivasan, A. J. Pierik and R. Lill, Crystal structures of nucleotide-free and glutathione-bound mitochondrial ABC transporter Atm1, *Science*, 2014, **343**, 1137-1140.

17. U. Muhlenhoff, S. Molik, J. R. Godoy, M. A. Uzarska, N. Richter, A. Seubert, Y. Zhang, J. Stubbe, F. Pierrel, E. Herrero, C. H. Lillig and R. Lill, Cytosolic monothiol glutaredoxins function in intracellular iron sensing and trafficking via their bound iron-sulfur cluster, *Cell Metab*, 2010, **12**, 373-385.
18. O. Stehling, A. A. Vashisht, J. Mascarenhas, Z. O. Jonsson, T. Sharma, D. J. Netz, A. J. Pierik, J. A. Wohlschlegel and R. Lill, MMS19 assembles iron-sulfur proteins required for DNA metabolism and genomic integrity, *Science*, 2012, **337**, 195-199.
19. K. Gari, A. M. Leon Ortiz, V. Borel, H. Flynn, J. M. Skehel and S. J. Boulton, MMS19 links cytoplasmic iron-sulfur cluster assembly to DNA metabolism, *Science*, 2012, **337**, 243-245.
20. V. D. Paul, U. Muhlenhoff, M. Stumpfig, J. Seebacher, K. G. Kugler, C. Renicke, C. Taxis, A. C. Gavin, A. J. Pierik and R. Lill, The deca-GX3 proteins Yae1-Lto1 function as adaptors recruiting the ABC protein Rli1 for iron-sulfur cluster insertion, *Elife*, 2015, **4**, e08231.
21. D. C. Odermatt and K. Gari, The CIA Targeting Complex Is Highly Regulated and Provides Two Distinct Binding Sites for Client Iron-Sulfur Proteins, *Cell Rep*, 2017, **18**, 1434-1443.
22. O. Stehling, J. Mascarenhas, A. A. Vashisht, A. D. Sheftel, B. Niggemeyer, R. Rosser, A. J. Pierik, J. A. Wohlschlegel and R. Lill, Human CIA2A-FAM96A and CIA2B-FAM96B integrate iron homeostasis and maturation of different subsets of cytosolic-nuclear iron-sulfur proteins, *Cell Metab*, 2013, **18**, 187-198.
23. E. Johansson and S. A. Macneill, The eukaryotic replicative DNA polymerases take shape, *Trends Biochem Sci*, 2010, **35**, 339-347.
24. J. R. Nelson, C. W. Lawrence and D. C. Hinkle, Thymine-thymine dimer bypass by yeast DNA polymerase zeta, *Science*, 1996, **272**, 1646-1649.
25. A. V. Makarova, J. L. Stodola and P. M. Burgers, A four-subunit DNA polymerase zeta complex containing Pol delta accessory subunits is essential for PCNA-mediated mutagenesis, *Nucleic Acids Res*, 2012, **40**, 11618-11626.
26. R. E. Johnson, L. Prakash and S. Prakash, Pol31 and Pol32 subunits of yeast DNA polymerase delta are also essential subunits of DNA polymerase zeta, *Proc Natl Acad Sci U S A*, 2012, **109**, 12455-12460.
27. A. G. Baranovskiy, A. G. Lada, H. M. Siebler, Y. Zhang, Y. I. Pavlov and T. H. Tahirov, DNA polymerase delta and zeta switch by sharing accessory subunits of DNA polymerase delta, *J Biol Chem*, 2012, **287**, 17281-17287.
28. T. J. Kelley, S. Moghaddas, R. Bose and S. Basu, Inhibition of immunopurified DNA polymerase-alpha from PA-3 prostate tumor cells by platinum (II) antitumor drugs, *Cancer Biochem Biophys*, 1993, **13**, 135-146.
29. F. Evanics, L. Maurmann, W. W. Yang and R. N. Bose, Nuclear magnetic resonance structures of the zinc finger domain of human DNA polymerase-alpha, *Biochim Biophys Acta*, 2003, **1651**, 163-171.
30. S. Klinge, R. Nunez-Ramirez, O. Llorca and L. Pellegrini, 3D architecture of DNA Pol alpha reveals the functional core of multi-subunit replicative polymerases, *EMBO J*, 2009, **28**, 1978-1987.
31. R. Dua, D. L. Levy and J. L. Campbell, Analysis of the essential functions of the C-terminal protein/protein interaction domain of Saccharomyces

cerevisiae pol epsilon and its unexpected ability to support growth in the absence of the DNA polymerase domain, *J Biol Chem*, 1999, **274**, 22283-22288.
32. J. Sanchez Garcia, L. F. Ciufo, X. Yang, S. E. Kearsey and S. A. MacNeill, The C-terminal zinc finger of the catalytic subunit of DNA polymerase delta is responsible for direct interaction with the B-subunit, *Nucleic Acids Res*, 2004, **32**, 3005-3016.
33. R. Chanet and M. Heude, Characterization of mutations that are synthetic lethal with pol3-13, a mutated allele of DNA polymerase delta in Saccharomyces cerevisiae, *Curr Genet*, 2003, **43**, 337-350.
34. D. J. Netz, C. M. Stith, M. Stumpfig, G. Kopf, D. Vogel, H. M. Genau, J. L. Stodola, R. Lill, P. M. Burgers and A. J. Pierik, Eukaryotic DNA polymerases require an iron-sulfur cluster for the formation of active complexes, *Nat Chem Biol*, 2011, **8**, 125-132.
35. R. Jain, E. S. Vanamee, B. G. Dzikovski, A. Buku, R. E. Johnson, L. Prakash, S. Prakash and A. K. Aggarwal, An iron-sulfur cluster in the polymerase domain of yeast DNA polymerase epsilon, *J Mol Biol*, 2014, **426**, 301-308.
36. Y. Suwa, J. Gu, A. G. Baranovskiy, N. D. Babayeva, Y. I. Pavlov and T. H. Tahirov, Crystal Structure of the Human Pol alpha B Subunit in Complex with the C-terminal Domain of the Catalytic Subunit, *J Biol Chem*, 2015, **290**, 14328-14337.
37. E. H. Kocabas, M., Metalloenzymes: use of recombinant protein expression and affinity tags to aid identification of native metal ion cofactors, *Biochem Anal Biochem*, 2013, **2**, 1000132.
38. H. M. Siebler, A. G. Lada, A. G. Baranovskiy, T. H. Tahirov and Y. I. Pavlov, A novel variant of DNA polymerase zeta, Rev3DeltaC, highlights differential regulation of Pol32 as a subunit of polymerase delta versus zeta in Saccharomyces cerevisiae, *DNA Repair (Amst)*, 2014, **24**, 138-149.
39. L. Giot, R. Chanet, M. Simon, C. Facca and G. Faye, Involvement of the yeast DNA polymerase delta in DNA repair in vivo, *Genetics*, 1997, **146**, 1239-1251.
40. E. I. Stepchenkova, E. R. Tarakhovskaya, H. M. Siebler and Y. I. Pavlov, Defect of Fe-S cluster binding by DNA polymerase delta in yeast suppresses UV-induced mutagenesis, but enhances DNA polymerase zeta - dependent spontaneous mutagenesis, *DNA Repair (Amst)*, 2017, **49**, 60-69.
41. R. D. Kuchta and G. Stengel, Mechanism and evolution of DNA primases, *Biochim Biophys Acta*, 2010, **1804**, 1180-1189.
42. M. Foiani, C. Santocanale, P. Plevani and G. Lucchini, A single essential gene, PRI2, encodes the large subunit of DNA primase in Saccharomyces cerevisiae, *Mol Cell Biol*, 1989, **9**, 3081-3087.
43. S. Francesconi, M. P. Longhese, A. Piseri, C. Santocanale, G. Lucchini and P. Plevani, Mutations in conserved yeast DNA primase domains impair DNA replication in vivo, *Proc Natl Acad Sci U S A*, 1991, **88**, 3877-3881.
44. M. L. Kilkenny, M. A. Longo, R. L. Perera and L. Pellegrini, Structures of human primase reveal design of nucleotide elongation site and mode of Pol alpha tethering, *Proc Natl Acad Sci U S A*, 2013, **110**, 15961-15966.
45. M. Foiani, A. J. Lindner, G. R. Hartmann, G. Lucchini and P. Plevani, Affinity labeling of the active center and ribonucleoside triphosphate binding site of yeast DNA primase, *J Biol Chem*, 1989, **264**, 2189-2194.

46. W. C. Copeland and T. S. Wang, Enzymatic characterization of the individual mammalian primase subunits reveals a biphasic mechanism for initiation of DNA replication, *J Biol Chem*, 1993, **268**, 26179-26189.
47. L. K. Zerbe and R. D. Kuchta, The p58 subunit of human DNA primase is important for primer initiation, elongation, and counting, *Biochemistry*, 2002, **41**, 4891-4900.
48. B. E. Weiner, H. Huang, B. M. Dattilo, M. J. Nilges, E. Fanning and W. J. Chazin, An iron-sulfur cluster in the C-terminal domain of the p58 subunit of human DNA primase, *J Biol Chem*, 2007, **282**, 33444-33451.
49. S. Klinge, J. Hirst, J. D. Maman, T. Krude and L. Pellegrini, An iron-sulfur domain of the eukaryotic primase is essential for RNA primer synthesis, *Nat Struct Mol Biol*, 2007, **14**, 875-877.
50. L. Liu and M. Huang, Essential role of the iron-sulfur cluster binding domain of the primase regulatory subunit Pri2 in DNA replication initiation, *Protein Cell*, 2015, **6**, 194-210.
51. L. Sauguet, S. Klinge, R. L. Perera, J. D. Maman and L. Pellegrini, Shared active site architecture between the large subunit of eukaryotic primase and DNA photolyase, *PLoS One*, 2010, **5**, e10083.
52. E. O'Brien, M. E. Holt, M. K. Thompson, L. E. Salay, A. C. Ehlinger, W. J. Chazin and J. K. Barton, The [4Fe4S] cluster of human DNA primase functions as a redox switch using DNA charge transport, *Science*, 2017, **355**.
53. Y. H. Kang, C. H. Lee and Y. S. Seo, Dna2 on the road to Okazaki fragment processing and genome stability in eukaryotes, *Crit Rev Biochem Mol Biol*, 2010, **45**, 71-96.
54. P. H. Wanrooij and P. M. Burgers, Yet another job for Dna2: Checkpoint activation, *DNA Repair (Amst)*, 2015, **32**, 17-23.
55. P. M. Burgers, Polymerase dynamics at the eukaryotic DNA replication fork, *J Biol Chem*, 2009, **284**, 4041-4045.
56. E. P. Mimitou and L. S. Symington, Nucleases and helicases take center stage in homologous recombination, *Trends Biochem Sci*, 2009, **34**, 264-272.
57. M. Levikova, D. Klaue, R. Seidel and P. Cejka, Nuclease activity of Saccharomyces cerevisiae Dna2 inhibits its potent DNA helicase activity, *Proc Natl Acad Sci U S A*, 2013, **110**, E1992-2001.
58. S. Kumar and P. M. Burgers, Lagging strand maturation factor Dna2 is a component of the replication checkpoint initiation machinery, *Genes Dev*, 2013, **27**, 313-321.
59. T. Formosa and T. Nittis, Dna2 mutants reveal interactions with Dna polymerase alpha and Ctf4, a Pol alpha accessory factor, and show that full Dna2 helicase activity is not essential for growth, *Genetics*, 1999, **151**, 1459-1470.
60. M. E. Budd, W. Choe and J. L. Campbell, The nuclease activity of the yeast DNA2 protein, which is related to the RecB-like nucleases, is essential in vivo, *J Biol Chem*, 2000, **275**, 16518-16529.
61. J. T. Yeeles, R. Cammack and M. S. Dillingham, An iron-sulfur cluster is essential for the binding of broken DNA by AddAB-type helicase-nucleases, *J Biol Chem*, 2009, **284**, 7746-7755.
62. S. Pokharel and J. L. Campbell, Cross talk between the nuclease and helicase activities of Dna2: role of an essential iron-sulfur cluster domain, *Nucleic Acids Res*, 2012, **40**, 7821-7830.

63. Y. Wu, A. N. Suhasini and R. M. Brosh, Jr., Welcome the family of FANCJ-like helicases to the block of genome stability maintenance proteins, *Cell Mol Life Sci*, 2009, **66**, 1209-1222.
64. M. F. White, Structure, function and evolution of the XPD family of iron-sulfur-containing 5'-->3' DNA helicases, *Biochem Soc Trans*, 2009, **37**, 547-551.
65. S. C. Wolski, J. Kuper and C. Kisker, The XPD helicase: XPanDing archaeal XPD structures to get a grip on human DNA repair, *Biol Chem*, 2010, **391**, 761-765.
66. B. Van Houten, J. Kuper and C. Kisker, Role of XPD in cellular functions: To TFIIH and beyond, *DNA Repair (Amst)*, 2016, **44**, 136-142.
67. E. Compe and J. M. Egly, TFIIH: when transcription met DNA repair, *Nat Rev Mol Cell Biol*, 2012, **13**, 343-354.
68. J. Rudolf, V. Makrantoni, W. J. Ingledew, M. J. Stark and M. F. White, The DNA repair helicases XPD and FancJ have essential iron-sulfur domains, *Mol Cell*, 2006, **23**, 801-808.
69. J. Kuper, S. C. Wolski, G. Michels and C. Kisker, Functional and structural studies of the nucleotide excision repair helicase XPD suggest a polarity for DNA translocation, *EMBO J*, 2012, **31**, 494-502.
70. L. Fan, J. O. Fuss, Q. J. Cheng, A. S. Arvai, M. Hammel, V. A. Roberts, P. K. Cooper and J. A. Tainer, XPD helicase structures and activities: insights into the cancer and aging phenotypes from XPD mutations, *Cell*, 2008, **133**, 789-800.
71. H. Liu, J. Rudolf, K. A. Johnson, S. A. McMahon, M. Oke, L. Carter, A. M. McRobbie, S. E. Brown, J. H. Naismith and M. F. White, Structure of the DNA repair helicase XPD, *Cell*, 2008, **133**, 801-812.
72. S. C. Wolski, J. Kuper, P. Hanzelmann, J. J. Truglio, D. L. Croteau, B. Van Houten and C. Kisker, Crystal structure of the FeS cluster-containing nucleotide excision repair helicase XPD, *PLoS Biol*, 2008, **6**, e149.
73. R. A. Pugh, C. G. Wu and M. Spies, Regulation of translocation polarity by helicase domain 1 in SF2B helicases, *EMBO J*, 2012, **31**, 503-514.
74. D. Constantinescu-Aruxandei, B. Petrovic-Stojanovska, J. C. Penedo, M. F. White and J. H. Naismith, Mechanism of DNA loading by the DNA repair helicase XPD, *Nucleic Acids Res*, 2016, **44**, 2806-2815.
75. T. P. Mui, J. O. Fuss, J. P. Ishida, J. A. Tainer and J. K. Barton, ATP-stimulated, DNA-mediated redox signaling by XPD, a DNA repair and transcription helicase, *J Am Chem Soc*, 2011, **133**, 16378-16381.
76. L. Prakash and S. Prakash, Isolation and characterization of MMS-sensitive mutants of Saccharomyces cerevisiae, *Genetics*, 1977, **86**, 33-55.
77. L. Prakash and S. Prakash, Three additional genes involved in pyrimidine dimer removal in Saccharomyces cerevisiae: RAD7, RAD14 and MMS19, *Mol Gen Genet*, 1979, **176**, 351-359.
78. S. Lauder, M. Bankmann, S. N. Guzder, P. Sung, L. Prakash and S. Prakash, Dual requirement for the yeast MMS19 gene in DNA repair and RNA polymerase II transcription, *Mol Cell Biol*, 1996, **16**, 6783-6793.
79. H. Kou, Y. Zhou, R. M. Gorospe and Z. Wang, Mms19 protein functions in nucleotide excision repair by sustaining an adequate cellular concentration of the TFIIH component Rad3, *Proc Natl Acad Sci U S A*, 2008, **105**, 15714-15719.

80. T. Seroz, G. S. Winkler, J. Auriol, R. A. Verhage, W. Vermeulen, B. Smit, J. Brouwer, A. P. Eker, G. Weeda, J. M. Egly and J. H. Hoeijmakers, Cloning of a human homolog of the yeast nucleotide excision repair gene MMS19 and interaction with transcription repair factor TFIIH via the XPB and XPD helicases, *Nucleic Acids Res*, 2000, **28**, 4506-4513.
81. S. Ito, L. J. Tan, D. Andoh, T. Narita, M. Seki, Y. Hirano, K. Narita, I. Kuraoka, Y. Hiraoka and K. Tanaka, MMXD, a TFIIH-independent XPD-MMS19 protein complex involved in chromosome segregation, *Mol Cell*, 2010, **39**, 632-640.
82. N. van Wietmarschen, A. Moradian, G. B. Morin, P. M. Lansdorp and E. J. Uringa, The mammalian proteins MMS19, MIP18, and ANT2 are involved in cytoplasmic iron-sulfur cluster protein assembly, *J Biol Chem*, 2012, **287**, 43351-43358.
83. A. A. Vashisht, C. C. Yu, T. Sharma, K. Ro and J. A. Wohlschlegel, The Association of the Xeroderma Pigmentosum Group D DNA Helicase (XPD) with Transcription Factor IIH Is Regulated by the Cytosolic Iron-Sulfur Cluster Assembly Pathway, *J Biol Chem*, 2015, **290**, 14218-14225.
84. S. K. Bharti, I. Khan, T. Banerjee, J. A. Sommers, Y. Wu and R. M. Brosh, Jr., Molecular functions and cellular roles of the ChlR1 (DDX11) helicase defective in the rare cohesinopathy Warsaw breakage syndrome, *Cell Mol Life Sci*, 2014, **71**, 2625-2639.
85. M. Petronczki, B. Chwalla, M. F. Siomos, S. Yokobayashi, W. Helmhart, A. M. Deutschbauer, R. W. Davis, Y. Watanabe and K. Nasmyth, Sister-chromatid cohesion mediated by the alternative RF-CCtf18/Dcc1/Ctf8, the helicase Chl1 and the polymerase-alpha-associated protein Ctf4 is essential for chromatid disjunction during meiosis II, *J Cell Sci*, 2004, **117**, 3547-3559.
86. R. V. Skibbens, Chl1p, a DNA helicase-like protein in budding yeast, functions in sister-chromatid cohesion, *Genetics*, 2004, **166**, 33-42.
87. M. L. Mayer, I. Pot, M. Chang, H. Xu, V. Aneliunas, T. Kwok, R. Newitt, R. Aebersold, C. Boone, G. W. Brown and P. Hieter, Identification of protein complexes required for efficient sister chromatid cohesion, *Mol Biol Cell*, 2004, **15**, 1736-1745.
88. J. L. Parish, J. Rosa, X. Wang, J. M. Lahti, S. J. Doxsey and E. J. Androphy, The DNA helicase ChlR1 is required for sister chromatid cohesion in mammalian cells, *J Cell Sci*, 2006, **119**, 4857-4865.
89. A. Inoue, T. Li, S. K. Roby, M. B. Valentine, M. Inoue, K. Boyd, V. J. Kidd and J. M. Lahti, Loss of ChlR1 helicase in mouse causes lethality due to the accumulation of aneuploid cells generated by cohesion defects and placental malformation, *Cell Cycle*, 2007, **6**, 1646-1654.
90. S. L. Gerring, F. Spencer and P. Hieter, The CHL 1 (CTF 1) gene product of Saccharomyces cerevisiae is important for chromosome transmission and normal cell cycle progression in G2/M, *EMBO J*, 1990, **9**, 4347-4358.
91. A. Farina, J. H. Shin, D. H. Kim, V. P. Bermudez, Z. Kelman, Y. S. Seo and J. Hurwitz, Studies with the human cohesin establishment factor, ChlR1. Association of ChlR1 with Ctf18-RFC and Fen1, *J Biol Chem*, 2008, **283**, 20925-20936.
92. S. Rudra and R. V. Skibbens, Sister chromatid cohesion establishment occurs in concert with lagging strand synthesis, *Cell Cycle*, 2012, **11**, 2114-2121.
93. C. P. Samora, J. Saksouk, P. Goswami, B. O. Wade, M. R. Singleton, P. A. Bates, A. Lengronne, A. Costa and F. Uhlmann, Ctf4 Links DNA Replication with

Sister Chromatid Cohesion Establishment by Recruiting the Chl1 Helicase to the Replisome, *Mol Cell*, 2016, **63**, 371-384.
94. V. Borges, D. J. Smith, I. Whitehouse and F. Uhlmann, An Eco1-independent sister chromatid cohesion establishment pathway in S. cerevisiae, *Chromosoma*, 2013, **122**, 121-134.
95. Y. Hirota and J. M. Lahti, Characterization of the enzymatic activity of hChlR1, a novel human DNA helicase, *Nucleic Acids Res*, 2000, **28**, 917-924.
96. J. M. Capo-Chichi, S. K. Bharti, J. A. Sommers, T. Yammine, E. Chouery, L. Patry, G. A. Rouleau, M. E. Samuels, F. F. Hamdan, J. L. Michaud, R. M. Brosh, Jr., A. Megarbane and Z. Kibar, Identification and biochemical characterization of a novel mutation in DDX11 causing Warsaw breakage syndrome, *Hum Mutat*, 2013, **34**, 103-107.
97. Y. Wu, J. A. Sommers, I. Khan, J. P. de Winter and R. M. Brosh, Jr., Biochemical characterization of Warsaw breakage syndrome helicase, *J Biol Chem*, 2012, **287**, 1007-1021.
98. P. van der Lelij, K. H. Chrzanowska, B. C. Godthelp, M. A. Rooimans, A. B. Oostra, M. Stumm, M. Z. Zdzienicka, H. Joenje and J. P. de Winter, Warsaw breakage syndrome, a cohesinopathy associated with mutations in the XPD helicase family member DDX11/ChlR1, *Am J Hum Genet*, 2010, **86**, 262-266.
99. S. L. Holloway, CHL1 is a nuclear protein with an essential ATP binding site that exhibits a size-dependent effect on chromosome segregation, *Nucleic Acids Res*, 2000, **28**, 3056-3064.
100. X. Sun, H. Chen, Z. Deng, B. Hu, H. Luo, X. Zeng, L. Han, G. Cai and L. Ma, The Warsaw breakage syndrome-related protein DDX11 is required for ribosomal RNA synthesis and embryonic development, *Hum Mol Genet*, 2015, **24**, 4901-4915.
101. R. P. Cunningham, H. Asahara, J. F. Bank, C. P. Scholes, J. C. Salerno, K. Surerus, E. Munck, J. McCracken, J. Peisach and M. H. Emptage, Endonuclease III is an iron-sulfur protein, *Biochemistry*, 1989, **28**, 4450-4455.
102. C. F. Kuo, D. E. McRee, C. L. Fisher, S. F. O'Handley, R. P. Cunningham and J. A. Tainer, Atomic structure of the DNA repair [4Fe-4S] enzyme endonuclease III, *Science*, 1992, **258**, 434-440.
103. M. M. Thayer, H. Ahern, D. Xing, R. P. Cunningham and J. A. Tainer, Novel DNA binding motifs in the DNA repair enzyme endonuclease III crystal structure, *EMBO J*, 1995, **14**, 4108-4120.
104. Y. Guan, R. C. Manuel, A. S. Arvai, S. S. Parikh, C. D. Mol, J. H. Miller, S. Lloyd and J. A. Tainer, MutY catalytic core, mutant and bound adenine structures define specificity for DNA repair enzyme superfamily, *Nat Struct Biol*, 1998, **5**, 1058-1064.
105. O. A. Lukianova and S. S. David, A role for iron-sulfur clusters in DNA repair, *Curr Opin Chem Biol*, 2005, **9**, 145-151.
106. E. M. Boon, A. L. Livingston, N. H. Chmiel, S. S. David and J. K. Barton, DNA-mediated charge transport for DNA repair, *Proc Natl Acad Sci U S A*, 2003, **100**, 12543-12547.
107. P. A. Sontz, N. B. Muren and J. K. Barton, DNA charge transport for sensing and signaling, *Acc Chem Res*, 2012, **45**, 1792-1800.
108. L. Eide, M. Bjoras, M. Pirovano, I. Alseth, K. G. Berdal and E. Seeberg, Base excision of oxidative purine and pyrimidine DNA damage in Saccharomyces

cerevisiae by a DNA glycosylase with sequence similarity to endonuclease III from Escherichia coli, *Proc Natl Acad Sci U S A*, 1996, **93**, 10735-10740.
109. I. Alseth, L. Eide, M. Pirovano, T. Rognes, E. Seeberg and M. Bjoras, The Saccharomyces cerevisiae homologues of endonuclease III from Escherichia coli, Ntg1 and Ntg2, are both required for efficient repair of spontaneous and induced oxidative DNA damage in yeast, *Mol Cell Biol*, 1999, **19**, 3779-3787.
110. L. Augeri, Y. M. Lee, A. B. Barton and P. W. Doetsch, Purification, characterization, gene cloning, and expression of Saccharomyces cerevisiae redoxyendonuclease, a homolog of Escherichia coli endonuclease III, *Biochemistry*, 1997, **36**, 721-729.
111. H. J. You, R. L. Swanson and P. W. Doetsch, Saccharomyces cerevisiae possesses two functional homologues of Escherichia coli endonuclease III, *Biochemistry*, 1998, **37**, 6033-6040.
112. K. L. Meadows, B. Song and P. W. Doetsch, Characterization of AP lyase activities of Saccharomyces cerevisiae Ntg1p and Ntg2p: implications for biological function, *Nucleic Acids Res*, 2003, **31**, 5560-5567.
113. H. J. You, R. L. Swanson, C. Harrington, A. H. Corbett, S. Jinks-Robertson, S. Senturker, S. S. Wallace, S. Boiteux, M. Dizdaroglu and P. W. Doetsch, Saccharomyces cerevisiae Ntg1p and Ntg2p: broad specificity N-glycosylases for the repair of oxidative DNA damage in the nucleus and mitochondria, *Biochemistry*, 1999, **38**, 11298-11306.
114. S. Senturker, P. Auffret van der Kemp, H. J. You, P. W. Doetsch, M. Dizdaroglu and S. Boiteux, Substrate specificities of the ntg1 and ntg2 proteins of Saccharomyces cerevisiae for oxidized DNA bases are not identical, *Nucleic Acids Res*, 1998, **26**, 5270-5276.
115. L. M. Griffiths, D. Swartzlander, K. L. Meadows, K. D. Wilkinson, A. H. Corbett and P. W. Doetsch, Dynamic compartmentalization of base excision repair proteins in response to nuclear and mitochondrial oxidative stress, *Mol Cell Biol*, 2009, **29**, 794-807.
116. M. Kolberg, K. R. Strand, P. Graff and K. K. Andersson, Structure, function, and mechanism of ribonucleotide reductases, *Biochim Biophys Acta*, 2004, **1699**, 1-34.
117. P. Nordlund and P. Reichard, Ribonucleotide reductases, *Annu Rev Biochem*, 2006, **75**, 681-706.
118. J. A. Cotruvo and J. Stubbe, Class I ribonucleotide reductases: metallocofactor assembly and repair in vitro and in vivo, *Annu Rev Biochem*, 2011, **80**, 733-767.
119. M. Huang, M. J. Parker and J. Stubbe, Choosing the right metal: case studies of class I ribonucleotide reductases, *J Biol Chem*, 2014, **289**, 28104-28111.
120. D. J. Netz, M. Stumpfig, C. Dore, U. Muhlenhoff, A. J. Pierik and R. Lill, Tah18 transfers electrons to Dre2 in cytosolic iron-sulfur protein biogenesis, *Nat Chem Biol*, 2010, **6**, 758-765.
121. Y. Zhang, L. Liu, X. Wu, X. An, J. Stubbe and M. Huang, Investigation of in vivo diferric tyrosyl radical formation in Saccharomyces cerevisiae Rnr2 protein: requirement of Rnr4 and contribution of Grx3/4 AND Dre2 proteins, *J Biol Chem*, 2011, **286**, 41499-41509.
122. Y. Zhang, H. Li, C. Zhang, X. An, L. Liu, J. Stubbe and M. Huang, Conserved electron donor complex Dre2-Tah18 is required for ribonucleotide

reductase metallocofactor assembly and DNA synthesis, *Proc Natl Acad Sci U S A*, 2014, **111**, E1695-1704.
123. H. Li, M. Stumpfig, C. Zhang, X. An, J. Stubbe, R. Lill and M. Huang, The diferric-tyrosyl radical cluster of ribonucleotide reductase and cytosolic iron-sulfur clusters have distinct and similar biogenesis requirements, *J Biol Chem*, 2017, **292**, 11445-11451.
124. D. R. Richardson, D. S. Kalinowski, S. Lau, P. J. Jansson and D. B. Lovejoy, Cancer cell iron metabolism and the development of potent iron chelators as anti-tumour agents, *Biochim Biophys Acta*, 2009, **1790**, 702-717.
125. A. Chabes, B. Georgieva, V. Domkin, X. Zhao, R. Rothstein and L. Thelander, Survival of DNA damage in yeast directly depends on increased dNTP levels allowed by relaxed feedback inhibition of ribonucleotide reductase, *Cell*, 2003, **112**, 391-401.
126. E. Guarino, I. Salguero and S. E. Kearsey, Cellular regulation of ribonucleotide reductase in eukaryotes, *Semin Cell Dev Biol*, 2014, **30**, 97-103.
127. N. Sanvisens, M. C. Bano, M. Huang and S. Puig, Regulation of ribonucleotide reductase in response to iron deficiency, *Mol Cell*, 2011, **44**, 759-769.
128. O. Tsaponina, E. Barsoum, S. U. Astrom and A. Chabes, Ixr1 is required for the expression of the ribonucleotide reductase Rnr1 and maintenance of dNTP pools, *PLoS Genet*, 2011, **7**, e1002061.
129. N. Sanvisens, A. M. Romero, X. An, C. Zhang, R. de Llanos, M. T. Martinez-Pastor, M. C. Bano, M. Huang and S. Puig, Yeast Dun1 kinase regulates ribonucleotide reductase inhibitor Sml1 in response to iron deficiency, *Mol Cell Biol*, 2014, **34**, 3259-3271.
130. J. Pijuan, C. Maria, E. Herrero and G. Belli, Impaired mitochondrial Fe-S cluster biogenesis activates the DNA damage response through different signaling mediators, *J Cell Sci*, 2015, **128**, 4653-4665.
131. N. Sanvisens, A. M. Romero, C. Zhang, X. Wu, X. An, M. Huang and S. Puig, Yeast Dun1 Kinase Regulates Ribonucleotide Reductase Small Subunit Localization in Response to Iron Deficiency, *J Biol Chem*, 2016, **291**, 9807-9817.
132. S. Puig, E. Askeland and D. J. Thiele, Coordinated remodeling of cellular metabolism during iron deficiency through targeted mRNA degradation, *Cell*, 2005, **120**, 99-110.
133. S. Puig, S. V. Vergara and D. J. Thiele, Cooperation of two mRNA-binding proteins drives metabolic adaptation to iron deficiency, *Cell Metab*, 2008, **7**, 555-564.
134. E. Pedro-Segura, S. V. Vergara, S. Rodriguez-Navarro, R. Parker, D. J. Thiele and S. Puig, The Cth2 ARE-binding protein recruits the Dhh1 helicase to promote the decay of succinate dehydrogenase *SDH4* mRNA in response to iron deficiency, *J Biol Chem*, 2008, **283**, 28527-28535.
135. S. V. Vergara, S. Puig and D. J. Thiele, Early recruitment of AU-rich element-containing mRNAs determines their cytosolic fate during iron deficiency, *Mol Cell Biol*, 2011, **31**, 417-429.
136. M. Martinez-Pastor, S. V. Vergara, S. Puig and D. J. Thiele, Negative feedback regulation of the yeast Cth1 and Cth2 mRNA binding proteins is required for adaptation to iron deficiency and iron supplementation, *Mol Cell Biol*, 2013, **33**, 2178-2187.

137. F. P. Guengerich, Introduction: Metals in Biology: alpha-Ketoglutarate/Iron-Dependent Dioxygenases, *J Biol Chem*, 2015, **290**, 20700-20701.
138. A. Salminen, A. Kauppinen and K. Kaarniranta, 2-Oxoglutarate-dependent dioxygenases are sensors of energy metabolism, oxygen availability, and iron homeostasis: potential role in the regulation of aging process, *Cell Mol Life Sci*, 2015, **72**, 3897-3914.
139. H. S. Kim, H. L. Kim, K. H. Kim, D. J. Kim, S. J. Lee, J. Y. Yoon, H. J. Yoon, H. Y. Lee, S. B. Park, S. J. Kim, J. Y. Lee and S. W. Suh, Crystal structure of Tpa1 from Saccharomyces cerevisiae, a component of the messenger ribonucleoprotein complex, *Nucleic Acids Res*, 2010, **38**, 2099-2110.
140. J. Henri, D. Rispal, E. Bayart, H. van Tilbeurgh, B. Seraphin and M. Graille, Structural and functional insights into Saccharomyces cerevisiae Tpa1, a putative prolylhydroxylase influencing translation termination and transcription, *J Biol Chem*, 2010, **285**, 30767-30778.
141. K. M. Keeling, J. Salas-Marco, L. Z. Osherovich and D. M. Bedwell, Tpa1p is part of an mRNP complex that influences translation termination, mRNA deadenylation, and mRNA turnover in Saccharomyces cerevisiae, *Mol Cell Biol*, 2006, **26**, 5237-5248.
142. C. Loenarz, R. Sekirnik, A. Thalhammer, W. Ge, E. Spivakovsky, M. M. Mackeen, M. A. McDonough, M. E. Cockman, B. M. Kessler, P. J. Ratcliffe, A. Wolf and C. J. Schofield, Hydroxylation of the eukaryotic ribosomal decoding center affects translational accuracy, *Proc Natl Acad Sci U S A*, 2014, **111**, 4019-4024.
143. R. S. Singleton, P. Liu-Yi, F. Formenti, W. Ge, R. Sekirnik, R. Fischer, J. Adam, P. J. Pollard, A. Wolf, A. Thalhammer, C. Loenarz, E. Flashman, A. Yamamoto, M. L. Coleman, B. M. Kessler, P. Wappner, C. J. Schofield, P. J. Ratcliffe and M. E. Cockman, OGFOD1 catalyzes prolyl hydroxylation of RPS23 and is involved in translation control and stress granule formation, *Proc Natl Acad Sci U S A*, 2014, **111**, 4031-4036.
144. M. J. Katz, J. M. Acevedo, C. Loenarz, D. Galagovsky, P. Liu-Yi, M. Perez-Pepe, A. Thalhammer, R. Sekirnik, W. Ge, M. Melani, M. G. Thomas, S. Simonetta, G. L. Boccaccio, C. J. Schofield, M. E. Cockman, P. J. Ratcliffe and P. Wappner, Sudestada1, a Drosophila ribosomal prolyl-hydroxylase required for mRNA translation, cell homeostasis, and organ growth, *Proc Natl Acad Sci U S A*, 2014, **111**, 4025-4030.
145. B. I. Fedeles, V. Singh, J. C. Delaney, D. Li and J. M. Essigmann, The AlkB Family of Fe(II)/alpha-Ketoglutarate-dependent Dioxygenases: Repairing Nucleic Acid Alkylation Damage and Beyond, *J Biol Chem*, 2015, **290**, 20734-20742.
146. G. Shivange, N. Kodipelli, M. Monisha and R. Anindya, A role for Saccharomyces cerevisiae Tpa1 protein in direct alkylation repair, *J Biol Chem*, 2014, **289**, 35939-35952.
147. M. A. Grodick, H. M. Segal, T. J. Zwang and J. K. Barton, DNA-mediated signaling by proteins with 4Fe-4S clusters is necessary for genomic integrity, *J Am Chem Soc*, 2014, **136**, 6470-6478.
148. A. A. Gorodetsky, A. K. Boal and J. K. Barton, Direct electrochemistry of endonuclease III in the presence and absence of DNA, *J Am Chem Soc*, 2006, **128**, 12082-12083.

149. A. K. Boal, E. Yavin, O. A. Lukianova, V. L. O'Shea, S. S. David and J. K. Barton, DNA-bound redox activity of DNA repair glycosylases containing [4Fe-4S] clusters, *Biochemistry*, 2005, **44**, 8397-8407.
150. A. K. Boal, J. C. Genereux, P. A. Sontz, J. A. Gralnick, D. K. Newman and J. K. Barton, Redox signaling between DNA repair proteins for efficient lesion detection, *Proc Natl Acad Sci U S A*, 2009, **106**, 15237-15242.
151. P. A. Sontz, T. P. Mui, J. O. Fuss, J. A. Tainer and J. K. Barton, DNA charge transport as a first step in coordinating the detection of lesions by repair proteins, *Proc Natl Acad Sci U S A*, 2012, **109**, 1856-1861.
152. M. E. Nunez, K. T. Noyes and J. K. Barton, Oxidative charge transport through DNA in nucleosome core particles, *Chem Biol*, 2002, **9**, 403-415.

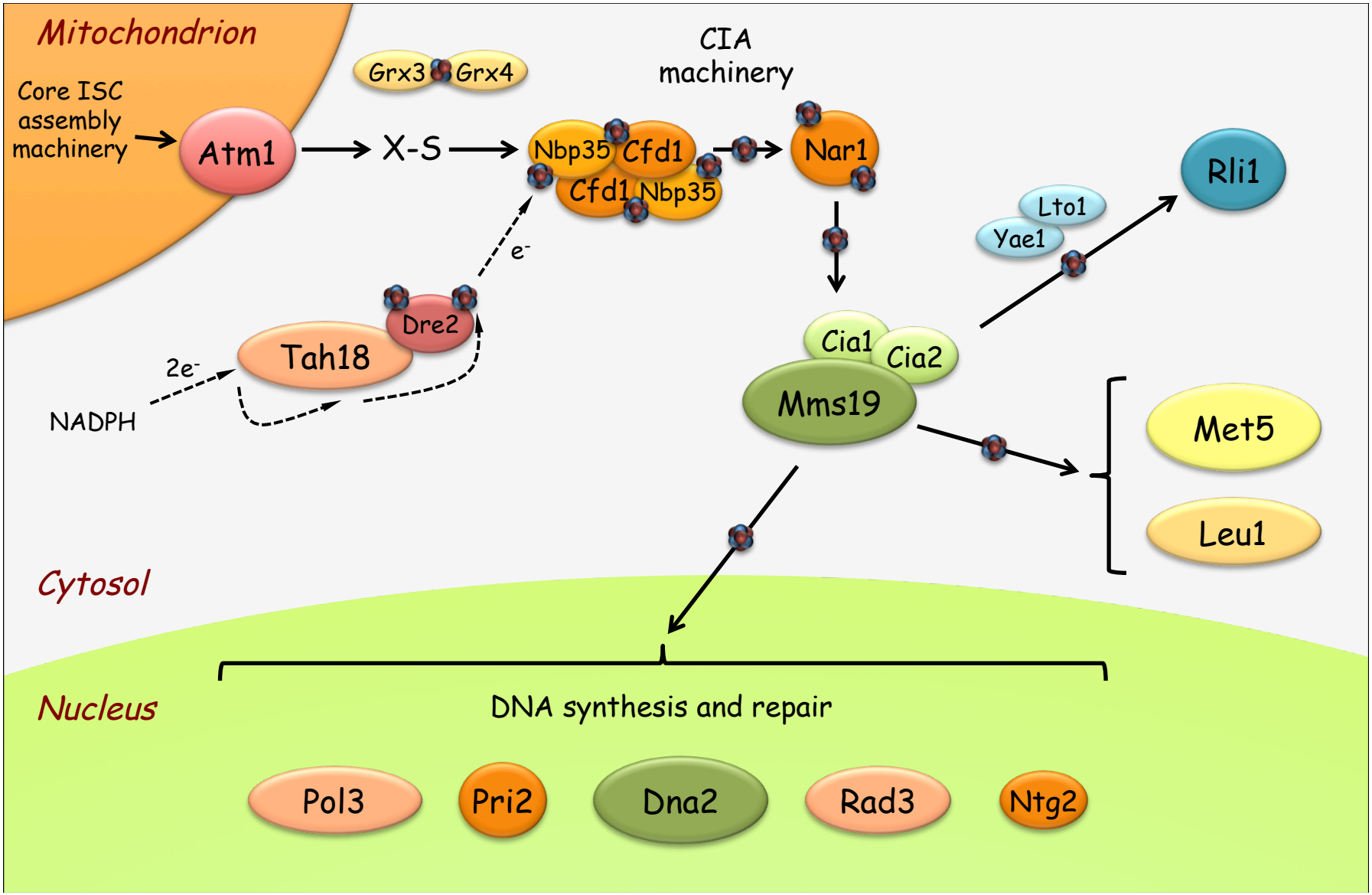
Mitochondrion
Core ISC assembly machinery
Atm1
X-S
Grx3
Grx4
CIA machinery
Nbp35
Cfd1
Cfd1
Nbp35
Nar1
Rli1
Lto1
Yae1
e-
Dre2
Tah18
2e-
NADPH
Cia1
Cia2
Mms19
Met5
Leu1
Cytosol
Nucleus
DNA synthesis and repair
Pol3
Pri2
Dna2
Rad3
Ntg2

Figure 1

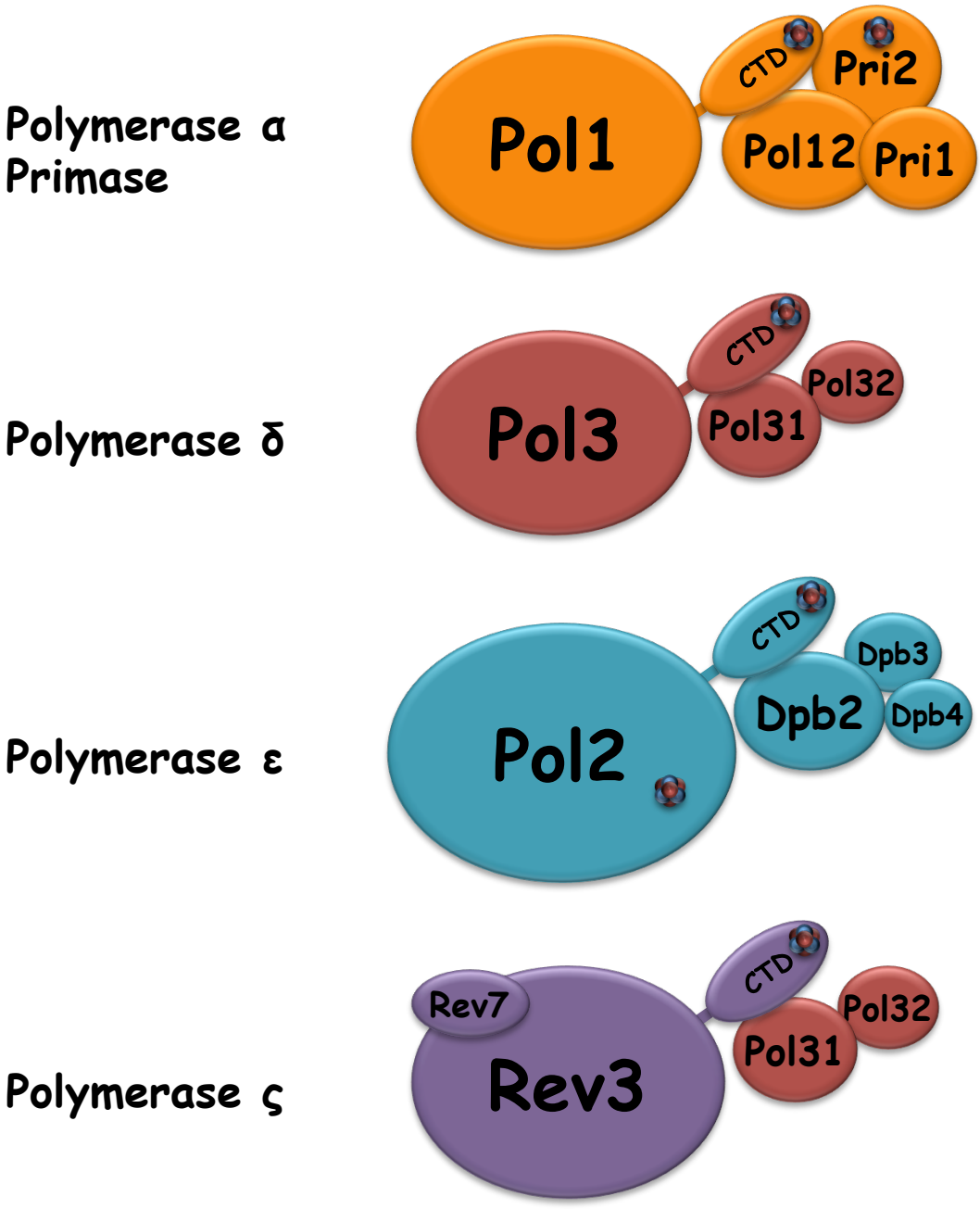
Polymerase α
Primase
Pol1
CTD
Pri2
Pol12
Pri1
Polymerase δ
Pol3
CTD
Pol32
Pol31
Polymerase ε
Pol2
CTD
Dpb3
Dpb2
Dpb4
Polymerase ς
Rev3
Rev7
CTD
Pol32
Pol31

Figure 2

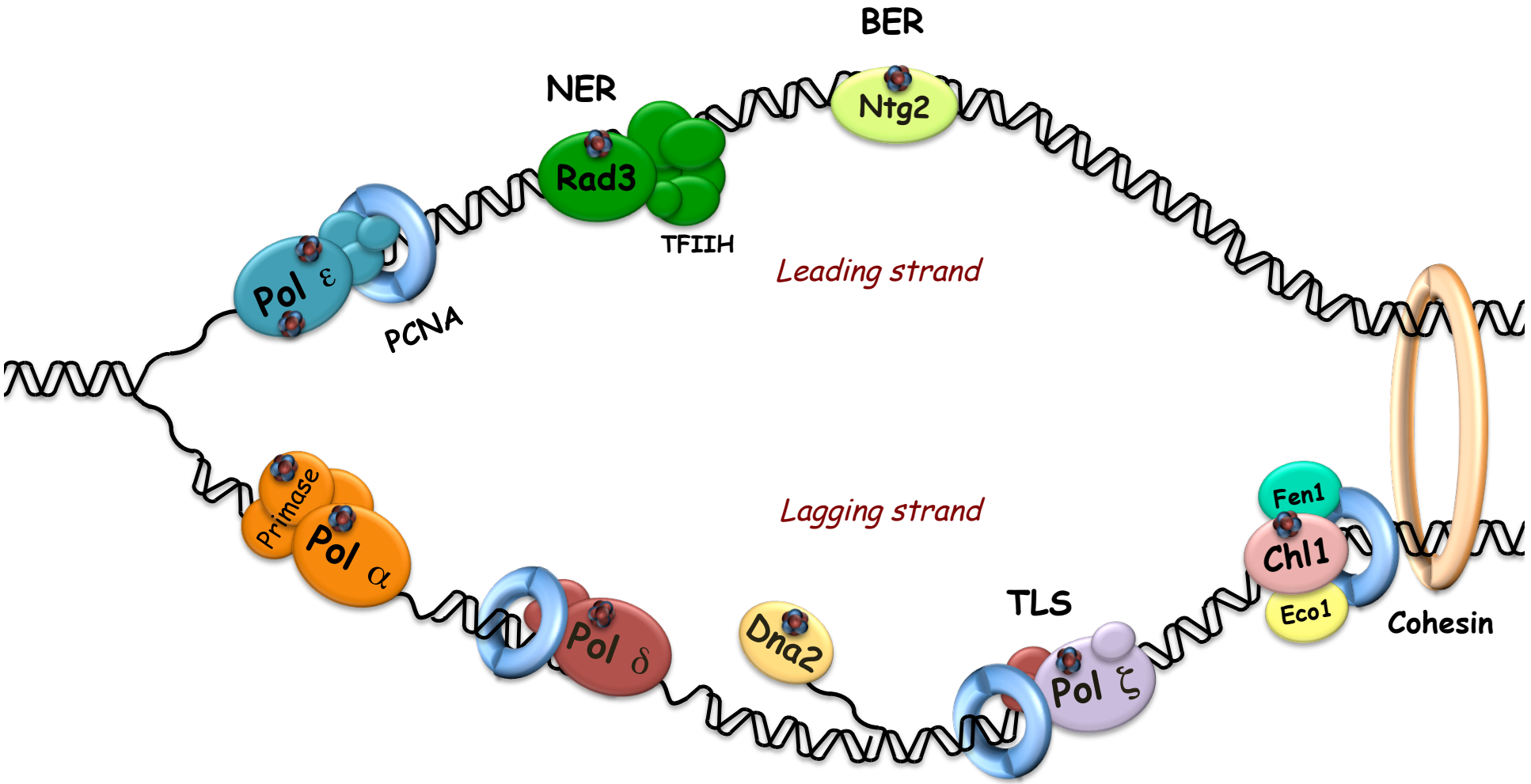
BER
NER
Ntg2
Rad3
TFIIH
Pol ε
PCNA
Leading strand
Lagging strand
Primase
Pol α
Pol δ
Dna2
TLS
Pol ζ
Fen1
Chl1
Eco1
Cohesin

Figure 3

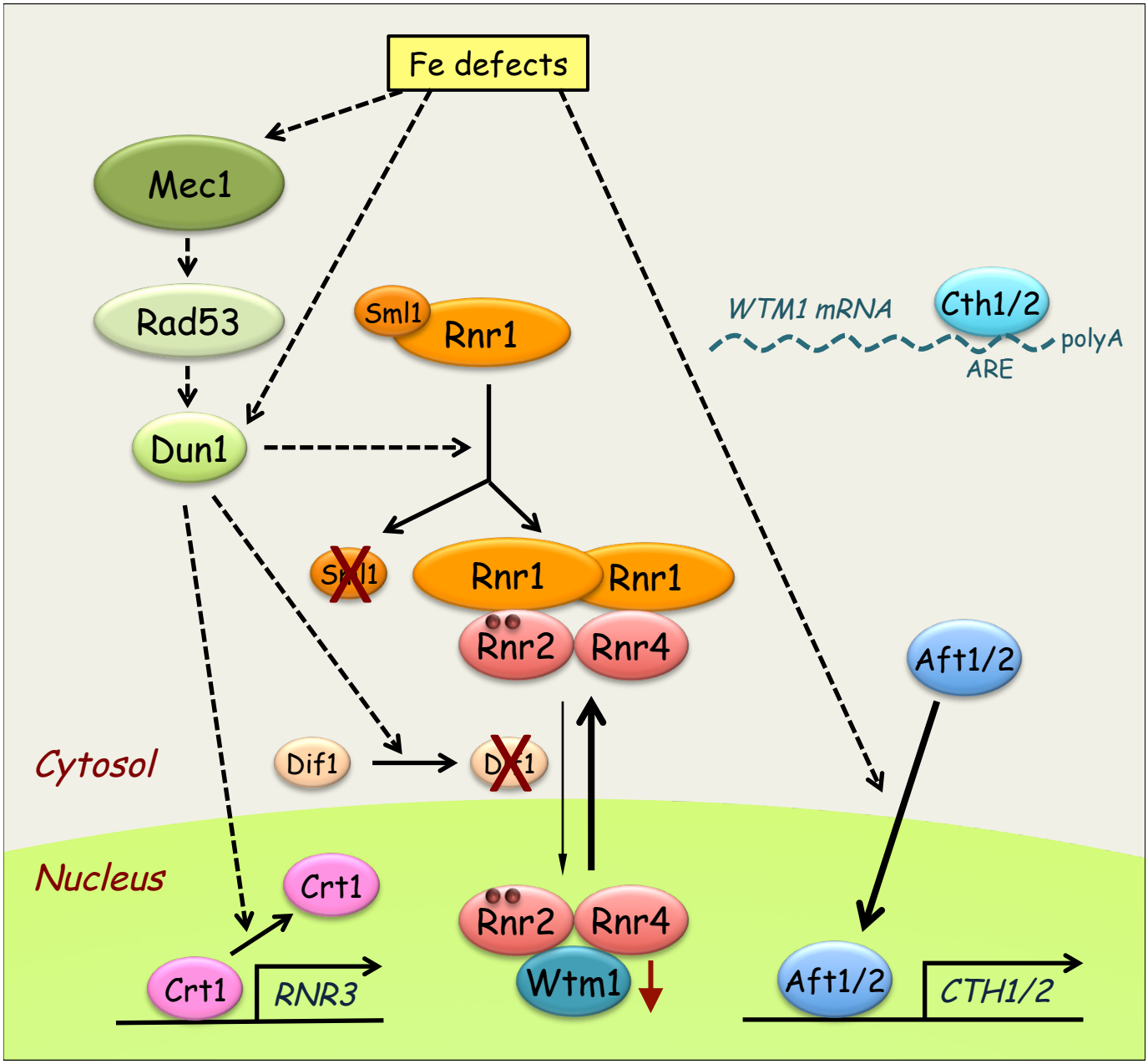
Fe defects
Mec1
Rad53
Dun1
Sml1
Rnr1
WTM1 mRNA
Cth1/2
polyA
ARE
Rnr1
Rnr1
Rnr2
Rnr4
Aft1/2
Cytosol
Dif1
Nucleus
Crt1
Crt1
RNR3
Rnr2
Rnr4
Wtm1
Aft1/2
CTH1/2

Figure 4

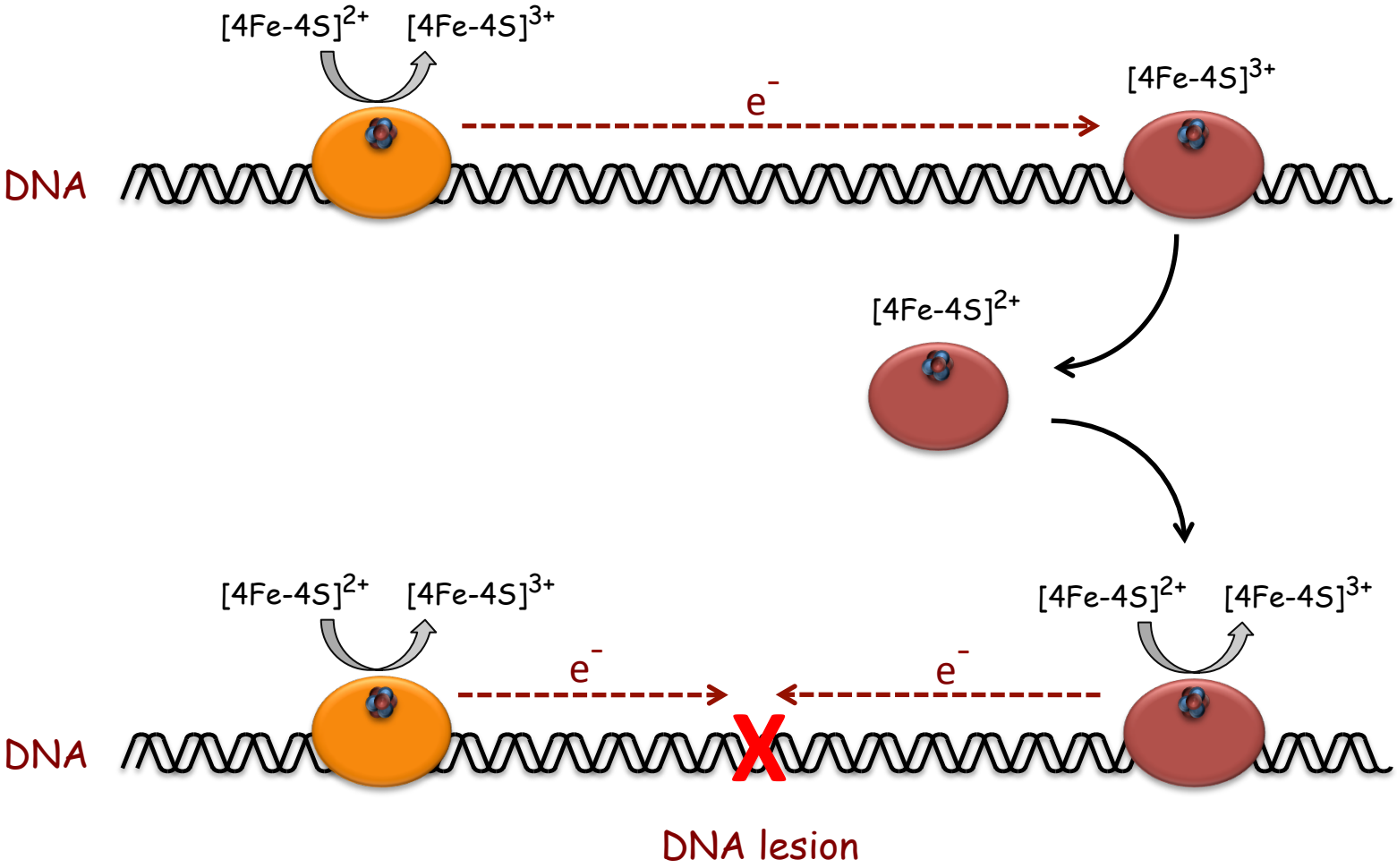
[4Fe-4S]2+
[4Fe-4S]3+
e-
[4Fe-4S]3+
DNA
[4Fe-4S]2+
[4Fe-4S]2+
[4Fe-4S]3+
[4Fe-4S]2+
[4Fe-4S]3+
e-
e-
DNA
DNA lesion

Figure 5