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REVIEW

Functional role of metalloproteins in genome stability

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Abstract **Cells contain a large number of metalloproteins that commonly harbor at least one metal ion cofactor. In metalloproteins, metal ions are usually coordinated by oxygen, sulfur, or nitrogen centers belonging to amino acid residues in the protein. The presence of the metal ion in metalloproteins allows them to take part in diverse biological processes, such as genome stability, metabolic catalysis, and cell cycle progression. Clinically, alteration of the function of metalloproteins in mammals is genetically associated with diseases characterized by DNA damage and repair defects. The present review focuses on the current perspectives of metal ion homeostasis in different organisms and summarizes the most recent understanding on magnesium, copper, iron, and manganese-containing proteins and their functional involvement in the maintenance of genome stability.**

Keywords metalloprotein, ROS, DNA damage, DNA repair, iron, copper

Introduction: Overview of metalloproteins and homeostasis of metal ions

Metalloproteins represent a class of proteins that contain a metal ion cofactor or clusters of metal ions (Waldron et al., 2009; Brown, 2010; Maret, 2010). They account for approximately half of all proteins present in cells (Waldron et al., 2009; Brown, 2010; Maret, 2010). Metal ions that commonly perform structural roles and act as cofactors in cellular reactions includes iron, manganese, cobalt, copper, zinc, molybdenum, cadmium, and tungsten. Magnesium ion, Mg^{2+}, is the most abundant metal in metalloenzymes that catalyze reactions involving ATP. However, in most cases, the magnesium ion is not bound to the protein but is often involved in loose bonds with phosphate-containing substrates, such as the pyrophosphate group of adenosine triphosphate (ATP) and is sometimes interchangeable with manganese (Waldron et al., 2009). Crystal structure of some metalloproteins reveals extremely conserved localization of metal ions in a "pocket," whose shape fits the substrate, and conforms to the nitrogen, oxygen, and sulfur centers of the protein's amino acid residues (Waldron et al., 2009; Banci and Bertini, 2013). There is growing evidence related to the important role of numerous metalloproteins that catalyze and facilitate the formation of reactive oxygen species (ROS) and free radicals. Free radicals alter and damage biomolecules, including proteins, lipids and DNA, triggering a number of human diseases (Maret, 2010; Zhang, 2014; Zhang et al., 2014a; Zhang and Liu, 2015; Zhang and Zhang, 2015a). Many of the proteins involved with DNA repair processes are metalloproteins. There is increasing evidence indicating the direct involvement of a number of metalloproteins in DNA damage and repair processes. The present review focuses on understanding the role of such metalloproteins involved in DNA damage and repair, including DNA polymerases (Lange et al., 2011; Zhang, 2014), DNA helicases (Brosh, 2013), DNA primases (Zhang, 2014), the small subunit of ribonucleotide reductase (RNR) (Zhang et al., 2014a, 2014b), aconitases (Kim et al., 2014; Zhang, 2014), superoxide dismutases (Keyer and Imlay, 1996), catalases (Kang et al., 2013), arginases (Mori, 2007), and cytochrome oxidases (Table1) (Zhang, 2014).

Elevated levels of essential metal ions are toxic and hence metal ion import, trafficking, availability, and export must be tightly regulated at the cellular level (Ma et al., 2009; Dlouhy and Outten, 2013). Organisms have evolved multi-layered mechanisms to regulate metal ion homeostasis that help them acquire the right metals, ensuring the physical and chemical properties of the metalloprotein (Zhang, 2014; Zhang and

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Table 1 Majormetalloproteins involved in the maintenance of genome stability

Protein	Metal ion(s)	Main function	Reference
Alcohol dehydrogenase (ADH)	Zinc	Facilitates the interconversion between alcohols and aldehydes or ketones with the reduction of nicotinamide/ adenine dinucleotide (NAD^+ to NADH)	Edenberg, 2007
Arginase	Manganese	Catalyzes the conversion of L-arginine into L-ornithine and urea	Dowling et al., 2008
Catalase	Iron	Catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2) and mitigates the toxic effects of H_2O_2 in cells	Zamocky et al., 2008
Cell adhesion molecule L1-related helicase (CHLR1)	Iron	Plays an important role in sister chromatid cohesion, DNA replication, and/or DNA repair	Shah et al., 2013
Cytochrome complex (cyt *c*)	Iron	Is an essential component for the functioning of the electron transport chain and in the initiation of apoptosis	Huttemann et al., 2012
Cytochrome *c*oxidase (C*c*O)	Iron and copper	It is an essential component for the functioning of the electron transport chain and affects several aspects of mitochondrial function	Srinivasan and Avadhani, 2012
DNA replication helicase/ nuclease 2 (DNA2)	Iron	Required for processing double-strand breaks (DSB), end resection, and processing Okazaki fragments	Cejka et al., 2010
DNA polymerases (Polα, δ and ε)	Iron	Initiating and processing DNA replication	Miyabe et al., 2011
DNA polymerase β	Magnesium	Catalyzes base excision repair required for DNA maintenance, replication and recombination	Sutton and Walker, 2001
DNA polymerase I	Magnesium	DNA replication and repair	Meyer et al., 2004
DNA primase	Iron	Catalyzes the synthesis of a short RNA primer complementary to the single-stranded DNA template	Schumacher et al., 2000; Wang et al., 2004
Fanconi anemia, complementation group J (FANCJ)	Iron	Promotes homologous recombination (HR) repair of damaged DNA	Kee and D'Andrea, 2010
Hemocyanin	Copper	Function in the transport or storage of O_2	Scudiero et al., 2007
Hemoglobin	Iron	Carries oxygen from respiratory organs to the rest of the body and acts as a biological Fenton reagent to promote heme degradation through the generation of ROS	Gourianov and Kluger, 2003; Goodarzi et al., 2014
Hexokinase	Magnesium	Catalyzes the phosphorylation of hexoses forming hexose phosphate	Aleshin et al., 1998
Manganese catalases	Magnesium	Catalyzing the decomposition of H_2O_2 to H_2O and O_2	Yoder et al., 2000
MMS19	Iron	Functions in DNA repair, chromosome segregation, and heterochromatin silencing	Stehling et al., 2013
Manganese superoxide dismutase (MnSOD)	Manganese	Detoxifies free radicles and protects cells from potential damage caused by excessive amounts of ROS	Candas and Li, 2014
Myoglobin	Iron	Primary oxygen-carrying pigment of muscle tissues	Garry and Mammen, 2007
Nitric oxide synthases (NOSs)	Iron	Catalyzes the production of nitric oxide (NO) from L-arginine	Rodrigo et al., 2013
P2 DNA polymerase IV	Magnesium	DNA replication and repair	Ling et al., 2001
Plastocyanin	Copper	Functions as an electron transfer agent between cytochrome *f* of the cytochrome b_6f complex from photosystem II and $P700^+$ from photosystem I	Peers and Price, 2006
DNA repair helicase RAD3		Mediates nucleotide excision repair (NER) process	Lee et al., 2000
Regulator of telomere elongation helicase 1 (RTEL1)	Iron	Functions in telomere-length regulation, DNA repair and in the maintenance of genomic stability	Uringa et al., 2011
Small subunit of ribonucleotide reductase (RNR)	Iron	Catalyzes the reductive synthesis of deoxyribonucleotides from their corresponding ribonucleotides	Zhang, 2014
Superoxide dismutase 1 (SOD1)	Copper and zinc	Functions in apoptotic signaling and in oxidative stress	Valentine et al., 2005; Yoon et al., 2009
Taq DNA polymerase	Magnesium	DNA replication and repair	Li et al., 1999
T7 DNA polymerase	Magnesium	DNA replication and repair	Doublié et al., 1998
Xanthine oxidase (XO)	Molybdenum	Catalyzes the oxidation of hypoxanthine to xanthine	Kelley et al., 2010
Xeroderma pigmentosum group D (XPD)	Iron	Mediates NER process	Cappelli et al., 1999

Zhang, 2015a). Bacteria possess a number of metal binding and metal-sensing regulators that are classified into families of metal de-repressors (ArsR–SmtB, CsoR–RcnR, and CopY), metal co-repressors (Fur, NikR, and DtxR), and metal activators (MerR) (Waldron et al., 2009). Metal ion homeostasis is determined by the affinities of cytosolic metal sensors for the metals they detect andhave been used to make inferences about the concentrations of metals available to proteins (Waldron et al., 2009). To date, very little is known about metal-sensing signaling pathways present in the plasma membrane of budding yeast *Saccharomyces cerevisiae*. However, a number of critical proteins have been identified in yeast that could regulate metal ion homeostasis, such as DNA binding transcription factors. This regulation is mediated by a variety of mechanisms including a series of metal-dependent events, such as changes in localization between the nucleus and cytosol, repression of the activation-domain function, and changes in DNA binding (Waldron et al., 2009). It has been reported that in *S. cerevisiae*, zinc responsive activator protein (Zap1) responds to zinc (Frey et al., 2011), metal binding activator 1 (Mac1), and Cu-regulated DNA binding protein 2 (Cup2) responds to copper (Dong et al., 2013), and activator of ferrous transport 1 and 2 (Aft1 and Aft2) responds to iron (Waldron et al., 2009). Mammalian cells maintain metal ion homeostasis by utilizing both high- and low-affinity transport (Zhang and Zhang, 2015a). Separate high-affinity systems for magnesium, manganese, iron, copper, and zinc are responsible for providing the element to the cell when it is in short supply (Rolfs and Hediger, 1999). Each system is controlled by metal responsive regulatory proteins, such as copper transport protein 1 (CTR1) for copper (Holzeret al., 2004), divalent metal transporter1 (DMT1) for iron (Torti and Torti, 2013), transient receptor potential cation channel subfamily M member 6 (TRPM6) and TRPM7 for magnesium (Gwanyanya et al., 2004), and ZRT/IRT-like protein 1 (ZIP1) for zinc (Franklin et al., 2003). In contrast, low-affinity systems play a housekeeping role, supplying metal ions when they are present abundantly in the environment. A number of low-affinity ion importers have been identified, namely copper transporter 2 (CTR2) for copper (Öhrvik et al., 2013), and zinc regulated transporter 2 (ZRT2) for zinc (Eide, 2006).

Role of magnesium-containing proteins in genome stability

Magnesium plays an important role in maintaining genomic stability owing to its stabilizing effect on DNA and chromatin structure. It functions as an essential cofactor in almost all enzymatic systems involved in the process of DNA replication (Hartwig, 2001). Additionally, magnesium is an essential cofactor for enzymes involved in nucleotide excision repair (NER), base excision repair (BER), and mismatch repair (MMR) (Arigony et al., 2013), facilitating the removal of DNA damage generated by environmental mutagens, endogenous processes, and DNA replication (Arigony et al., 2013).

DNA polymerases

DNA polymerases are a class of enzymes that mediate DNA replication, a process that creates DNA molecules by assembling nucleotides and assists in the DNA repair process by building DNA blocks (Sutton and Walker, 2001). Based on the amino acid sequence homology and crystal structure analysis, DNA polymerases are classified into seven families, namely A, B, C, D, X, Y, and RT (Gardner and Kelman, 2014). To date, a large number of DNA polymerases with functional metal ions have been crystallized. Some of them include, Klenow fragment produced by DNA polymerase I in *Escherichia coli* (family A) (Meyer et al., 2004), *Taq* DNA polymerase (Klentaq1) (family A) (Li et al., 1999), T7 DNA polymerase (family A) (Doublié et al., 1998), bacteriophage RB69 DNA polymerase (family B) (Wang et al., 1997), DNA polymerase β (family X) (Sawayaet al., 1997), P2 DNA polymerase IV (Dpo4) (family Y) (Ling et al., 2001), and human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) (family RT) (Jacobo-Molinaet al., 1993). Though sequence similarity across different DNA polymerase families is very low, all the deduced crystal structures share a common feature in possessing two metal ions (usually Mg^{2+}) coordinated in the active site by conserved acidic residues (Yang et al., 2004). This conserved metal binding site in divergent DNA polymerases emphasizes the importance of metal ions in assisting DNA replication and repair (Yang et al., 2004).

Hexokinases

Hexokinases are enzymes that catalyze the phosphorylation of hexoses, forming hexose phosphate (Aleshin et al., 1998). In most organisms, the enzyme utilizes glucose as the substrate and for msglucose-6-phosphate as the product (Cárdenas et al., 1998). Hexokinases are widely found among a variety of species including bacteria, yeast, plants, and mammals (Cárdenas et al., 1998). Four classes of hexokinases are present in mammalian cells designated as hexokinases I, II, III, and IV or as hexokinases A, B, C, and D (Cárdenas et al., 1998). Interestingly, hexokinase plays an important role both in glycolysis and in the control of apoptosis (Kim et al., 2006). Inhibition of hexokinase activity by inhibitors, such as 2-deoxyglucose (2-DG), 3-bromopyruvate (3-BrPA), and lonidamine (LND), yielded similar effects to those of glucose deprivation in terms of the activation of AMP-activated protein kinase (AMPK), inactivation of mammalian target of rapamycin (mTOR), and cell cycle arrest and cell death (El Mjiyad et al., 2011). In addition, mitochondrial hexokinases,

such as hexokinase I and II, mediate cell survival through growth factors and Akt (also known as protein kinase B (PKB)) (Gottlob et al., 2001). Decreased mitochondrial hexokinase has been observed in association with apoptosis induced by growth factor deficiency and UV irradiation (Gottlob et al., 2001). This suggests a possible modulation of the association of hexokinase with mitochondria by Akt, thereby preventing the release of cytochrome *c*, a critical component that initiates apoptotic cascade (Gottlob et al., 2001).

Crystal structure of hexokinases revealed a common ATP binding site and an Mg^{2+} ion surrounded by variable sequences (Mulichak et al., 1998; Yang et al., 2004). Functional analysis also indicated the requirement of Mg^{2+} for hexokinase activity (Kaji and Colowick, 1965; Bachelard, 1971; Purich and Fromm, 1972). As shown in reaction (1), hexokinases catalyze the transfer of the gama-phosphoryl group of an ATP molecule bound to a magnesium ion to the oxygen at the C-6 of glucose, producing glucose-6-phosphate and ADP (Cárdenas et al., 1998). In the process, hexokinase undergoes an induced-fit conformational change when it binds to glucose, which ultimately prevents the hydrolysis of ATP (Cárdenas et al., 1998).

$$\mathrm{Hexose-CH_2OH+MgATP^{2-}} \rightarrow \mathrm{Hexose-CH_2O-pO_3^{2-}+MgADP^{-}+H^{+}} \quad (1)$$

Role of copper-containing proteins in genome stability

Copper-containing proteins commonly contain one or more copper ions as prosthetic groups (Shleev et al., 2005). Impairment of the function of copper-containing proteins, such as copper-zinc superoxide dismutase (CuZnSOD, also known as SOD1), cytochrome *c* oxidase, hemocyanin, and plastocyanin, generates ROS or free radicals and is associated with the DNA damage process.

SOD1

The structure of SOD1 proteins from different species is highly conserved in the fully metallated state (Moreira et al., 2013). Human SOD1 is a 32-kDa homodimer and each monomer contains a β-barrel motif, a binuclear Cu/Zn binding site, and an intramolecular disulfide bond (Valentine et al., 2005). The copper site is required for the enzymatic activity of SOD1 protein that catalyzes the disproportionation of superoxide anion to generate dioxygen and hydrogen peroxide (Yoon et al., 2009). This catalysis is a two-step process: One molecule of superoxide first reduces the cupric ion to form dioxygen (2), and then a second molecule of O_2^- reoxidizes the cuprous ion to form hydrogen peroxide (3) (Valentine et al., 2005).

$$\mathrm{O_2^- + Cu^{2+}ZnSOD \rightarrow O_2 + Cu^{+}ZnSOD} \quad (2)$$

$$\mathrm{O_2^- + 2H^{+} + Cu^{+}ZnSOD \rightarrow H_2O_2 + Cu^{+}ZnSOD} \quad (3)$$

A number of studies have revealed an important role of SOD1 in apoptotic signaling and oxidative stress, especially as part of the mitochondrial death pathway and cardiac myocyte apoptosis signaling (Yu et al., 2006; Nishitoh et al., 2008; Soo et al., 2009; Barbosa et al., 2010). Interestingly, wild-type SOD1 ($SOD1^{WT}$) was found to be anti-apoptotic, while mutant SOD1 (mutSOD1) proteins were pro-apoptotic both *in vitro* and *in vivo* (Pasinelli et al., 2004; Tafuri et al., 2015). Both $SOD1^{WT}$and mutSOD1 bind to the anti-apoptotic protein Bcl-2 (Pasinelli et al., 2004). Reduced expression of Bcl-2 was detected in transgenic mice expressing $SOD1^{G93A}$ mutation along with increased expression of pro-apoptotic proteins Bax and Bad (Chung et al., 2015).

Mammalian SOD1 is localized in the intermembrane space or to the outer mitochondrial membrane, where superoxide anions (O_2^-) are generated (Tafuri et al., 2015). *In vitro*, SOD1 reacts chemically with hydrogen peroxide (H_2O_2), peroxynitrite (NO_3^-), or hypochlorite (ClO^-) and in the process becomes oxidatively damaged (Valentine et al., 2005). Clinically, SOD1 induces critical ischemia-reperfusion injury, a component of heart attack that is specific to the myocardium (Ansley and Wang, 2013). During this process, SOD1 induces the release of ROS, which contributes to cell damage and cell death via a direct effect on the cell as well as by inducing an apoptotic signaling (Ansley and Wang, 2013). In addition, mice lacking SOD1 have serious developmental defects, such as muscle mass loss, macular degeneration, hepatocellular carcinoma, and shortened lifespan (Shefner et al., 1999).

Cytochrome *c* oxidase

Cytochrome *c* oxidase (C*c*O) is a heme-copper containing enzyme that is widely found in bacteria and eukaryotic mitochondria (Horn and Barrientos, 2008). It couples the oxidation of cytochrome *c* by molecular oxygen to the translocation of protons across the membrane (Horn and Barrientos, 2008). The C*c*O complex contains two hemes, a cytochrome a and cytochrome a_3, and two copper centers (Cu_A and Cu_B) (Shapleigh et al., 1992; Horn and Barrientos, 2008). Cytochrome a_3 and Cu_B form a binuclear center that serves as the site for oxygen reduction (Cooper et al., 1997). Cytochrome *c*, a component of the electron transport chain (cytochrome bc1 complex), docks near the Cu_A binuclear center and transfers an electron to it and is oxidized back to cytochrome *c* containing Fe^{3+} (Cruciat et al., 2000). The reduced Cu_A binuclear center transfers an electron to cytochrome a, which in turn transfers an electron to the cytochrome a_3-Cu_B binuclear center (Brunori et al., 2005). As shown in reaction (4), C*c*O receives an electron each from four cytochrome *c* molecules and transfers them to anoxygen

molecule, converting O_2 to two molecules of H_2O (Brunori et al., 2005).

$$4Fe^{2+} - \text{cytochrome } c + 8H^{+}_{in} + O_2 \rightarrow 4Fe^{3+} - \text{cytochrome } c + 2H_2O + 4H^{+}_{out} \quad (4)$$

As a mitochondrial protein with a critical function in the electron transport chain, C*c*O activity affects many aspects of mitochondrial function involved in a wide variety of diseases, including cancer, neurodegenerative diseases, bone and skeletal diseases, and diabetes (Srinivasan and Avadhani, 2012). There is growing evidence suggesting increased mitochondrial ROS production and cellular toxicity related to impaired C*c*O function contributing to diseases (Srinivasan and Avadhani, 2012). However, the mechanistic role of the function of C*c*O in the above process remains a subject of debate. Some studies suggest the involvement of an intrinsic apoptosis pathway induced by C*c*O due to its mitochondrial localization (Srinivasan and Avadhani, 2012). For instance, in heart failure and myocardial infarction, upregulation of C*c*O was reported, which could have promoted cell death, as indicted by TUNEL-positive cells and activated caspase-3 detected in the analysis (Wu et al., 2009).

Hemocyanin

Hemocyanins are a class of metalloproteins containing two copper atoms that reversibly bind a single oxygen molecule. They are present in a majority of mollusks and some arthropods (van Holde et al., 2001). Similar to hemoglobin, hemocyanins also function to transport or store O_2 (Scudiero et al., 2007). However, hemocyanins are directly suspended in the hemolymph and are not bound to blood cells (van Holde et al., 2001). Each of the two copper atoms embedded at the core in hemocyanin is associated with three histidine residues (Sivakamavalli and Vaseeharan, 2015)

Studies indicate the production of ROS by hemocyanin via an unclear mechanism (Jiang et al., 2007). Hemocyanin found in the blood of *Concholepas concholepas* and *Megathura crenulata* have powerful anti- tumor effects in cells (Moltedo et al., 2006; Sarker and Zhong, 2014). Mice treated with hemocyanin isolated from *C. concholepas* showed decreased tumor growth and incidence, thereby prolonging cell survival (Moltedo et al., 2006). Keyhole limpet hemocyanin (KLH) derived from *M. crenulata* has been reported to possess promising anticancer activity against the proliferation of breast cancer, pancreatic cancer, and prostate cancer cells (Sarker and Zhong, 2014).

Plastocyanin

Plastocyanins are present in plants, algae, and cyanobacteria. They transfer electrons between cytochrome f of the cytochrome b_6f complex from photosystem II and $P700^+$ from photosystem I (Peers and Price, 2006). As shown in reactions (5) and (6), plastocyanin ($Cu^{2+}Pc$) is first reduced by cytochrome f to generate $Cu^{+}Pc$, which then binds to $P700^+$ and is oxidized to form $Cu^{2+}Pc$.

$$Cu^{2+}Pc + e^- \rightarrow Cu^{+}Pc \quad (5)$$

$$Cu^{+}Pc \rightarrow Cu^{2+}Pc + e^- \quad (6)$$

X-ray crystallographic structures of plastocyanins have identified a hydrophobic surface surrounding the exposed histidine of the copper binding site present in all plastocyanins that are believed to be the recognition/binding sites for other proteins involved in electron transfer (Peers and Price, 2006). In plants, copper deficiency or genetic limitation of its delivery to the chloroplast decreases the plastocyanin content, as the protein is less stable in the absence of Cu (Ravet and Pilon, 2013). Consequently, the efficiency of electron transport rate throughout the photosynthetic apparatus decreases. In the absence of downstream electron acceptors, this leads to ROS accumulation in the light owing to the photo-reduction of oxygen at PSII (Ravet and Pilon, 2013). Moreover, photosynthetic electron transport is essential for the maintenance of high $NADPH/NADP^+$ and GSH/GSSG ratios, and this reduced state of the stroma can facilitate ROS removal systems, such as glutathione dependent peroxidases (Ravet and Pilon, 2013).

Role of iron-containing proteins ingenomestability

Organisms encode a large number of iron-containing proteins that are extensively involved in maintaining genome stability, especially with regard to DNA replication and repair (Zhang, 2014; An et al., 2015; Chen et al., 2015; Zhang and Zhang, 2015b; Zhang et al., 2015). These iron-containing proteins include hemoproteins, catalases, the small subunit of ribonucleotide reductases (RNRs), and numerous iron-sulfur (Fe-S) cluster proteins (Zhang, 2014; An et al., 2015).

Hemoproteins

Hemoproteins are a class of metalloproteins containing heme as the prosthetic group (Zhang, 2014). Notable examples of hemoproteins include hemoglobin, myoglobin, cytochromes, and nitric oxide synthases (NOSs), all of which have diverse biological functions including oxygen transport, oxidative catalysis, and electron transport (Zhang, 2014).

Hemoglobin is present in red blood cells and primarily functions as a carrier of oxygen from respiratory organs to the rest of the body (Gourianov and Kluger, 2003). Hemoglobin acts as a biological Fenton reagent promoting heme degradation through the production of ROS (Goodarzi et al., 2014). Hemoprotein-mediated oxidative stress is thought

to be involved in the pathophysiology of numerous diseases, such as blast pressure injury and crush injury(D'Agnillo et al., 2000).

Myoglobin is a hemoprotein that is restricted to cardiomyocytes and oxidative skeletal muscle fibers (Garry and Mammen, 2007). It shares many structural similarities with hemoglobin. A significant number of studies have indicated that the dysfunction of myoglobin causes oxidative stress and increased nitric oxide (NO) production (Plotnikov et al., 2009; Kamga et al., 2012; Totzeck et al., 2014). Studies in myoglobin-deficient mice have demonstrated that myoglobin functions as a scavenger of nitric oxide or ROS (Schlieper et al., 2004).

Cytochromes are a class of iron-containing hemoproteins responsible for the generation of ATP via electron transport in the mitochondria (Liu et al., 2014). According to spectrochemical characteristics, cytochromes are mainly classified into five groups, namely *a*, *b*, *c*, *d*, and P450 (Liu et al., 2014). All these cytochromes are involved in the generation of ROS (Zhang, 2014). For instance, the disruption of NADH-cytochrome *b5* reductase, a membrane-bound protein that serves as an electron carrier in several oxidative reactions of reductases, possibly increased ROS production (Zhang, 2014). Cytochrome *c* serves multiple functions, such as being an integral component of mitochondrial electron transport and in initiating apoptosis (Huttemann et al., 2012). Under normal physiological conditions, cytochrome *c* is localized in the mitochondrial intermembrane space. Upon receiving an apoptotic signal, it is released into the cytosol and can initiate the activation cascade of caspases triggering apoptosis (Elmore, 2007). This mechanism is involved in the physiology of a number of diseases, including cancer, neurological disorders, cardiovascular disorders, and autoimmune diseases (Favaloro et al., 2012).

NOSs are a family of enzymes that catalyze the conversion of L-arginine to NO. NO is closely linked to ROS production, which in turn promotes oxidative DNA damage (Rodrigo et al., 2013). Various studies have identified the association of NOSs activity with tumor cell proliferation rate and with the expression of signaling components associated with cancer development, such as the estrogen receptor (Xu et al., 2002). It has been suggested that high levels of NOS expression may be cytotoxic for tumor cells, whereas low levels can promote tumor growth (Xu et al., 2002).

Catalases

Catalases are a class of enzymes that catalyzes the decomposition of H_2O_2 to H_2O and O_2, thereby mitigating the toxic effects of H_2O_2 in cells (Zamocky et al., 2008; Li et al., 2015). In eukaryotic organisms, most catalases exist as tetramers of 60 or 75 kDa subunits, and each subunit consists of a porphyrin heme group buried deep within the structure (Zamocky et al., 2008). Altered catalase activity has been associated with a number of diseases, including cancer, diabetes, and Parkinson's disease. Catalase is frequently downregulated in tumor cells leading to the accumulation of H_2O_2, causing DNA damage and/or cell death. In addition, the PI3K/Akt/mTor signaling pathway regulates the expression of catalase in breast cancer cells (Glorieux et al., 2014). A number of studies have suggested the role of low levels of H_2O_2 as a cellular messenger in insulin signaling. Mutations in catalase gene increased the concentration of H_2O_2, which could damage the normally catalase-poor pancreatic β-cells, suggesting that it may be a risk factor for type 2 diabetes (Góth, 2008). Reduced catalase activity has been observed in the substantia nigra and putamen of Parkinsonian brains (Ambani et al., 1975). Further studies have revealed the protective effect of catalase on neuronal cells against cell death through mechanisms involving the elimination of oxidative damage (Peng et al., 2005).

Small subunit of RNR

RNRs are a class of proteins that utilize radical chemistry to catalyze the reductive synthesis of deoxyribonucleotides from their corresponding ribonucleotides, thereby ensuring accurate DNA synthesis and genomic integrity (Zhang et al., 2014b; Sanvisens et al., 2014). Structurally, eukaryotic RNR is made up of a large subunit (α or R1) and a small subunit (β or R2), both of which together form a functional complex known as $(\alpha_2)_3(\beta_2)_n$, where n is 1 or 3 (Sanvisens et al., 2014). The smaller subunit requires an iron to form a diferric-tyrosyl radical cofactor ($Fe^{III}{}_2$-Y·) in order to initiate nucleotide reduction (Zhang et al., 2014b). Studies have suggested that an imbalanced dNTP pool could enhance dNTP misincorporation and further, by inhibiting the proofreading function of DNA polymerases, lead to increased DNA mutations, DNA breaks, and cell death (Zhang, 2014; Zhang et al., 2014b). Activation of DNA damage checkpoint was associated with altered protein levels of RNR subunits (Zhang, 2014). When cells completed DNA replication and/or repair, degradation of ribonucleotide reductase M2 (RRM2), the small subunit of RNR in mammals, was mediated through two E3 ubiquitin ligase complexes, namely, the Skp1/Cullin/F-box (SCF) and the anaphase-promoting complex (APC) (Zhang, 2014). RRM2B (also known as p53R2), is an important RNR subunit that exists as a p53-inducible and p53-dependent molecule. In response to DNA damage, it forms an active RNR holoenzyme with RRM1 and facilitates DNA repair by activating the ATM/ATR-CHK checkpoint pathway (Nakano et al., 2000; Harper and Elledge, 2007). Additionally, DNA damage could induce the expression of *RRM2B* in a p53-dependent manner (Uramoto et al., 2006). Similarly, the expression of yeast *RNR* genes, particularly *RNR3*, is induced via the activation of the Mec1-Rad53-Dun1 damage checkpoint kinase cascade (Zhang et al., 2014b).

Role of iron-sulfur (Fe-S) cluster proteins in genome stability

Fe-S cluster proteins utilize a group of ancient cofactors composed of iron and sulfur in different and interchangeable stoichiometries, which are usually ligated to cysteines of associated proteins (Johnson et al., 2005; Rouault, 2015). Numerous Fe-S cluster proteins are known that sustain genomic stability, including the three DNA polymerases (Polα, Polδ, and Polε), the regulator subunit of DNA primase, and DNA helicases (Zhang, 2014).

DNA polymerases and primases

Eukaryotic organisms commonly use three conserved polymerases (Polα, Polδ, and Polε) for initiating and processing DNA replication (Miyabe et al., 2011). Polα is closely associated with the small and the large primase subunits (PRIM1 and PRIM2) on the template at the origin of replication to initiate the synthesis of short RNA primers. Polδ and Polε utilize these RNA primers to synthesize the lagging and leading strands, respectively (Schumacher et al., 2000; Wang et al., 2004). In addition, eukaryotes also contain Polζ, (a B family Pol), which functions in the extension step of translesion DNA synthesis, but with lower fidelity compared to that of other polymerases (Acharya et al., 2006). Interestingly, all of these DNA polymerases and primases are Fe-S cluster proteins, requiring a Fe-S cluster to form their corresponding active holoproteins (Netz et al., 2012). A number of studies have demonstrated that the stability and activity of these nuclear DNA polymerases depends on the cytosolic and mitochondrial Fe-S cluster biogenesis machineries, since they serve as sulfur donors for DNA polymerases (Rouault, 2012).

DNA helicases

DNA helicases are enzymes that unwind the duplex DNA to provide a single strand of DNA for replication, repair, and recombination processes (Lohman, 1992). For a number of DNA helicases, the Fe-S cluster is essential for the helicase activity (Zhang, 2014). Notable examples include xeroderma pigmentosum group D (XPD), Fanconi anemia complementation group J (FANCJ), radiation repair 3 (RAD3), cell adhesion molecule L1-related helicase (CHLR1), regulator of telomere elongation helicase 1(RTEL1), and DNA replication helicase/nuclease 2 (DNA2) (Zhang, 2014). Of these DNA helicases, XPD is required for nucleotide excision repair (NER) (Cappelli et al., 1999). FANCJ plays an important role in a homologous recombination (HR) pathway of double-strand break (DSB) repair pathway (Kee and D'Andrea, 2010). RAD3 is an ATP-dependent DNA helicase involved in NER of DNA, damaged by UV irradiation, bulky adducts, or cross-linking agents (Lee et al., 2000). CHLR1 is essential for DNA replication, DNA damage repair and for the establishment of cohesion between sister chromatids (Shah et al., 2013). RTEL1 is involved in telomere-length regulation, DNA repair, and in the maintenance of genomic stability (Uringa et al., 2011). DNA2 is a protein required for DSB end resection and for the processing of Okazaki fragments (Cejka et al., 2010). Defects or mutations in these helicases can result in characteristic human genetic disorders in which genomic instability and predisposition to cancer are common features (van Brabant et al., 2000). For instance, mutations in XPD can result in xeroderma pigmentosum, Cockayne syndrome (Zhang et al., 2010), or trichothiodystrophy (van Brabant et al., 2000).

Role of other Fe-S cluster proteins in genome stability

Analysis of the maturation and biogenesis of Fe-S cluster proteins in the mitochondria and cytosol was performed using the iron-sulfur cluster (ISC) machinery and cytosolic iron-sulfur cluster assembly (CIA) machinery, respectively (Sipos et al., 2002; Lill et al., 2012). Eukaryotic organisms exhibited a high degree of conservation, both in components and biogenesis mechanisms of the ISC and CIA pathways (Zhang, 2014). All members of the CIA machinery, including Nbp35 (NUBP1 in mammals), Cfd1 (NUBP1 in mammals), Cia (CIAO1 in mammals), Nar1 (NARFL in mammals), Cia2, and Mms19, are possibly involved in DNA replication and repair (Stehling et al., 2013). Disruption of MMS19 has been reported to affect DNA repair, chromosome segregation, and heterochromatin silencing (Stehling et al., 2012). In addition, both the human and yeast MMS19 proteins interact with numerous Fe-S proteins, such as Polδ, DNA primase, Dna2, XPD, RTEL1, and FANCJ (Gari et al., 2012), which are widely involved in DNA replication and repair processes. These results further implicate the importance of MMS19 in maintaining genome stability.

Role of manganese-containing proteins in genome stability

In manganese-containing proteins, a manganese ion is present in the active site of the metalloenzyme and plays a crucial role in enzymatic activity. Some of these manganese-containing proteins include manganese superoxide dismutase (MnSOD), manganese catalases, and arginase (Schiavone and Hassan, 1988), all of which play functional roles in the maintenance of genome stability.

Manganese-containing superoxide dismutase (MnSOD)

In mammals, MnSOD (also known as SOD2), is an essential mitochondrial antioxidant enzyme that protects the cell from potential damage caused by excessive amounts of ROS (Candas and Li, 2014). MnSOD demonstrates an antiapoptotic role against oxidative stress, ionizing radiation, and

inflammatory cytokines (Candas and Li, 2014). Alteration of MnSOD function is genetically associated with many diseases, including cancer and heart disease. For instance, MnSOD suppresses tumor cell growth, while the over-expression of *MnSOD* enhanced the invasiveness of tumor metastasis (Behrend et al., 2005). *MnSOD* knockout mice die shortly after birth, while heterozygous mice have a normal lifespan but exhibit minimal phenotypic defects and suffer increased DNA damage with higher incidence of cancer (Li et al., 1995). Further, in ischemia-reperfusion injury, MnSOD has been reported to be critical for ROS release during oxidative stress (Kim et al., 2006), contributing to cell damage and death, both by exerting a direct effect on the cell and by initiating apoptotic signaling (Candas and Li, 2014).

Manganese catalases

Manganese catalases are a class of manganese-containing metalloenzymes that are widespread among prokaryotes. Similar to iron-containing catalases, they mediate the catalytic decomposition of H_2O_2 to H_2O and O_2 (Yoder et al., 2000). The structure of manganese catalaseas depicted by X-ray crystallography reveals the presence of two manganese ions bound at the core of the quad-helix (Wu et al., 2004). Several manganese catalase genes have been identified from genomic databases, and a few of them have demonstrated to play roles in microaerophilic oxidative stress and in maintaining genome stability (Whittaker, 2012).

Arginase

Arginase is a manganese-containing enzyme that catalyzes the conversion of L-arginine into L-ornithine and urea (Dowling et al., 2008). Arginase competes with NOS for the common substrate L-arginine, leading to the uncoupling of NOS to produces uperoxide and decreased NO (Dowling et al., 2008). Upregulation of arginase results in increased ROS production (Zhou et al., 2015). Furthermore, aberrant expression of arginase is reported in a number of pathological processes, including heart failure, Chagas disease, and hypertension (Zhou et al., 2015).

Role of other metal ion-containing proteins in genome stability

In addition to the metalloproteins mentioned above, cells also contain a large quantity of zinc, cobalt, nickel, cadmium, molybdenum, and tungsten-containing proteins. Some of them have been implicated to be involved in the maintenance of genome stability. Alcohol dehydrogenases (ADH) are a group of zinc-containing proteins that facilitate the inter-conversion between alcohols and aldehydes or ketones with the reduction of nicotinamide adenine dinucleotide (NAD^+ to NADH) (Edenberg, 2007). Ethanol exposure of HeLa-ADH1B cells resulted in a significant increase of DNA damage and activation of the Fanconi anemia-breast cancer susceptibility (FA-BRCA) dependent DNA damage response network (Abraham et al., 2011). Xanthine oxidase (XO), a molybdenum-containing enzyme, functions to catalyze the oxidation of hypoxanthine to xanthine (Kelley et al., 2010). Substrate-derived electrons at the Mo-cofactor of XO reduce O_2 at the FAD-cofactor, both univalently, generating superoxide, and divalently, forming hydrogen peroxide (H_2O_2) (Kelley et al., 2010), which can cause cellular damage and/or cellular toxicity.

Conclusions and future prospects

Almost half of all proteins present in cells are metalloproteins requiring metal ions for their function. A majority of these metalloproteins play important functional roles in the maintenance of genome stability. Functional alteration of a few metalloproteins has been reported to be involved in the generation of ROS that could damage DNA, proteins, and lipids. Metalloproteins, such as DNA polymerases/primases, helicases, and the small subunit of RNRs, are critical components directly involved in DNA replication and repair processes. In recent decades, great advances have been made in understanding the maturation and structure of metalloproteins and their functional mechanisms. However, some key points involved in metal ion sensing, acquisition, and mismetallation are still unclear. For less common metals, it remains to be understood as to how cells in different organisms correctly distinguish between inorganic elements. As for mismetallation, most metalloproteins are active with only one metal, although they can bind other metals, both *in vitro* and in *vivo*. It remains a challenge to understand how cells control metallation and avoid mismetallation. Given that mutations or dysfunction of many metalloproteins are genetically associated with multiple diseases, a complete understanding of the underlying mechanisms related to these critical issues could help us treat these diseases in the future.

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Author contributions

Chunqiang Zhang, Fan Zhang, and Ping Zhou prepared and organized the sections pertaining to “Magnesium-containing proteins,” “Copper-containing proteins,” and “Manganese-containing proteins,” respectively. Caiguo Zhang prepared and organized the section on “Magnesium-containing proteins” and wrote the paper.

Compliance with ethics guidelines

The authors declare no conflicts of interest. This article does not

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