

REVIEW

Molecular structure and functions of zinc binding metallothionein-1 protein in mammalian body system

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Abstract: Zinc a major trace element; perform diverse roles in genetics and physiology in almost every vital body system of the mammalian body. Zinc regulates the expression of almost all essential genes responsible for performing pivotal functions in mammal cells through provision of structural integrity to the major transcriptional factors Zn finger Proteins (ZnF) and gene regulation for production of metallothionein protein. Zinc performed at least eight vital functions in living organisms including gene regulation e.g., as a promoter through metal response elements, structural i.e. zinc-finger motifs, catalytic e.g., metalloenzymes, DNA and RNA polymerase, DNA replication, Growth promotion, antioxidant, regulate functions of central nervous system and also act as hepato-protectant and detoxifying agent. Almost all of these vital functions are regulated through metallothionein protein, a cysteine rich Zn binding protein. These functions are basic mechanism for sustaining life. Therefore, this review paper was planned with the objective to highlight the important functions of Zn inside the mammal's body with particular reference to the metallothionein protein. Bioinformatics study performed for estimation of conservation and evolution of this important protein shows its greater conservancies in six important mammalian species.

Keywords: Zinc, metalloenzymes, metallothionein, zinc finger motif, transcription factors, conserved motifs of MT-1 protein.

INTRODUCTION

The ubiquitous existence of Zn (zinc) in animal, Plant and microbial cells illustrates various functions. The Zn physiological role is proven through interaction of this element with certain enzymes e.g., growth cessation is associated with Zn nutritional deficiency caused by inhibition of the DNA polymerase activity and consequently effecting DNA synthesis. The enzymes RNA (I, II, and III) polymerases are all Zinc dependent enzymes (metalloenzymes). With the depletion of cellular Zn a drastic decrease was observed in the synthesis of ribosomal, transfer and messenger RNA level. At the genetic level the Zn role in the synthesis of metallothionein is well known. This metallothionein synthesis is mostly controlled by the transcriptional regulation. Zn induces the transcription of MT gene, just like glucocorticoids, epinephrine and glucagon induce its transcription through elevation of cellular cAMP. A recent research study concluded that 12 base pairs promoter region fragment of the metallothionein (MT) gene regulates the expression of heterologous genes, which confirm the zinc role in the expression of particular

genes. These findings demonstrate important functions of Zn at molecular level which controlled vital functions in mammal body. Zinc regulates the expression of almost all essential genes responsible for pivotal functions in mammal cells through provision of structural integrity to the major transcriptional factors. Zn a trace element, which is vital for almost every living being because of its diverse functions, notably, act as an integral component of protein structure, also act as a cofactor in various enzymes which catalyse certain important biological reactions (Ackland ML 2006). Zinc has predominantly a versatile nature of functions regarding gene expression including enzymatic, structural and gene regulatory. Hence this overabundance of properties categorises zinc in a distinct class with respect to other elements regarding its importance in biological systems. In this review, various functions and roles of Zn playing in mammalian cells will be discussed in detail.

Zn role in Metallothionein production

Zinc is vital for the smooth functioning of cells and its structural integrity to perform important functions including gene expression (Cousins 1996; Frausto Da Silva JJR 1991; Ohalloran 1993). There is Zn pool in the body which regulate these functions through transporters

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across the intracellular sites and at the plasma membranes (Cousins 1998). The most important gene expression controlled by Zn level is the expression of metallothionein gene, MT is one of the cysteine rich Zn binding-proteins, linked with almost all of these metabolic changes in the body (Davis and Cousins 2000). The fundamental functions of Zn in biological system comprised of as catalytic e.g., metallo-enzymes, physical as Zn finger motifs and gene regulation as promoters with metal response elements (Cousins 1994). But the most important function of Zn in the mammalian body system is the regulation of genes responsible for the production of metallothionein (MT) proteins. This low molecular weight protein is responsible for diverse roles in biological systems. MTs are also known as housekeeping proteins due to its ubiquitous nature in mammalian body. Metallothionein production in body is mostly induced by certain stimuli including heavy metals toxicity, radiation and oxidative stress. Metallothionein has many isoforms (M-I to M-IV) which are further categorised into various isoforms. Every isoform of MT protein perform specific function in the mammalian body system, the most importantly are apoptosis, antioxidant and immunity in certain organs (Thirumorthy N 2011).

In large animals, small intestine is a primary organ for the absorption of dietary zinc. The homeostatic control of Zn absorption is well known through dietary zinc supply. The clinical manifestations such as growth retardation, anorexia, skin lesions and impaired immunity were observed with the reduced supply or intake of dietary zinc. Most of these effects occurred through variation in genetic expression and regulation, even though these particular genes are not yet found which are linked with these clinical manifestations. Therefore, specific research is needed to be conducted to find out the specific genes affected due to dietary Zn deficiency and manifests these clinical symptoms in mammals. However the literature supported this hypothesis that dietary intake of Zn causes regulation of gene expression which produces metallothionein protein through multiple metallothionein response elements positioned in the metallothionein gene promoter region (Cousins 1994; Ohalloran 1993). The Zn dependent Zn binding protein also known as Zn finger (ZnF) proteins is a major class of transcription factors. About 3-10% of the total genome of human was found conserved for the number of genes coded for proteins with zinc-binding domains (Andreini *et al.* 2006; Blasie and Berg 2002). Zn finger (ZnF) is a dynamic class of significant proteins; this wide-ranging family perform versatile functions in biological systems. Because of their diverse nature, all ZnF proteins could not be define with a single and simple definition, but the most collective approach through which we can define this diverse class collectively is a small and functional domain which requires coordination by at least one Zn ion. The zinc ion gives structural integrity to these proteins and serves as

stabilizer and basically having no role in binding of any targets. The “finger” is basically a tributary structure of both the α -helix and β -sheet. Zn ion is responsible to hold together these two helices. ZnF domains are basically interactors; they bind RNA, DNA, Proteins and other small molecules. The diverse functions are really extraordinary which included transcriptional activation, DNA recognition, RNA packaging protein assembly and folding, lipid binding and apoptosis regulation (Laity *et al.* 2001).

Another important function of Zn is the expression regulation of metallothionein protein through untranslated promoter region with multiple response elements (MREs). Accumulation of cellular MT protein is dependent on gene expression as well as on protein degradation, while both of these depend on Zn dietary intake. In turn these MT proteins the action of ZnF transcription factors regulate various cellular functions including apoptosis, gene expression, differentiation and proliferation. To activate transcription of metallothionein gene, the most important transcription factor namely metal responsive transcription factor 1 (MTF 1) is essentially required to bind with metal responsive element (MRE) located in 5UTR promotor. In vitro the metallothionein factor 1 binding with MRE is only induced by the Zn ions reflecting the importance of Zn in MTF 1 transcription factor and hence this transcription factor perform its role as Zn sensor-protein (Otsuka 2001). Metallothionein are well known proteins in the field of medical sciences because of their protective functions in heavy metal toxicities and also in ROS (reactive oxygen species). MTs also perform their function in the homeostasis of certain elements such as Zn (Suzuki *et al.* 1993). The most important role of MTs is the transcriptional regulation; hence provide further insight to study its cellular response mechanism against heavy metals. Many proteins having diverse physiological features were studied through DNA assays (binding), where MRE sequences were used as probes (Koizumi 1998). Only Zn binding-MT responsive element factor were verified as metal responsive element factor 1 (MTF 1), considered crucial for the heavy metal dependent (MT) genes expression. The role of Zn in MT gene expression was confirmed by (Liuzzi *et al.* 2001), figs. 1 and 1A demonstrate that dietary intake of Zn increase the expression of MT genes in rats tissue. Potentially Zn stores in the tissue as MT. The metallothionein proteins are the most substantial intracellular Zn binding proteins which playing important functions in mammal cells as mentioned above (Andrews 1990). Thus, it could be conferred from these findings that dietary intake of Zn is critically needed for the production of Metallothionein proteins which perform pivotal role in the gene expression of certain specific genes responsible for manifesting many important functions in the mammalian body (fig. 3).



Fig. 1: Differential expression of rat kidney metallothionein (MT) mRNA in response to zinc intake. Representative Northern analyses of pooled total RNA (15 mg/lane) are shown. (A) Rats were fed, 1 (2Zn), 30 pair-fed (PF), 30 ad libitum (1Zn) and 180 (11Zn) mg Zn/kg diet for 2wk (n 5 5/group). (B) Rats were administered an oral dose of either saline [9 g/L NaCl (2)] or zinc [70mg/kg body (1)] 2h before being killed. b-Actin was used as an RNA loading control (Liuzzi et al. 2001).

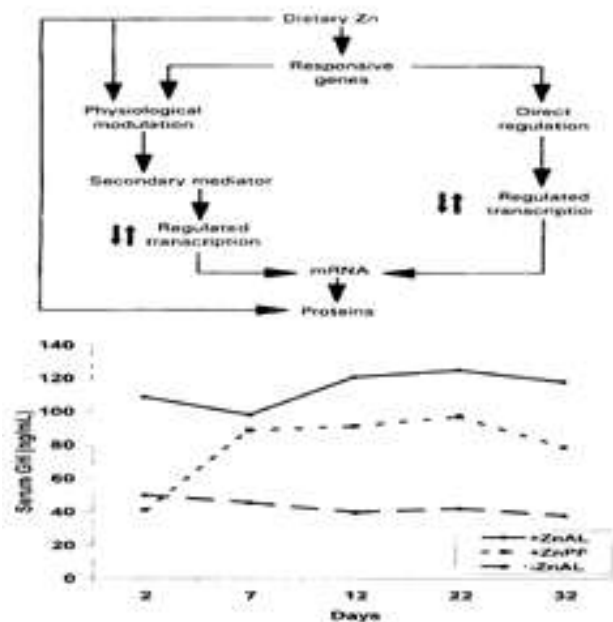


Fig. 2: Serum growth hormone concentrations rats fed zinc-adequate or deficient diets. Sprague-Dawley rats were fed a zinc-adequate diet (60 mg/kg: +ZnAL) or a zinc-deficient diet (1.3mg/kg: -ZnAL) or Pair 32 d. and serum GH concentrations were measured. Data are from Roth and Kirchgessner 1997.

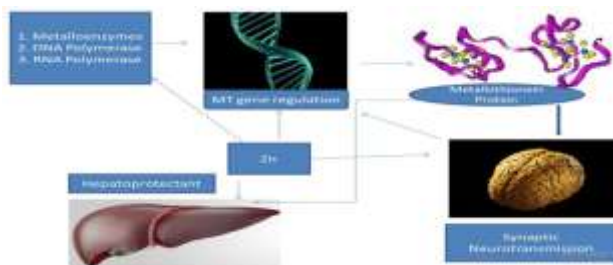


Fig. 3: Overview of Zn functions in different body organs

Bioinformatics of Metallothionein protein

The sequences of amino acids of Metallothionein 1 protein were obtained from NCBI (<https://www.ncbi.nlm.nih.gov>) for sex (06) mammalian species including

(Homo sapiens NP_005937.2, Bos Taurus NP_001035582.1, Canis lupus familiaris NP_001003173.1, Sus scrofa NP_001001266.1, Rattus norvegicus NP_620181.1 and Pan troglodytes NP_001288207.1. The sequences were downloaded through TBtools (toolbox for biologists) v.0.58 software in fasta format. Protein sequences of all six species (Multiple sequence alignment) was aligned through MUSCLE sequencing alignment (multiple sequence comparison through log expectation), the phylogenetic tree (neighbourhood-joining) was built, MEGA version 7.0.26 (Philadelphia, PA, USA) software was used for both Multiple sequence alignment and phylogenetic tree construction (Kumar et al. 2016). For the analysis of protein structure and function, the motif was searched and conserved domains were identified through online MEME suite website (Bailey et al. 2009), CDD NCBI and TBtools (Marchlerbauer et al. 2017; Marchlerbauer et al. 2015).

Biological evolution and conservation estimates of MT-1 Protein

The multiple sequences alignment of the metallothionein 1 protein was performed using sex common mammalian species including human, cattle, domesticated dog, domestic pig, brown or common rat and chimpanzee (fig. 6). The sequence alignment of the primary protein sequences showed high similarity in the protein structure (sequences) among the tested species; based on this structural similarity we can say that protein function is also similar among these mammals tested species. The results of phylogenetic tree, constructed for metallothionein protein (fig. 4) and MEME suit tool used for searching common motifs in super-secondary structure (figs. 4 and 7) showed that Human, chimpanzee and domestic dog was found the most closely related species in terms of metallothionein protein sequence, while pig, cattle and mice branches were found far away from the human sequence. Sequences of cattle, pig and brown rate were found very closes to each. Only two significant motifs were found among the sex mammalian species, this low numbers of significant motifs is may be because of its short length sequence (figs. 4 and 7), this further indicate the similarity in function at super-secondary structure level. The NCBI's Conserved Domain Database (CDD) was performed with the aims to locate the evolutionary conserved protein domains superfamily. Two specific hits were found conserved in all sex mammalian species which representing two domain superfamilies (fig. 5). It means each tested mammalian species possess metallothionein 1 protein. The protein sequences of the metallothionein 1 protein of all mammalian species were extracted from the NCBI database (<https://www.ncbi.nlm.nih.gov/>).

Catalytic role of Zn e.g. metalloenzymes

Zinc being relatively non-hazardous crucial trace element (Joshi and Shrivastava 2004) which is very much needed for smooth function of human metabolic system (Rink

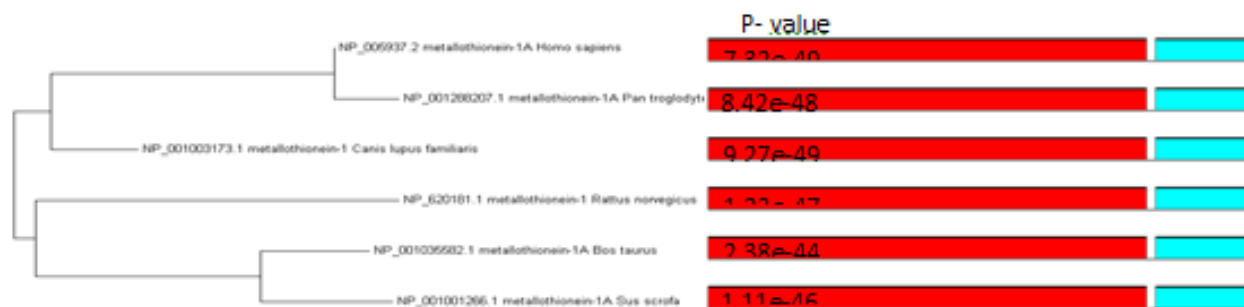


Fig. 4: The phylogenetic tree and structure motif of Six tested mammalian species. The position, significance and strength of concerned motif site were shown through colour blocks. These colours were given by MEME suit online system, performed for the motif analysis. The “red and blue line” indicated the specific conserved motif sites. The p value also known as “combined match p value” is a probability that a motif under test would have a match to the random sequence with an equal or greater score to the largest value found in the sequence under test.

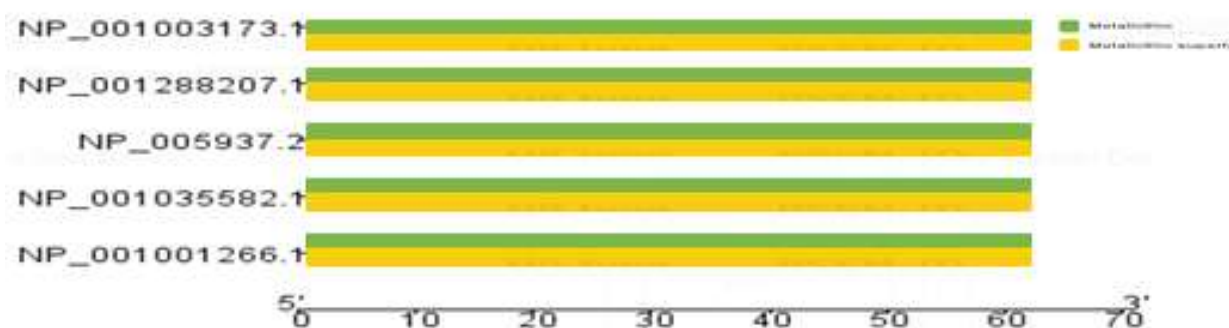


Fig. 5: Structure domain families of Metallothionein protein in six mammalian species, two colours are two different blocks with particular hit which represents two different super families.

and Gabriel 2000; Tomas. *et al.* 2009). In cellular metabolism Zn is pervasive, and is an important part of one or more catalytic sites of either one or more enzyme categories (Coyle *et al.* 2002). Besides direct role of Zn in gene expression modulation through promotor region of gene regulation with special reference to the genes responsible for metallothionein production, the indirect mechanisms affected by zinc status also have a substantial control on gene regulation. The altered zinc status caused modulation in metabolic status through a pathway which is basically considering one of the indirect effects of Zn. These metabolic status modulations include changes in enzymatic activities or Zn dependent signalling molecules. This modulation in turn causes alteration of gene expression through feedback stimulation. As these indirect effects are not directly affected by Zn therefore these are very difficult to study, but it is very evident that these indirect effects are closely associated with dietary Zn intake i.e. Zn supplementation. Almost more than two hundred enzymes need Zn as an active functional constituent and these enzymes perform the most important metabolic functions in the mammalian body. Even though Zn metalloenzymes perform diverse functions, there is significant correlation between the loss of enzyme activity and the genes dependent with dietary Zn intake. Total 39 dietary regulated expressed genes

sequences were identified which were regulated by the Zn intake. This gene expression machinery was affected by numerous ways such as chromatic structure, template DNA function, various transcription factors and also RNA polymerases. Modulation in one or more than one of these Zn dependent processes were proven to be linked with Zn deficiency (Falchuk 1998). Hence, we can conclude from this literature that Zn metalloenzymes have a very eminent role in the overall metabolic processes in the mammal body system. It paves the way to formulate various hypotheses regarding Zn roles in various Zn metalloenzymes systems and in turn regulation of specific genes which are linked with these enzymes system. The most important is its role in DNA and RNA polymerases, which are the basic requirement of the most important function for sustaining life through DNA replication.

Zn role in nervous system (Central)

Zn is second after iron in its vital functions performed by trace elements in mammalian body system. The highest concentration is found in brain for regulation of physiological functions of central nervous system (Singh *et al.* 1995; Vijayta and Dhawan 2005). Zn deposited in neocortical layers, it is estimated that 10 percent of the total Zn accumulated in the brain is placed in glutamate comprising synaptic vesicles which is activated during

release of synaptic neurotransmitter (Bitanihirwe BK 2009; Frederickson *et al.* 2004). After release Zn becomes bound with various membrane receptors and ions, these cationic divalent ions are basically responsible molecules for neurotransmission (Baranano *et al.* 2001; Nakashima. A. S 2009; Smart *et al.* 2004). Thus, we can conclude that Zinc is a crucial element for successful passage of neurotransmission. Furthermore, Zn protects the neural cell membrane stability as an antioxidant agent through regulation of metallothioneins expression and also causes inhibition of lipid peroxidation of brain cells (Powell, 2000). Zinc is present in brain in two different “pools” one is the larger and other is smaller pool. The larger pool is bounded with protein and is vital component of various Zn metalloenzymes and metalloproteins. The metalloproteins are basically ubiquitous in nature and is present in almost every soft tissue and cell organelles. The smaller pool also known as synaptic Zn is only 5 percent of the overall Zn but its role is of greater significance. This Synaptic Zn is entirely stored in presynaptic vesicles of neurons located in forebrain portion of the CNS. This Zn is present either free or weakly bound Zn²⁺ ion in the extracellular fluid (Sandstead, 1994). Zn is mostly concentrated in synaptic vesicles and act as a messenger when it receives any signal during neural stimulation to perform a neural activity (Choi. Y 2001; Kresse *et al.* 2005; Michal Hershinkel 2007). Thus accurate regulation of Zn homeostasis is essential for whole organism in general and for central nervous system in particular. (Małgorzata Joanna Gdula-Argasińska and Bogdan 2014) discussed in detail about the effect of Zn on aetiology, pathogenesis, control and remedy of some specific disorders of CNS. Literature further exhibit that most of these central nervous system disorders are linked with Zn Imbalance and this imbalance is not only due to deficient dietary intake but also resulted due to improper Zn transport proteins and impairment of Zn dependent metabolic pathways. Again, this is a very important research area to find out the specific metabolic pathways which are linked with Zn transporter genes family to maintain Zn homeostasis in the body cells. Depression, Alzheimer’s disease and aging connected loss of mental functions are considered degenerative processes of CNS which are linked with Zn dyshomeostasis. Intensive investigation is going on to find the specific and precise zinc function and its binding-proteins in the pathogenesis of central nervous system but it is well documented that proper Zn supplement is very helpful in the prevention and treatment of various brain disorders. Hence it could be concluded that Zn plays a significant role in regulation central nervous system functions particularly combating many fatal CNS dysfunction.

Zn role in gene regulation

Generally, the nutrients perform a vital role in gene regulation, particularly Zn significantly affect the expression of important genes and the dietary deficiency of this important micronutrient significantly affect the

expression of specific genes responsible for proper function of vital body system including gastrointestinal system. GIT being absorptive and secretory organs is directly prone to both acute and chronic effects of Zn positions, an acute effect is manifested as result of deficiency through dietary Zn intake and chronic effects are due to improper homeostasis in the body. For the optimum performance of GIT system, the cells pass through rapid growth and development phases based on gene expression. Therefore Zn metabolism for maintaining its homeostasis through expression of genes is one of the vital functions of GIT system (Cousins 1996). Further research is needed to find out Zn metabolism and homeostasis, as very little information is available about molecular and cellular mechanisms of Zn responsive elements for maintaining health with special reference to genes involved. A typical example of Zn direct association with the transcription factors of gene is its linkage with promoter region of metallothionein genes as cis acting DNA transcription factors also known as metal response elements. Metal response elements are crucial for the transcription of metallothionein genes which are in turn proportional to the presence of Zn level in the system. Zn is an important micronutrient. In the area of nutrition research, the regulation of gene expression through dietary nutrients is well recognised. This type of gene expression regulated by nutrients is mainly due to the direct interaction of these nutrients with cellular components, usually transcription factors which modulate transcription and also level of mRNA expression. Zinc just like retinoids, sterols, fatty acids and calcitriol binds with particular transcription factors as ligands and modulate function of a gene or a gene family. Thus Zn can modify transcription of a gene through occupancy of a specific site reserved for the enhanced for the expression of this gene (Cousins 1998). Thus, this literature proved that dietary Zn is an integral part of particular gene expression regulatory component i.e. transcription factor in the mammalian cell through acting as ligand and binds with specific sites in particular transcription factors.

Zinc and growth hormone

The serious manifestation of Zn deficiency both in humans and animals is growth retardation. The probable reason of this adverse effect of Zn deficiency is due to disruption of the growth regulated by endocrine system of the body. It is well documented that circulating level of growth hormone decreases with deficiency of dietary zinc (Ming Chuan Cha 1997). The pituitary gland holds the highest level of Zn concentration as compared to other organs of the body with believe that Zn is the causative factor for the enhance function of pituitary hormone. It is proved that the origin of growth hormone is pituitary gland furthermore GH is the basic hormonal regulator of the body growth, therefore various research findings proved that zinc deficiency caused cessation of growth



Fig. 6: Multiple sequence alignment of metallothionein protein within sex different mammalian species. The degree of similarity has been explained through three different types of background shades, the black colour shadow reflects (100%) similarity; the grey with black scripts shows (80%) similarity; the texts having grey with white colours shows (60%) similarity while white colour text indicates no conserve sequence (similarity) within tested species protein sequences.

hormone and consequently somatic growth. Deficiency of Zn causes failure of growth hormone secretion from the pituitary gland in human as also in rats as shown in fig. (Kirchgessner Roth 1997). Thus, these findings confirmed the direct role of Zn in secretion of GH. These findings proved the role of dietary Zn as a basic micronutrient for the normal body growth in mammals.

Zinc role in DNA replication, transcription and protein syntheses

Zn is a vital micronutrient for growth, development and differentiation of the mammalian cells. Deficiency of Zn in the mammal cells cause genetic, teratological and various other clinical manifestations. Research conducted on zinc function; in *Euglena gracilis* have demonstrated its important functions in transcription, replication and translation of the genes. Zinc withdrawal from this single celled eukaryotic organism modify complete morphology and utility of RPases, DNAs and also other regulatory elements (proteins) particularly histone proteins which perform important function in the expression of certain genes. Expression or suppression of these genes causes synthesis or degradation of particular types of proteins. This function of Zn is of significant importance in cell metabolism. The same nature of research needs to be performed in vertebrates. Zn role in DNA and RNA polymerases has been well studied and was found that Zn is a fundamental component of the many polymerases. This research further revealed the gene transcription role of intrinsic zinc in polymerases as multidimensional. In RPases, zinc ions perform three important functions including gene regulation through recognition of specific promoter and initiation of RNA chain, the second role is catalytic and the last one is role is structural. Zn finger motif including xenopus transcription factor A class is a concrete evidence of the Zn role in gene regulation as for these protein Zn is the functional and integral component of these elements, these findings provide a conclusion that Zn directly participate in the regulation of particular genes. More research studies regarding the structural and mechanical roles of Zn on both the polymerases and regulatory proteins i.e. ZnF proteins regulating gene expression will not only highlight Zn role in cellular growth and development, but will justify its role in certain clinical abnormalities due to deficiency of Zn. The

synthesis of RNA is one of the integral part of gene expression and zinc ions are needed for the catalytic function of the RNA polymers particularly RNA nucleotide transferases which are also known as zinc metalloenzymes. The dietary deficiency of zinc causes reduction in the gene activity. Zn is one of the structural components of many metallo enzymes particularly those involved in gene replication such as both DNA and RNA polymerases. Therefore deficiency of Zn leads to depress the synthesis of DNA and consequently cell division which is needed for the growth and development of body system (Falchuk 1998).

Zinc as an antioxidant

The Zn role as antioxidant in various biochemical systems of mammalian body is well proven under various circumstances of oxidative stress. Zn mood of action as antioxidant could be broadly categorised into two classes based on its effects namely as chronic effects and acute effects. Former is low Zn intake for long term, which subsequently activates other substances to act as antioxidant. Thus, in chronic category the Zn indirectly acts as antioxidant. Following chronic exposure to Zn, activation of metallothionein proteins has been reported. These proteins are group of light molecular weight ranged from 6000 to 7000 (kDA). Metallothionein are metals binding-proteins containing range from 60 to 68 amino acids residues, including 30 percent cysteine amino acid (Saito C 2010). Induction of metallothionein proteins upon chronic exposure of Zn is different is different organs such as reported in liver (Jihen *et al.* 2009), in blood, brain (Malhotra and Dhawan 2008), and in intestine (Dhawan and Chadha 2010). The second category is acute effects of Zn which is again classified into 02 sub categories first one is sulfhydryl and the second one is an antagonism group of redox active transition metals. Literature further confirms the Zn role as an excellent hepatoprotection agent and therefore Zn is recommended to be used prophylactically in case of toxicity caused by pesticides. Although the potential efficacy of Zn is well known in dealing with liver toxicity caused by various toxins such as chlorpyrifos but the detail molecular mechanism of Zn mode of action is still unexplored. Hence this aspect of Zn functions needs to be explored (Malhotra and Dhawan 2008). Another

protective role of Zn is in the production of Phosphotriesterases, this is one of the metalloenzymes classes. The catalytic active site of this metalloenzyme contains Zn cation ions, which detoxify most of toxins particularly pesticides (Raushel 2002). Zinc, being located at the catalytic centre of Phosphotriesterases metalloenzyme, improves stability of this enzyme as compared to other metal cations (Daniel *et al.*, 2004). These findings confirmed the role of Zn as hepatoprotectant and also as a detoxifying agent during pesticides toxicity.

CONCLUSION

It could be inferred from the above literature that dietary Zn plays vital roles in cellular function at various stages particularly in

1. Metallothionein proteins, which in turn perform a pivotal role in the gene expression of certain specific genes responsible for manifesting many important functions in the mammalian body.
2. Regulation the function of central nervous system particularly combating many fatal CNS dysfunctions.
3. Gene expression in mammalian cell through acting as ligand and binds with specific sites in particular transcription factors.
4. Maintaining growth and development, dietary Zn is the basic micronutrient for the normal body growth in mammals.
5. DNA replication, Transcription and Protein syntheses particularly through production of DNA and RNA polymerases
6. Hepatic protection and detoxification particularly during pesticides toxicity.
7. The bioinformatics analysis (biological evolution and conservation estimates) confirmed that metallothionein I protein (Zn binding protein) is conserved among the tested mammalian species and hence we can conclude that function is same in these mammalian species.

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REFERENCES

Ackland MLM (2006). Zinc deficiency and its inherited disorders a review. *Gene Nutrition*, **1**(1): 41-49.
 Andreini C, Banci L, Bertini I and Rosato A (2006). Counting the zinc-proteins encoded in the human genome. *Journal of Proteome Research*, **5**(1): 196-201.
 Andrews GK (1990). Regulation of metallothionein gene expression. *Prog. Food Nutr. Sci.*, **14**(2-3): 193-258.

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW and Noble WS (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Research*, **37**(2): W202-208.
 Baranano DE and Ferris CD HS (2001). Snyder neural messengers, *Trends Neurosci.*, **24**(2): 99-106.
 Bitanhirwe BK CM (2009). Zinc: The brain's dark horse. *Synapse*, **63**(11): 1029-1049.
 Blasie CA and Berg JM (2002) Structure-based thermodynamic analysis of a coupled metal binding-protein folding reaction involving a zinc finger peptide. *Biochemistry*, **41**(50): 15068-15073.
 Choi Y HVC and SA Lipton (2001). Three pairs of cysteine residues mediate both redox and Zn²⁺ modulation of the nmda receptor. *J. Neurosci.*, **21**(2): 392-400
 Cousins (1994). Metal elements and gene expression. *Annu. Rev. Nutr.*, **14**(1): 449-469.
 Cousins (1996). Zinc . *In: Present Knowledge of Nutrition Filer LJ & Ziegler EE eds. International Life Sciences Institute Nutrition Foundation, ILSI Press, Washington, D.C., USA, pp.293-306.*
 Cousins RJ (1998). A role of zinc in the regulation of gene expression. *Proc. Nutr. Soc.*, **57**(2): 307-311.
 Coyle P, Mathew G, Game PA, Myers JC, Philcox JC, Rofe AM and Jamieson GG (2002). Metallothionein in human oesophagus, Barrett's epithelium and adenocarcinoma. *Br. J. Cancer*, **87**(5): 533-536.
 Daniel R, Nathalie V, David C and Masson (2004) E, Fr'ed' erique RENAULT, Marie-Th' er'ese FROMENT and Patrick of the activesite metal cation to the catalytic activity and to the conformational stability of phosphotriesterase: Temperature-and pH-dependence, *Biochem. J.*, **380** (3): 627-633.
 Davis SR, Cousins RJ (2000) Metallothionein expression in animals: A physiological perspective on function. *The Journal of Nutrition*, **130** (5): 1085-1088
 Dhawan DK and Chadha VD (2010) Zinc: A promising agent in dietary chemoprevention of cancer. *Indian J. Med. Res.*, **132** (6): 676-682.
 Falchuk K (1998). The molecular basis for the role of zinc in developmental biology. *Mol. Cell Biochem.*, **188**(1-2): 41-48.
 Frausto Da Silva JJR WR (1991). The biological chemistry of the elements. Clarendon Press, Oxford.
 Frederickson CJ, Maret W and Cuajungco MP (2004). Zinc and excitotoxic brain injury: A new model. *Neuroscientist*, **10**(1): 18-25
 Jihen H, Imed M, Fatima H, and Abdelhamid K (2009). Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver of the rat: histology and Cd accumulation. *Food Chem Toxicol.*, **46**(11):3522-7.
 Joshi VM and Shrivastava M (2004). Partial ischemic necrosis of the uterus following a uterine brace compression suture. *BJOG: An international journal of Obstetrics and Gynaecology*, **111**(1): 279-280

- Kircheggner Roth HPM (1997). Course of concentration changes of growth hormone, IGF-1, insulin and C-peptide in serum, pituitary and liver of zinc-deficient rats. *J. Anim. Phys. Anim. Nutr.* **77**(2):91-101.
- Otsuka, Fuminori, and Shinji Koizumi (1998). Mechanism of heavy metal-inducible gene transcription. In: *Toxicant-Receptor Interactions*, **10**(1):155-170.
- Kresse W, Sekler I, Hoffmann A, Peters O, Nolte C, Moran A and Kettenmann H (2005). Zinc ions are endogenous modulators of neurotransmitter-stimulated capacitative Ca²⁺ entry in both cultured and in situ mouse astrocytes. *Eur. J. Neurosci.*, **21**(6): 1626-1634.
- Kumar S, Hash CT, Thirunavukkarasu N, Singh G, Rajaram V, Rathore A, Senapathy S, Mahendrakar MD, Yadav RS and Srivastava RK (2016). Mapping Quantitative Trait Loci Controlling High Iron and Zinc Content in Self and Open Pollinated Grains of Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]. *Front Plant Sci.*, **7**(1): 1636.
- Laity JH, Lee BM and Wright PE (2001). Zinc finger proteins: New insights into structural and functional diversity. *Curr. Opin. Struct. Biol.*, **11**(1): 39-46.
- Liuzzi JP, Blanchard RK, Cousins RJ (2001) Differential regulation of zinc transporter 1, 2 and 4 mRNA expression by dietary zinc in rats. *J. Nutr.*, **131**(1): 46-52.
- Małgorzata Joanna Gdula-Argasinska Ts, Włodzimierz Opoka, Tyszka-Czochara Agata and Bogdan L (2014). The role of zinc in the pathogenesis and treatment of central nervous system (CNS) diseases. Implications of zinc homeostasis for proper CNS function. *Acta. Pol. Pharm.*, **71**(3): 369-377.
- Malhotra A and Dhawan DK (2008) Zinc improves antioxidative enzymes in red blood cells and hematology in lithium-treated rats. *Nutr. Res.*, **28**(1):43-50.
- Marchlerbauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR (2017). CDD/SPARCLE: Functional classification of proteins via subfamily domain architectures. *Nucleic Acids Res.*, **45**(D1):D200-D203.
- Michal Hershfinkel WFS and Israel Sekler (2007). The zinc sensing receptor a link between zinc and cell signalling. *Mol. Med.*, **13**(7-8): 331-336.
- Ming Chuan Cha AR (1997). Zinc deficiency inhibits the direct growth effect of growth hormone on the tibia of hypophysectomized rats. *Biol. Trace Elem. Res.*, **59**(1-3): 99-111.
- Nakashima. A. S DR (2009) Zinc and cortical plasticity. *Brain. Res. Rev.*, **59**(2): 347-73.
- Ohalloran TV (1993). Transition metals in control of gene expression. *Science*, **261**(5122): 711-725.
- Otsuka F (2001). Molecular mechanism of the metallothionein gene expression mediated by metal-responsive transcription factor 1. *J. Health Sci.*, **47**(6):513-519.
- Raushel FM (2002). Bacterial detoxification of organophosphate nerve agents. *Curr. Opin. Microbiol.*, **5**(3):288-95.
- Rink L and Gabriel P (2000). Zinc and the immune system. *Proc. Nutr. Soc.*, **59**(4): 541-552.
- Saito C YH, Artigues A, Villar MT, Farhood A, Jaeschke H (2010) Mechanism of protection by metallothionein against acetaminophen hepatotoxicity, *Toxicol. Appl Pharmacol.*, **242**(2): 182-190.
- Sandstead H H. 1994. Understanding zinc: recent observations and interpretations. *J. Lab. Clin. Med.*, **124**(3):322-327
- Singh B, Dhawan D, Chand B, Mangal PC and Trehan PN (1995). Trace element distribution in rat brain following lead and lithium supplementation -- a study using an EDXRF spectrometer. *Applied Radiation and Isotopes*, **46**(1): 59-64.
- Smart TG, Hosie AM and Miller PS (2004) Zn²⁺ ions: Modulators of excitatory and inhibitory synaptic activity. *Neuroscientist*, **10**(5): 432-442.
- Suzuki, K. T., and Kuroda, T. (1995). Transfer of copper and zinc from ionic and metallothionein-bound forms to Cu, Zn superoxide dismutase. *Res. Commun. Mol. Pathol. Pharmacol.*, **87**(3):287-296.
- Thirumoorthy N ASS, KT Manisenthil Kumar, M Senthil kumar, N K Ganesh and Malay Chatterjee (2011). A Review of Metallothionein Isoforms and their Role in Pathophysiology. *World J. Surg. Oncol.*, **9**(54): 1-7
- Tomas. EM, Tore H and Olle B (2009). Molecular dynamics study of zinc binding to cysteines in a peptide mimic of the alcohol dehydrogenase structural zinc site. *Phys. Chem. Chem. Phys.*, **11**(6): 975-983
- Goel A, Dani V, Dhawan D K. 2005. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. *Chem. Biol. Interact.*, **156** (2-3): 131-140.