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Review Paper

Fish metallothionien gene expression: A good bio-indicator for assessment of heavy metal pollution in aquatic ecosystem

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Abstract

Metallothionein (MT) gene encodes metal regulatory proteins or metallothionien which regulate the heavy metal concentration in all vertebrates including fishes. These proteins have cysteine rich residues that bind with metal ions by metal-thiolate bonding and maintain homeostasis of metal ions in organisms. The different isoforms of MT have reported in all vertebrates as MT-1, MT-2, MT-3 and MT-4. MT-1 and MT-2 are well reported in fishes. The present manuscript reviews the expression and regulation of the gene encoding metal regulatory protein or metallothionien in fishes. The regulation of essential metals in fishes is maintained by the metal responsive element (MRE), which on binding with transcription factor-1 (MTF-1) starts the transcription and controls the expression of MT gene. Thus the expression level of metallothionein gene in the fishes can be used as a potential bio-indicator for assessment of heavy metal pollution in the aquatic system. It provides protection to the fishes from oxidative stress induces by metal ions and can also represent the stress condition of aquatic ecosystem due to heavy metal pollutants.

Keywords: MTs and its isoforms, MT gene expression and Regulation, MT functions.

Introduction

In the present time, the metal pollution in surface water, drinking water and other food products is major concern. The rapid industrialization, mining, agricultural activity and vehicles discharge huge amount of health hazards chemicals containing Cr, Cu, Cd, Pb, As and Hg in the environment. These metals directly come into the aquatic environment such as river and ponds due to discharge of untreated industrial wastes. In India many rivers such as Kali, Ramganga, Ganga, Yamuna and many others have been reported to be polluted mainly through discharged of untreated wastes into rivers by many industries[1]. The accumulation of heavy metals takes place either into the tissue of aquatic organisms or persists into the sediment that results gradual increment of heavy metal concentration in the ecosystem which linked to biogeochemical cycle and causes toxicity in the aquatic flora and fauna[2].

The regulation of metal ions by aquatic organisms is up to a certain concentration and protects themselves from adverse effects. However, continuous exposure of heavy metals into the aquatic body disturbs the regulatory processes and initiates metal accumulation.

Fishes are considered to be a sensitive pollution indicator of aquatic body because they are directly in contact with surface water and can store and metabolize the water borne pollutants[3,4]. The regulation of heavy metals in fishes is regulated by metal binding protein called metallothionien (MT). It regulates the essential metals such as Zn and Cu, protect aquatic biota from metal toxicity and oxidative stress[5].

The MT protein encoded by MT gene and its transcription is started by binding of metals on metal responsive elements (MRE) region that binding transcription factor-1 (MTF-1)[6]. The changes in level of MT gene expression in fishes have been used as a biomarker for detecting the metal pollution in water bodies[7].

Fishes are directly in contact with surface water and being used for the assessment of water quality of aquatic system and as such can serve as bio-indicators of aquatic environment[8,9]. In the present day the possible use of MT gene expression in fishes as a biomarker for assessment of heavy metal stress in aquatic environment is an important aspect for researchers.

Metallothionien gene in fishes

MT gene encodes low molecular weight (6-7 K Da) protein called Metallothionein[10]. It is a cytosolic protein which contains cysteine residues that chelate with metal ions such as Zn, Cd, Cu, and Se by metal- thiolate bonding[11]. It protects organisms from oxidative stress induces by metal ions[12].

During this process the cysteine binding metal ions are liberated into the medium by oxidizing of cysteine into cystine that activates the MTs synthesis[13].

MT and its different isoforms in fishes

There are four different isoforms of MT known in all vertebrates viz MT-1, MT-2, MT-3 and MT-4 [14]. These isoforms are found in different parts of the body and performs different role. Among these isoforms MT-1 and MT-2 are major isoforms, being ubiquitous and found in all tissue[15]. MT-3 and MT-4 are minor isoforms basically found in specialized tissue such as MT-3 is found in nervous system and glia, MT-4 in squamous epithelial cells[16,17]. MT-1 and MT-2 are widely reported in all vertebrates, including fish. MT-1 and MT-2 isoforms have been repoted in many fish species such as the common carp (*C. carpio*), icefish (*Chionodraco hamatus*), and pike (*Esox lucius*)[18-20]. The Pisces MT was first reported in fish *Pleuronectes platessa* and its two different isoforms MT-A and MT-B were reported in Rainbow trout[21,22]. The two isoforms MT-1 and MT-2 have been also reported in goldfish *Carassius auratus*[23]. Many authors working in the field of metallothionein have described different MT isoforms in many vertebrates including fish species.

Expression and regulation of MT gene

Expression of MT gene is induced by various inducers such as oxidative stress, metal exposure, cytokines, hormonal stress like glucocorticoids and thyroid etc[24,25]. Each inducer works through a particular MT gene promoter for initiating specific response such as oxidative stress, heavy metal, cytokines, glucocorticoid and thyroid, require antioxidant response eliments (ARE), metal response elements (MRE), cytokine response eliment (CRE), glucocorticoid response elements (GRE), and thyroid response elements (TRE) respectively for transcription of MT gene. MT gene transcription is initiated by the binding of metal transcription factor-1 (MTF-1) to metal responsive element (MRE) promoter through activation of heavy metals[26,27]. Generally, the MT expression level is dose dependent of heavy metals. However, there is no such relation found in the case of excessive over dose of heavy metals[28]. Different fish species show different MTs expression level and distinct tolerance to exposure of different heavy metals and they may also be inducing[29].

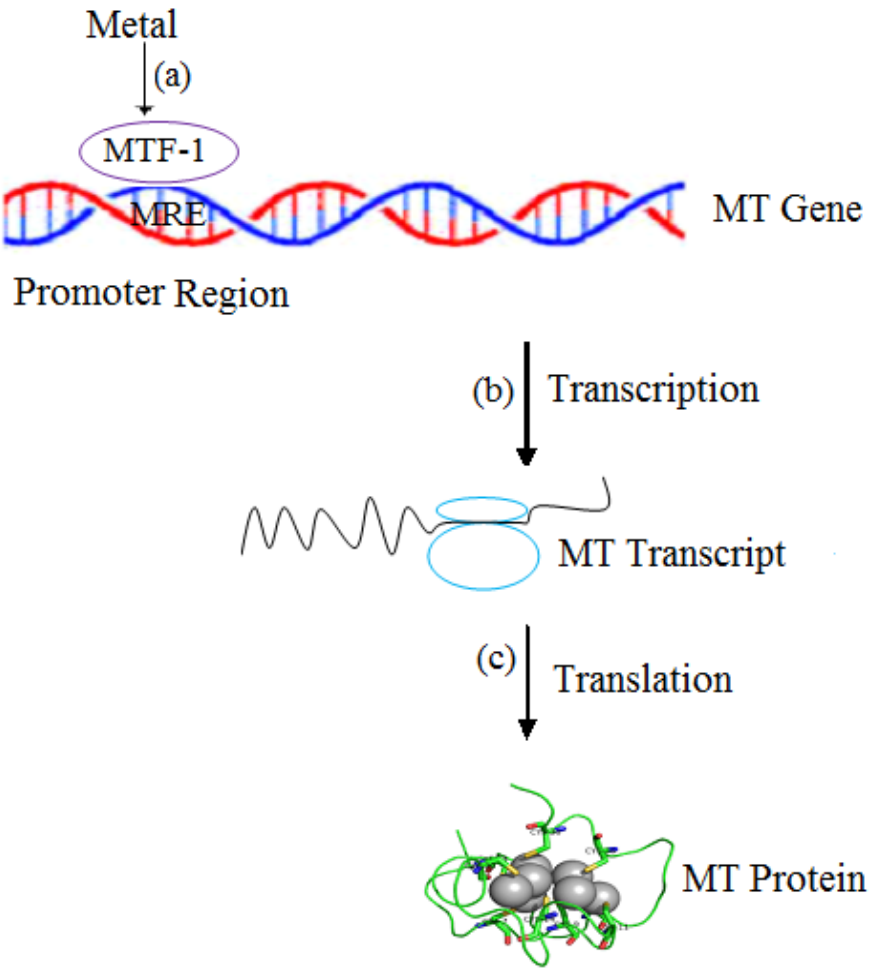

Figure-1: Expression and Regulation of MT gene.
(a) Metal induced MTF-1 binds with MRE promoter region (b) Binding of MTF-1 with MRE starts the transcription and produce MT transcript (c) Translation of MT transcript to produce MT protein which protects cells from oxidative damage.

Function of MT

In the past few years many researchers have suggested the functions of metallothionien in cells of aquatic animals including fish species. Some reported functions of metallothionien in aquatic animals summarized below.

Free radicals scavenger: The free radicals scavenging activity of MT have reported in several animal cell lines and also in epithelium cell line of *Cyprinus carpio* fish by cadmium exposure[30-32]. The MT expression and free-radical scavenging activity was also observed in *Channa punctatus* in response to exposure of some chemicals and stresses[33].

Metal detoxification and homeostasis: MT plays a significant role in detoxification and homeostasis of essential and non-essential metals[34,35]. The synthesis of MTs several folds during oxidative stress that prevent cells from cytotoxicity and DNA damage [32,36,37].

Inhibition of apoptosis: The significant roles of MTs have been reported in the process of apoptosis[38]. The inhibition of apoptosis by hepatic MT has been reported in *Carassius auratus gibelio* by regulating caspase-3 activity[39].

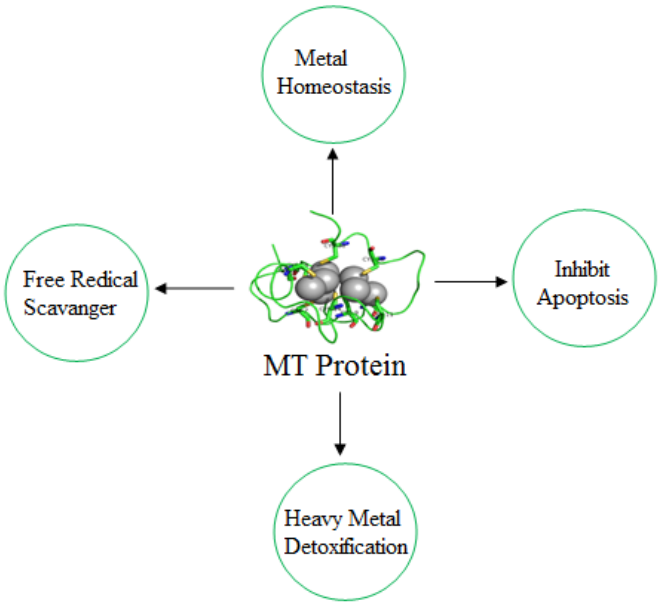

Figure-2: The main functions of Metallothionein in fishes.

In development: In a study, MT mRNA is found highest level in ovary of *H. mylodon*[40]. The MT mRNAs may play a significant role in homeostasis of metal which is primary needs of embryogenesis and early development of larva in polluted aquatic environment [41].

Bio-indicator: MT gene expression act as a bioindicator for evaluating the heavy metal pollution in the aquatic environment. MTs gene have recently gained attention for the implication of their role as bioindicator for monitoring the heavy metal pollution of aquatic ecosystem[40,42,43,]. Nevertheless, the application of MTs as bioidicator due to change of expression level of MT gene through a number of factors such as pH, temperature, season, sex, age and reproductive cycle[41, 44].

Conclusion

This review shows the important role played by MTs in heavy metal tolerance. The exposure of heavy metals induces the MT gene expression for regulation of accumulated heavy metals and protects the cells from oxidative damage especially during development. In addition, the results of fish MT gene expression can be used as sensitive bio-idicator for assessment of heavy metal pollution in the aquatic environment. In recent time the developing countries are suffering from increasing the heavy metal concentration in aquatic system such as river and ponds. The relationship between MT gene expression and exposure of exogenous pollutants can be contributed to improve the strategies for assessing the fish health by MT gene expression in the polluted aquatic environment. Therefore, for this to be achieved it should be collected more information about different isoform of MT gene, their function and various sources which induces MT expression in fishes.

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