Metallothionein genes in the sea urchin Paracentrotus lividus

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Metallothioneins (MTs) constitute a heterogeneous superfamily of cysteine rich proteins, which coordinate divalent (Zn2+, Cd2+) or monovalent (Cu+) metal ions.

Several functions have been proposed for these peptides, ranging from toxic metal protection to physiological metal homeostasis, free radical scavenging, oxidative stress protection, antiapoptotic defense, control of the redox status of the cell and also a role during development.

Regarding the MT system in vertebrates’ nearest kin, little information is available at present. Recently MTs were also characterized in cephalochordates.

Hence in order to shed some light on MT origin and functional differentiation through evolution, we studied MT genes in sea urchin *P. lividus*. Here we report the characterization of five different sea urchin MT genes (PlMT4 through PlMT8) and their regulation pattern during development.

By Southern blot hybridization using MT4 through MT8 cDNA fragments as probes (Ragusa et al, 2013), we determined the number of MT genes in the genome, showing the presence of at least two different PlMT8 genes.

Using primers based on *P. lividus* (v2.0) genome sequences, we amplified by PCR, cloned and sequenced the MT genes.

PlMT gene structures are different from both vertebrates and cephalochordates. The genes are composed of 4 exons separated by 3 introns, the last intron is into the 3'UTR. In PlMT7 gene there are two predicted polyadenylation signals and in fact two species of mRNA transcripts exist.

By RT-qPCR we showed that MT4 to MT6 are not expressed during development, while MT7 mRNA level rises throughout embryonic development and MT8 rises until gastrula stage and decreases thereafter.

Analyzing MT promoters *in silico*, a considerable number of transcription factor binding elements can be identified, comprising putative metal response elements (MRE), antioxidant response elements (ARE), but their copy number and positions are different between constitutive (MT7-8) and induced (MT4/6) genes.

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