To investigate the regulation of *metallothionein* genes *(HsMTs)* of *Hyriopsis schlegelii*, 1,121-bp and 1,270-bp regions of the *HsMT1* and *HsMT2* promoters were cloned and analyzed, respectively. The two promoters shared partially conserved features and possessed distinct characteristics such as the number or position of metal response elements (MREs). Further analysis of the *HsMT1* and *HsMT2* promoters was performed by the reporter assay using the luciferase gene. Both promoters were activated by various metals, and presented different levels of metal ions inducibility in human hepatoblastoma cells. Deletion mutant assays demonstrated that both the longest promoter regions achieved the maximum inducibility, and the metal inducibility was dependent on the presence of the MRE in *HsMT1* and the distal MRE in *HsMT2*. In addition, we cloned a putative metal responsive transcription factor (hereby designated as *HsMTF-like*) and studied its effect on *HsMTs* expression in human hepatoblastoma cells. An *in vivo* assay demonstrated that HsMTF-like activates basal *HsMTs* transcription level, and the MRE in the *HsMTs* promoter mediates this activation process. Moreover, this basal transcription level can be further boosted by zinc treatment. In conclusion, the regulation mechanism for MT activation in *H. schlegelii* should be evolutionarily conserved.

Metal response elements (MREs) play important roles in metallothionein (MT) transcription of *Hyriopsis schlegelii*.