Marine Genomics 4 (2011) 61–68

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

The metallothionein genes of *Mytilus galloprovincialis*: Genomic organization, tissue expression and evolution

Serena Aceto *, Giulia Formisano, Francesca Carella, Gionata De Vico, Luciano Gaudio

Department of Biological Sciences, University of Naples Federico II, 80134 Napoli, Italy

ARTICLE INFO

Article history:
Received 7 December 2010
Received in revised form 3 January 2011
Accepted 3 January 2011
Available online 22 January 2011

Keywords:
Metallothionein genes
Mytilus galloprovincialis
Real-Time PCR
Introns
Molecular evolution

ABSTRACT

Recently, increasing interest has been directed to the study of metallothioneins (MTs), which are small proteins that are able to bind metal ions. The induction of MT synthesis after exposure to metal or other environmental contaminants in a large number of aquatic invertebrates makes these proteins good biomarkers in water monitoring programs. Within bivalves, the species *Mytilus galloprovincialis* and *Mytilus edulis* represent model organisms for these types of studies, as well as for molecular studies regarding the expression and characterization of MT encoding genes.

In the present paper, we focused on the genomic characterization, evolutionary, and tissue-expression analyses of the *MT-10*, *MT-10 Intronless*, and *MT-20* genes in *M. galloprovincialis*. The comparison of the genomic sequences showed the presence of long nucleotide stretches within the introns of the *MT* genes that are conserved between *M. galloprovincialis* and *M. edulis*. These non-coding conserved sequences may contain regulatory motifs. Real-Time RT-PCR experiments revealed that, at the basal conditions, the *MT-10* and *MT-10 Intronless* genes are expressed at levels considerably higher than the *MT-20* gene, mainly in the digestive gland and gill tissue. The strong induction of the *MT-20* gene expression detected in a field-collected sample is associated with the up-regulation of both the *MT-10* and *MT-10 Intronless* genes. Evolutionary analysis revealed signals of localized positive selection that, together with the tissue-expression data, support a possible functional diversification between the MTs encoded by the *MT-10* and *MT-10 Intronless* genes.

1. Introduction

Metallothioneins (MTs) are low molecular weight, cysteine-rich proteins that are able to bind heavy metals. They are involved in cellular responses to different stimuli, such as heavy metal exposure, hormone administration, the presence of oxyradical-generating compounds and hyperthermia (Dondero et al., 2005; Leignel et al., 2005; Gourgou et al., 2010). Based on the distribution of the cysteine-motifs, MTs can be classified into three classes. Class I includes the MTs of mammals, crustaceans and bivalves and presents up to 9 C-X-C motifs; class II includes the MTs of insects, nematodes, sea urchins, and fungi; and class III, characterized by the (E-C)n-G motif, includes the MTs of plants and yeast (Leignel and Laulier, 2006). The cysteine-motifs permit the formation of metal thiolate clusters (α- and β-domains) within the protein, which enables it to bind metal ions (Mackay et al., 1993; Haq et al., 2003).

During the last decade, many researchers focused on the study of the MT family within bivalves, with emphasis on Mytilidae. MTs are considered good biomarkers of environmental pollution, due to their capability to bind and confine metal ions, and Mytilidae species are useful sentinel-organisms in the marine environment because they filter and accumulate particles present in the water (Cajaraville et al., 2000; Amiard et al., 2006). In the mussel species *Mytilus edulis* and *Mytilus galloprovincialis*, molecular studies highlighted the presence of different MT isoforms that are encoded by two multigene families: *MT-10* and *MT-20* (Mackay et al., 1993; Barsyte et al., 1999; Grattarola et al., 2006), showing differential basal and induced expressions (Lemoine et al., 2000; Fasulo et al., 2008). In both species, *MT-10* mRNA is expressed at basal levels, whereas *MT-20* expression is very low under basal conditions. In addition, Cd, Zn, Cu, and Hg ions induce the transcription of *MT-10* genes, while Cd and Hg exposure and the concomitant presence of Cu and H_2O_2 increase the expression of *MT-20* genes (Dondero et al., 2005; Lemoine et al., 2000).

Within *M. edulis*, *MT-10* and *MT-20* genes show a conserved gene structure composed of three exons and two introns with conserved positions (Leignel and Laulier, 2006). This organization is conserved in the *MT-1* and *MT-2* genes of the tropical green mussel *Perna viridis* (Khoo and Patel, 1999) and in the *MT-I* gene of *M. galloprovincialis* (Ceratto et al., 2002), which belongs to the *MT-20* gene family. In addition, in *M. edulis, M. galloprovincialis,* and in the hydrothermal mussel *Bathymodiolus thermophilus*, the presence of a small, intron-free *MT-10* gene was reported, with correct ORF and cysteine-motifs positions (Leignel et al., 2005; Zorita et al., 2007).

* Corresponding author. Department of Biological Sciences, University of Naples Federico II, via Mezzocannone 8, 80134, Napoli, Italy. Tel.: +39 0812535190/015; fax: +39 0812535035.
E-mail address: serena.aceto@unina.it (S. Aceto).

1874-7787/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.margen.2011.01.001

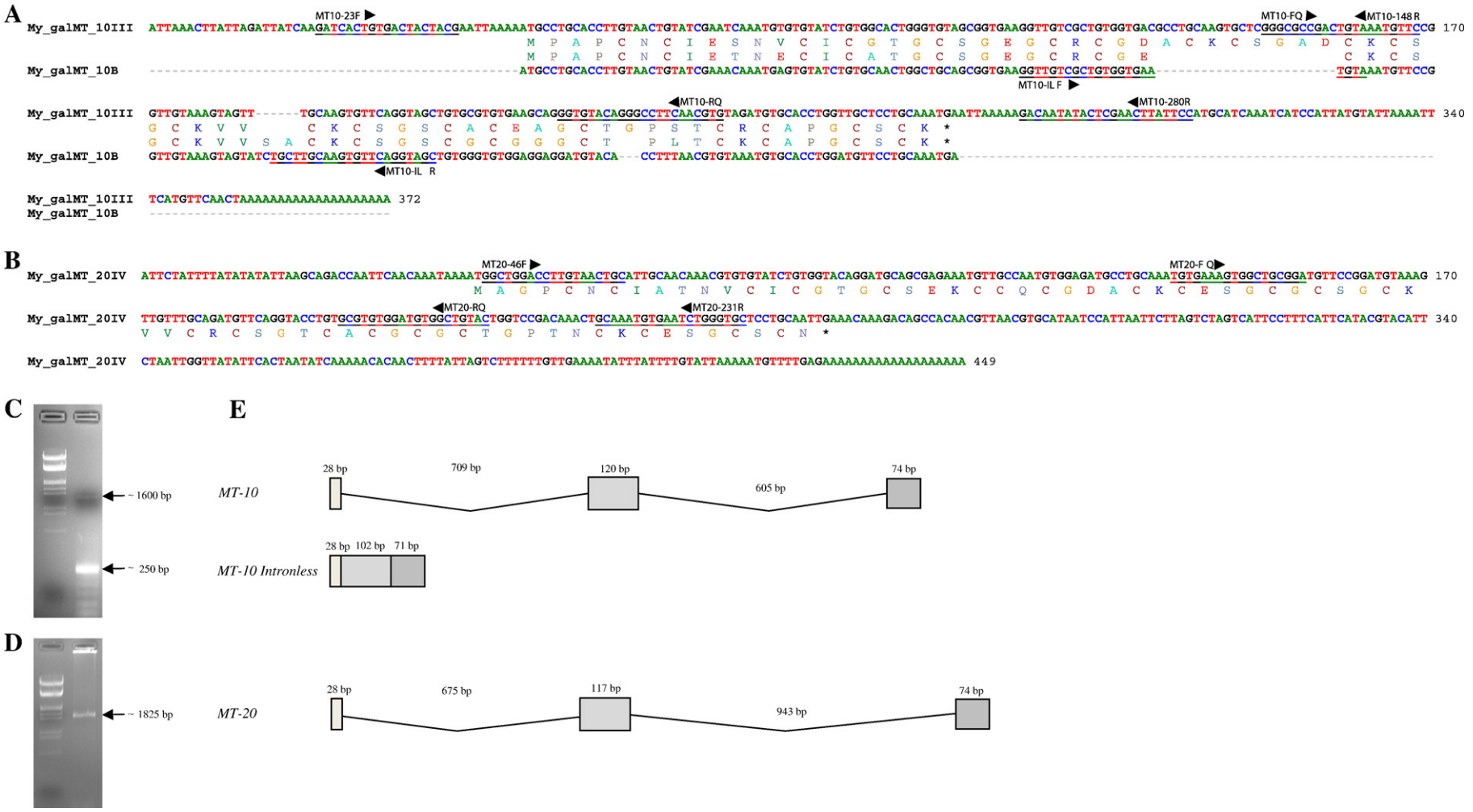

Fig. 1. Genomic organization of the *MT-10*, *MT-10 Intronless*, and *MT-20* genes of *M. galloprovincialis*. (A) Nucleotide alignment of the *MT-10III* and *MT-10B* (*Intronless*) cDNAs of *M. galloprovincialis* present in the GenBank. (B) Nucleotide sequence of the *MT-20IV* cDNA of *M. galloprovincialis* present in the GenBank. The underlined positions correspond to the primer sequence. Primer names are reported and the arrows indicate the primer direction. (C) Agarose gel electrophoresis of the amplification reaction of the genomic DNA of *M. galloprovincialis* using the primer pair MT10-23F/MT10-280R and (D) MT20-46F/MT20-231R. The runs were conducted using the Marker III (Fermentas) as molecular weight marker. (E) Schematic diagram of the genomic structure of the *MT-10*, *MT-10 Intronless*, and *MT-20* genes of *M. galloprovincialis* analyzed in the present work. Boxes represent exons, and lines represent introns. The numbers indicate the length of the corresponding region (in base pairs).