Mytilus galloprovincialis ferritin: gene and cDNA sequence analysis

M. Korlević[1], E. Durmiši[2], B. Treursić[2], A. Baričević[1] and B. Hamer[1]

[1] Center for Marine Research, Ruđer Bošković Institute, Giordano Paliaga 5, 52210 Rovinj, Croatia. Email: marino.korlevic@irb.hr, baricevic@irb.hr, hamer@cim.irb.hr

[2] Juraj Dobrila University of Pula, Zagrebačka 30, 52100 Pula, Croatia. Email: edurmisi@unipu.hr, barbara.treursic@unipu.hr

INTRODUCTION:

Ferritin is a main iron storage protein that has a central role in iron metabolism. Typical ferritin is composed of 24 subunits forming a protein shell with a storage cavity that can accommodate up to 4000 Fe atoms. During iron uptake, ferritin tends to lower Fe^{2+} concentration, thus competing with Fenton reaction and limiting formation of toxic reactive oxygen species (ROS) and vice versa (Arosio *et. al.*, 2009). We sequenced and analysed the ferritin cDNA from the Mediterranean mussel *Mytilus galloprovincialis*.

METHODS:

- RNA extraction
- Reverse transcription of total RNA
- Rapid amplification of cDNA ends (RACE)
- Cloning
- Sequencing
- Bioinformatic analysis

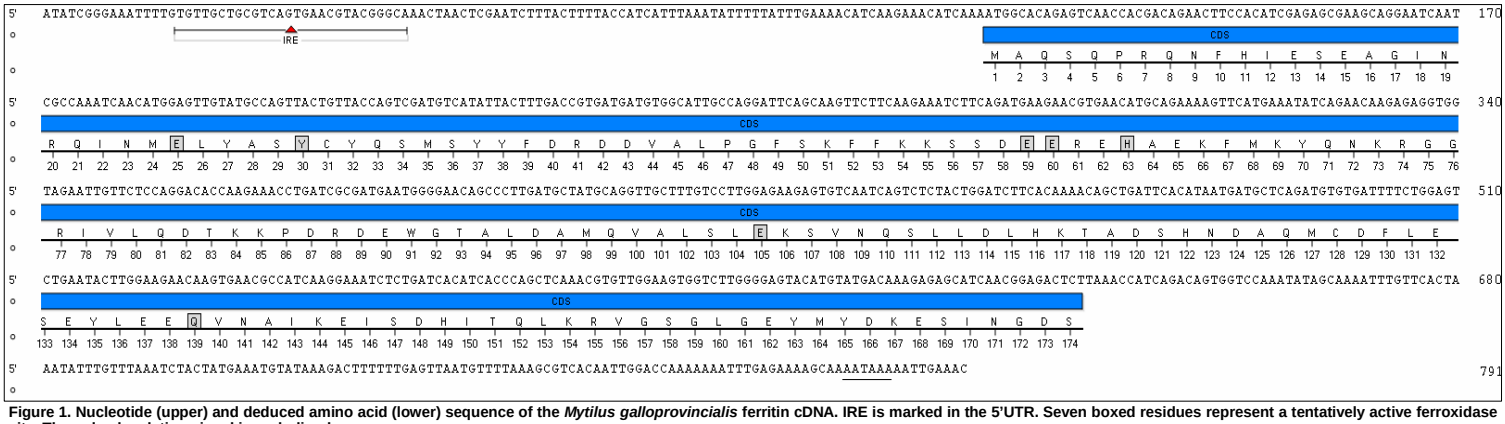

Figure 1. Nucleotide (upper) and deduced amino acid (lower) sequence of the *Mytilus galloprovincialis* ferritin cDNA. IRE is marked in the 5'UTR. Seven boxed residues represent a tentatively active ferroxidase site. The polyadenylation signal is underlined.

RESULTS:

A 791-bp long ferritin cDNA containing a 514-bp open reading frame (ORF), 5' and 3' untranslated regions (UTR) was characterized from *Mytilus gallopriviciallis*. In the 5'UTR, the highly conserved iron response element (IRE) was determined (17-44-bp; Fig. 2). Within the 3'UTR, a polyadenylation signal (AATAAA) is located. The putative ORF encodes a 174 amino acid polypeptide with a theoretical pI/Mw 4.88/20.114 kDa. Seven tentative ferroxidase active site residues were determined (Fig. 1).

The *Mytilus galloprovincialis* ferritin evolutionary relationship is indicating homology with other mollusc ferritins and vertebrate H-ferritin (Fig. 3).

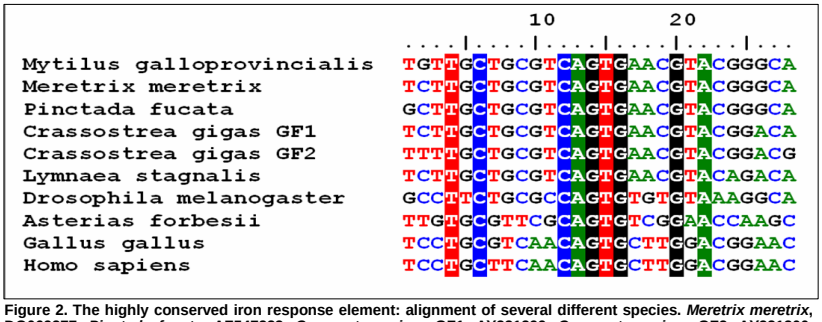

Figure 2. The highly conserved iron response element: alignment of several different species. *Meretrix meretrix*, DQ069277, *Pinctada fucata*, AF547223, *Crassostrea gigas* GF1, AY321299, *Crassostrea gigas* GF2, AY321300, *Lymnaea stagnalis*, X56778, *Drosophila melanogaster*, U91524, *Asterias forbesii*, AF001984, *Gallus gallus*, NM_205086, *Homo sapiens*, NM_002032. The homologous residues are shaded (Wang *et al.*, 2009).

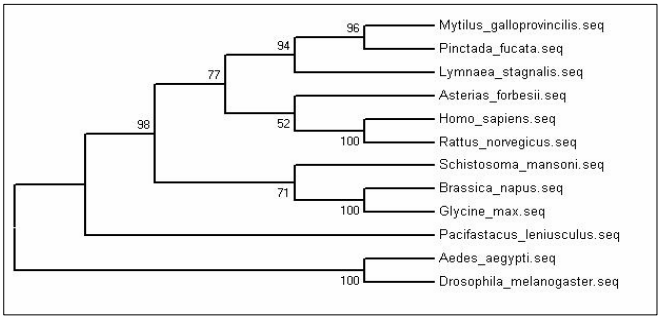

Figure 3. The phylogenetic tree (Neighbor-joining analysis) of ferritin amino acid sequences from different organisms: *Pinctada fucata*, AF547223, *Lymnae stagnalis*, CAA40096, *Asterias forbesii*, AAB60883, *Homo sapiens*, AAA52437, *Rattus norvegicus*, AAB39890, *Schistosoma mansoni*, AAA29881, *Brassica napus*, AAB53099, *Glycine max*, AAC18928, *Pacifastacus leniusculus*, CAA62186, *Aedes aegypti*, AAA99996, *Drosophila melanogaster*, AAB70121. Bootstrap values, given as a percentage of 1000 replicates, are noted on the tree. Evolutionary analyses were conducted in MEGA5 (Zhang *et al.*, 2003).

DISCUSSION AND CONCLUSION:

Ferritin expression is regulated by the interaction of iron response protein (IRP) and iron response element (IRE) located in the 5'UTR of the ferritin mRNA. During high iron level IRP is inhibited, thus allowing the translation of ferritin mRNA. The presence of the highly conserved IRE (Fig. 2.) in the sequenced cDNA is suggesting the same regulatory mechanism in *Mytilus galloprovincialis* (Wang *et al*., 2009). Ferritin is acting as an antioxidative protein by inhibiting the formation of reactive oxygen species through the Fenton reaction. The presence of the seven highly conserved residues (Fig. 1) in the sequenced cDNA is showing that the mussel ferritin has a ferroxidase capacity. Ferroxidation is the main step in iron storage but also in the inhibition of reactive oxygen species (ROS) formation (Orino *et* al., 2001).

Ferritin as an iron storage and antioxidat protein has the potential to be a possible biomarker of oxidative stress.

REFERENCES:

1.Arosio, P., Ingrassia, R., Vacadini, P., 2009. Ferritins: A family of molecules for iron storage, antioxidation and more. Biochimica et Biophysica Acta 1790, 589-599.

2.Orino, K., Lehman, L., Tsuji, Y., Ayaki, H., Torti, S.V., Torti, F.M., 2001. Ferritin and the response to oxidative stress. Biochemical Journal 357, 241-247.

3.Wang, X., Liu, B., Xiang, J., 2009., Cloning, characterization and expression of ferritin subunit from clam *Meretrix meretrix* in different larval stages. Comparative Biochemistry and Physiology Part B 154, 12-16.

4.Zhang, Y., Meng, Q., Jiang, T., Wang, H., Xie, L., Zhang, R., 2003. A novel ferritin subunit involved in shell formation from the pearl oyster (*Pinctada fucata*). Comparative Biochemistry and Physiology Part B 135, 43-54.