Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXV

Transcriptional contribution of transposable elements in relation to salinity conditions in teleosts: are TE silencing mechanisms involved?

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The evolutionary success of organisms is strictly linked to the genome composition and in particular **transposable elements** (TEs) represent one of the most intriguing components. Fish are an interesting taxon comprising species adapted to a wide range of environments. The aim of this second year of PhD is to analyze the transcriptional contribution of TEs in the gill transcriptomes of three fish species exposed at different salinity conditions. Therefore, three species were considered: the giant marbled eel ***Anguilla marmorata*** and the chum salmon ***Oncorhynchus keta***, that are both diadromous, and thus they have to face changes in salinity in a defined stage of their life cycle (during reproduction) and the marine medaka ***Oryzias melastigma***, that is an euryhaline organism sensu stricto. The analyses revealed an interesting activity of TEs in the case of juvenile eels, commonly adapted to salty water, when exposed to brackish and freshwater conditions. Moreover, the evaluation of the expression of genes involved in TE silencing mechanisms (six in heterochromatin formation, 14 known to be part of the NuRD complex, and four of the Argonaute subfamily) unveiled that they are active. Combining gene expression with molecular modelling analyses the three-dimensional (3D) assembly of the **TRIM33/KRAB-like complex** was predicted obtaining specific structural insights. Intriguingly, our results evidenced for the first time a *KRAB-like* domain specific of actinopterygians that together with TRIM33 might allow the functioning of NuRD complex. Therefore, this complex, so far known to be mainly active in tetrapod TE silencing, acts also in fish species and might be responsible for the observed TE transcriptional variation.

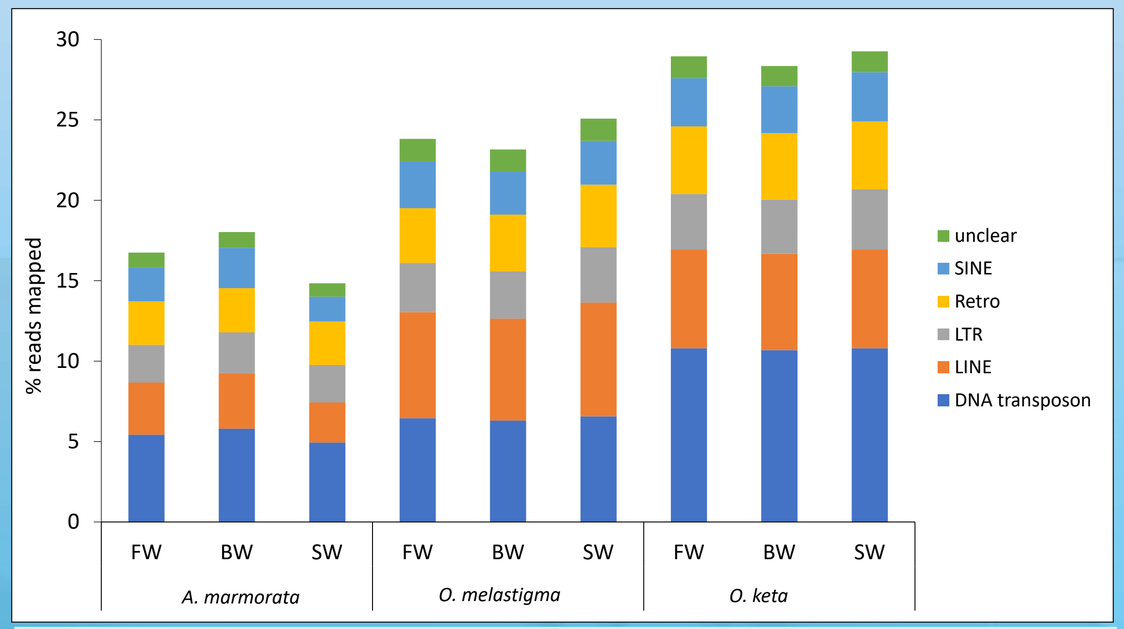

Figure 1. Transcriptional contribution of TE in *A. marmorata*, *O. melastigma*, and *O. keta* gill transcriptomes. FW: freshwater; BW: brackish water; SW: salt water. An appreciable difference in the percentage of TE mapped reads emerged comparing the SW with FW and BW conditions of eels. Since specimens used for this experiment were at juvenile stage and thus adapted to live in seawater, the exposure at low salinity levels might have determined the increase in TE transcription observed. The major impact in these changes was due to the expression of SINE. This finding is also in line with our previous results highlighting a role of this kind of TEs in the catadromous behavior of eels.

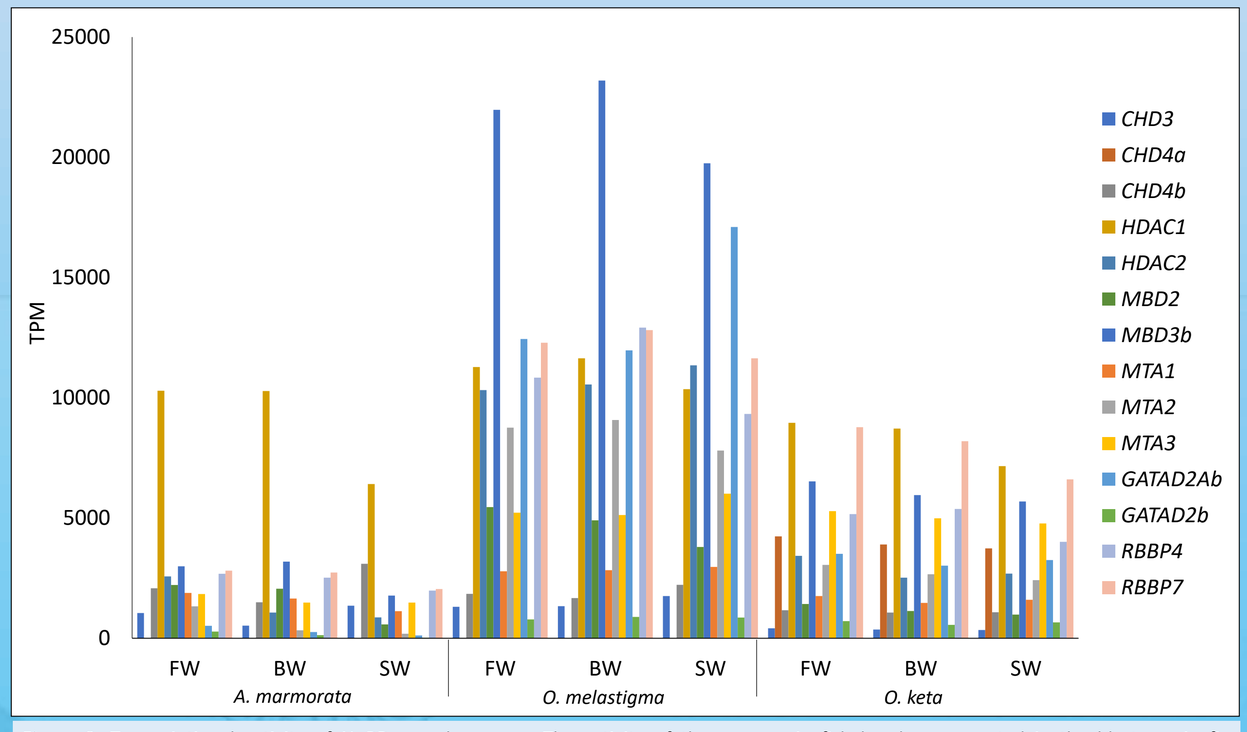

Figure 2. Transcriptional activity of NuRD complex genes. The activity of these genes in fish has been reported in the blastema in fin regeneration and these data agree with recent works showing the expression of NuRD complex genes also in adult tissues. The expression of genes investigated showed a major variability in the giant marbled eel than marine medaka and chum salmon. This trend reflected that obtained for TE transcription suggesting a possible relationship between NuRD complex and TEs also in fish.

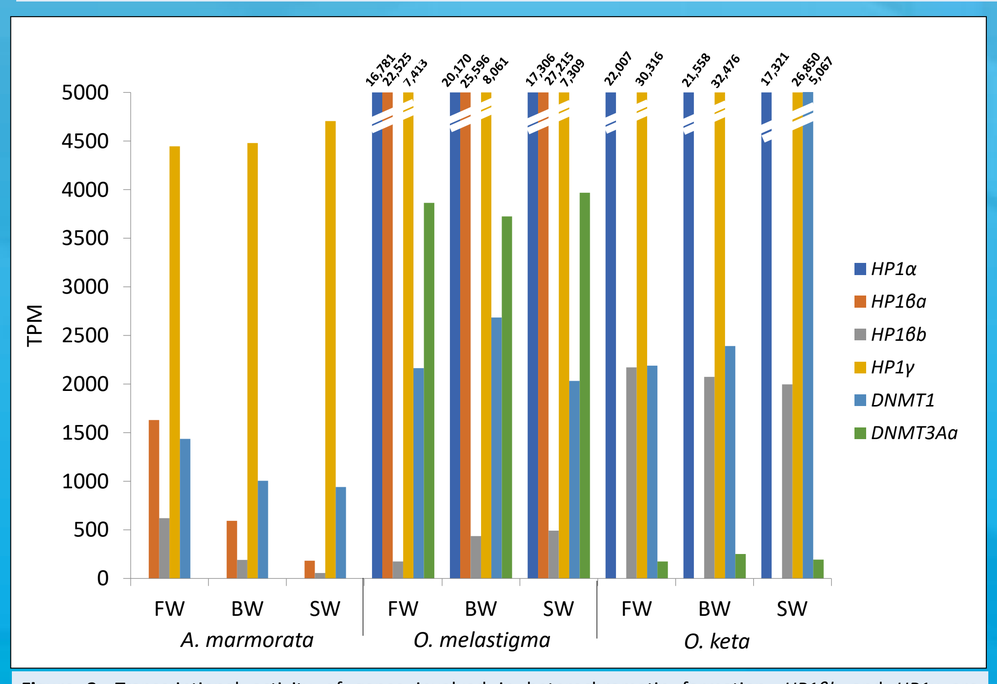

Figure 3. Transcriptional activity of genes involved in heterochromatin formation. *HP1βb* and *HP1γ* are transcriptionally active in all species and tested conditions. Due to the high number of mobile elements present in the genome, the high expression levels of *HP1γ* in chum salmon might explain the low difference detected in total TE contribution levels, compared to the other two species. Between the two genes selected for *DNMTs*, the expression analysis suggested that DNMT1 might be the candidate protein in TE methylation.

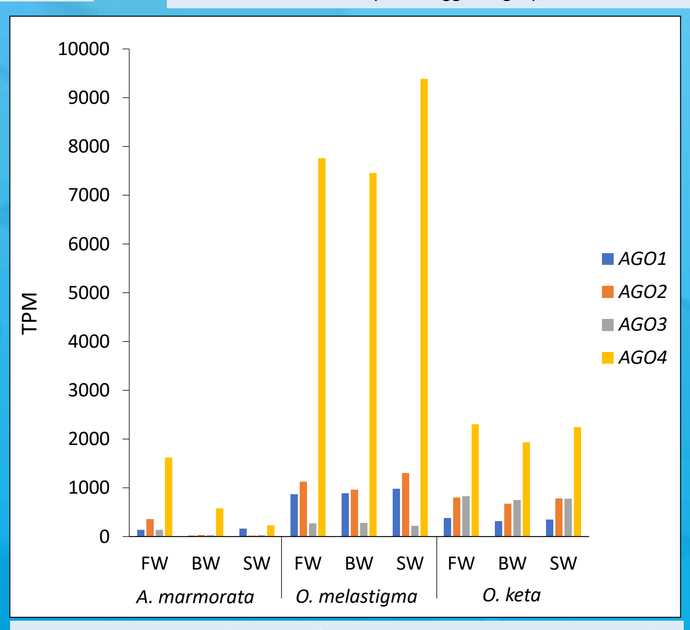

Figure 4. Transcriptional activity of *Argonaute* genes. In *A. marmorata*, the expression of these genes was variable and *AGO4* showed a decreasing level from FW to SW. This gene and *AGO2* showed an expression trend similar to that of TEs. AGO4 has been shown to play a role in transposon silencing in gonads and our results suggested that this function might also be conserved in somatic tissues. A slicing activity for AGO2 has been reported and the endosiRNAs derived seem to be involved in the transposon repression.

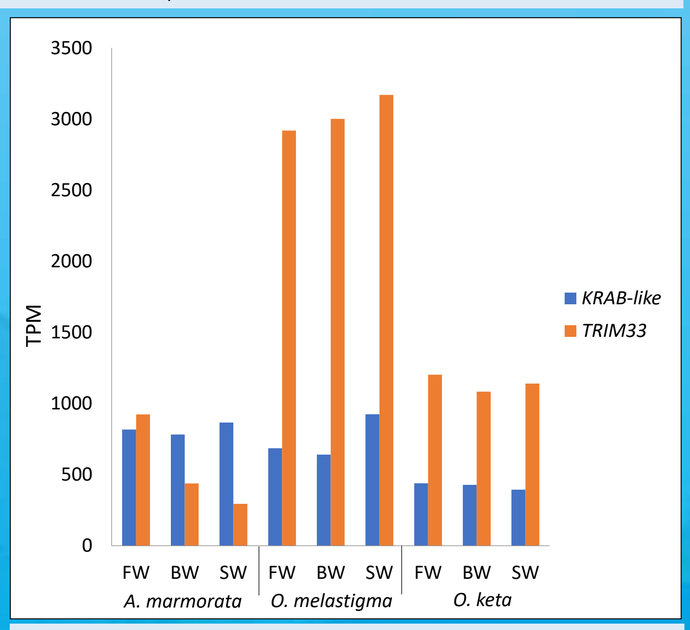

Figure 5. Transcriptional activity of *KRAB*-like and *TRIM33* genes. The transcriptional expression levels of *KRAB-like* were uniform between the three tested conditions in the two diadromous species, differently from *O. melastigma* that showed a higher activity in SW condition. For *TRIM33* the same trend reported for NuRD complex related genes was highlighted.

In tetrapods, the NuRD complex is recruited at TE sequence through the involvement of KRAB-ZFPs and TRIM28. In fish lineage, these components have not been identified. So, we investigated the possibility that TRIM33 and our KRAB-like could play the same function of TRIM28 and KRAB-ZFPs of tetrapods. Indeed, TRIM33 is a protein belonging to the Tripartite Motif family, it shows the same domain architecture of TRIM28 and is spread among ray-finned fish. Moreover, we identified a similar sequence to KRAB domain and ZF motifs named KRAB-like showing these features and widely spread in ray-finned fish.

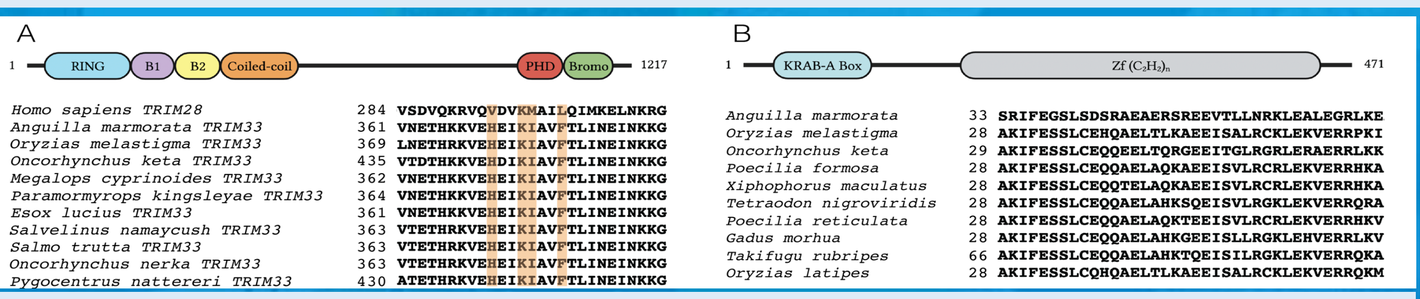

Figure 6. Multiple sequence alignment and 3D structures of the KRAB domain contained in the KRAB-like protein and that of TRIM33 protein. (A) In the upper side, a schematic representation of the domain architecture is reported for the *A. marmorata* TRIM33 protein. In the lower side, multiple sequence alignment related to the coiled-coil region of *H. sapiens* TRIM28 protein and of ten actinopterygian TRIM33 sequences is showed. The main interacting residues at the interface are highlighted in orange. (B) In the upper side, a schematic representation of the domain architecture is reported for the *A. marmorata* KRAB-like protein. In the lower side, the N-terminal region multiple sequence alignment of ten actinopterygian KRAB-like sequences is reported. The KRAB-A Box is showed in light blue.
The KRAB-like sequence here reported showed a KRAB-like domain at the N terminus and several Zinc Finger motifs at the C terminus. This protein domain architecture is similar to that of tetrapod KRAB-ZFPs.

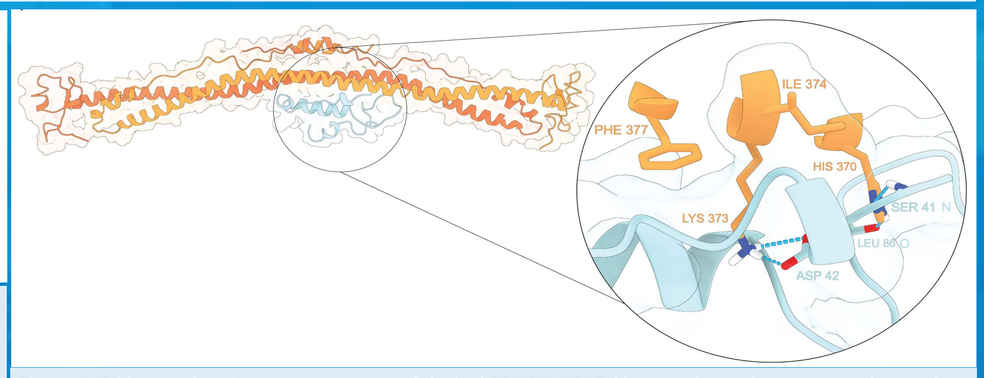

Figure 7. Ribbon and surface representation of docked TRIM33-KRAB-like complex with a zoom on the residues H370/K373/I374/F377. Chain A and chain B of TRIM33 are colored in dark and light orange, respectively.
The docking analysis supported the ability of TRIM33 and KRAB-like proteins to assemble in a complex in actinopterygians, comparable to that of human TRIM28/KRAB-ZFP93.

2° year of PhD 2020/2021:

Pubblications: (1) Carotti, E., Carducci, F., Canapa, A., Barucca, M., Greco, S., Gerdol, M., Biscotti, M. A. Transposable Elements and Teleost Migratory Behaviour. *Int J Mol Sci*. **2021,** 22, 602. (2) Carducci, F.; Carotti, E.; Gerdol, M.; Greco, S.; Canapa, A.; Barucca, M.; Biscotti, M.A. Investigation of the activity of transposable elements and genes involved in their silencing in the newt Cynops orientalis, a species with a giant genome. *Sci. Rep*. **2021**, 11, 14743. (3) Carotti, E., Carducci, F., Greco, S., Gerdol, M., Di Marino, D., Perta, N., La Teana, A., Canapa, A., Barucca, M., Biscotti, M. A. Transcriptional contribution of transposable elements in relation to salinity conditions in teleosts and silencing mechanisms involved. *Int. J. Mol. Sci.* **2022**, 23, 5215.

Poster presentations: (1) Carotti, E., Carducci, F., Canapa, A., Barucca, M., Greco, S., Gerdol, M., & Biscotti, M. A. TRANSPOSABLE ELEMENTS AND GENES INVOLVED IN THEIR SILENCING MECHANISMS IN DIADROMOUS FISH SPECIES. *66th Congress of the GEI-Italian Society of Development and Cell Biology (GEI-SIBSC),* 22 June **2021** (*Virtual*); (2) Carotti, E., Carducci, F., Canapa, A., Barucca, M., Greco, S., Gerdol, M., & Biscotti, M. A. The sound of silencing: NuRD complex and transposable elements activity in diadromous fish species. *The Mobile Genome: Genetic and Physiological Impacts of Transposable Element.* 29 Aug-1 Sep **2021** (*Virtual Conference*).