Studying evolution at the protein level:
Combining biochemistry and systems biology approaches to unravel the mechanisms underlying the evolution of the transcription factor Brachyury

Director of studies: Giovanna Benvenuto

Department Research Infrastructures for Marine Biological Resources

Many processes in cell biology, development and tissue homeostasis depend on temporal and spatial regulation, in which key molecules are subjected to differential expression and/or turnover control. In the last decades, advances in systems biology and genomics have significantly increased our understanding of how gene regulatory networks (GRN) operate to control cell type specification, tissue identity and developmental processes. However, while these studies were very successful in describing logics of such processes and sometimes also provide explanation of evolutionary changes, these analyses often take into account transcriptional regulation only (e.g. by measuring only RNA level of expression and localization), somehow neglecting the fact that transcription factors (TF), which are responsible for transcription regulation, are proteins and as such are subjected to differential and/or turnover control which might be different from the ones of their corresponding RNAs. Similarly, the role played in evolution by changes at the protein level (changes in trans, as opposed to the changes on cis-elements), are often neglected.

Brachyury is the founding member of the T-box transcription factor family and one of the genes that have been extensively investigated functionally in diverse animal species. Brachyury was first identified genetically in mouse and initial studies focused on its role in the mesoderm formation and notochord differentiation in various chordates. In sea urchins, like in most invertebrates, brachyury is expressed in the endoderm. *brachyury* gene expression patterns for *Strongylocentrotus purpuratus* are entirely consistent with data from other echinoderm species. Recent studies in the Arnone's lab at SZN showed a very peculiar situation concerning the endodermal expression of the protein Brachyury in *S. purpuratus* during gastrulation. In fact, while the gene is expressed only transiently at the blastopore cells before they ingress through convergent extension in the blastocoel to form the primitive gut, the protein Brachyury appear to be retained by all the endodermal cells that had experienced significant steady state levels of *brachyury* mRNA before ingression. In other words, throughout gastrulation, the protein Brachyury is present in the nuclei of gut cells, while the mRNA is not. This discrepancy between mRNA and protein expression appears to be a novelty of the *S. purpuratus*, as a complete overlap between protein and mRNA exists in the sea urchin *Lytechinus variegatus*, and in the two Mediterranean species so far tested (*Arbacia lixula* and *Paracentrotus lividus*). The mechanisms by which this novelty was achieved, as well as the consequences in terms of transcriptional regulation and downstream target expression are completely unknown.

This project aims at elucidating (1) the mechanism by which Brachyury protein is retained by gut cells during gastrulation in *S. purpuratus* while it is not in *P. lividus* (by measuring protein turnover in vivo using a tandem fluorescent protein timer approach and testing wild type and mutated forms of this protein in both species) and (2) the different role of the Brachyury transcription factor in the developing gut of these two sea urchin species (by a systems level analysis of the downstream targets of Brachyury in the two species using both RNA-Seq and the recently developed Assay for Transposase Accessible Chromatin with high-throughput sequencing, ATAC-Seq).