Evolution of a Gene Regulatory Network controlling gut patterning:
Comparing upstream control and chromatin 3-D organization around *xlox/pdx1* and *cdx* genes in sea urchin, sea star and amphioxus

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In the recent decade, several systems level studies demonstrated the power of developmental Gene Regulatory Networks (GRN) in describing the causal progression of regulatory states (generated by a unique combination of transcription factors, TF) in embryonic space and time, necessary to specify different cell types and ultimately body plans, by providing testable predictions that are not resolvable with simplistic views of gene regulation. They also highlight the potential of comparative GRN approaches to study evolution of specification processes and body plans, providing an explanation of how changes in genome sequence can cause changes in developmental processes. Echinoderm embryos provide the rich source of morphological variation necessary to address relevant evolutionary developmental (evo-devo) questions such as the evolution of novelties. This potential has been already well illustrated by the comparison of the GRNs controlling endomesoderm specification in sea urchins and sea stars and, together with the up-to-date technological advances in genomics and transcriptomics, has encouraged in the very recent years the molecular exploration of additional echinoderm species.

The embryonic gut of bilaterians forms through the invagination or involution of endodermal cells, which results in the formation of a tripartite structure composed of esophagus, stomach and intestine. Previous studies in the Arnone's lab have highlighted conservation of TFs and signaling molecule expression domains along the developing gut of echinoderms and vertebrates. Among TFs, two ParaHox genes, *xlox* (*pdx-1* in vertebrates) and *cdx*, play a central role in the GRN controlling posterior gut patterning in both sea urchins and vertebrates. Moreover, the conservation of the relative expression of these genes along the antero-posterior axis of the gut in most bilaterians supports an ancestral role in gut patterning. Interestingly, the exploration of the gut GRN in two echinoderm classes, sea urchins and sea stars, highlighted differences and similarities with respect to vertebrates. More recent studies corroborate the conclusion that, despite the extreme conservation of topology of expression of these two genes within the posterior gut of these animals, the GRNs around them appear to be subject of considerable rewiring even if they contain the same groups of orthologous genes. An obvious, and general question that arises is which actually are the constrains that maintain such a strong association between these genes and the domain of their expression even when the GRNs that connect them get extensively rewired.

To address these questions, this project aims at analyzing the gene regulatory network linkages operating upstream of *xlox* and *cdx* genes in both sea urchins (*Strongylocentrotus purpuratus*) and sea stars (*Patiria miniata*), using amphioxus (*Branchiostoma lanceolatum*) for comparison with chordates. Main objectives of this project will be (1) the identification of cis-regulatory elements and open chromatin epigenomic profiling of the enhancers upstream of *xlox* and *cdx*, in sea urchins, sea stars and amphioxus (by in silico analysis and transposase- accessible chromatin sequencing, ATAC-seq); (2) the reconstruction of the GRNs upstream of these genes (by species-specific or inter-species cis-regulatory and knock-down transgenic analyses).