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EVOLUTION OF A GENE REGULATORY NETWORK CONTROLLING GUT PATTERNING: COMPARING UPSTREAM CONTROL AND CHROMATIN ORGANIZATION AROUND XLOX/PDX1 AND CDX GENES IN SEA URCHIN, SEA STAR AND AMPHIOXUS

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ABSTRACT

ParaHox genes *Lox* and *Cdx* have a conserved role in embryonic development of many metazoan taxa. The two genes control the development of the gut and this control is done in time and location specific manner. The clustering of these genes on the chromosome is important for their temporal and spatial expression patterns. In the species with an intact cluster, the genes and their expression show temporal and spatial collinearity, while in the species where such cluster is broken, temporal linearity is altered. This raises questions as to the importance of genomic organization of these genes in the nucleus. This thesis employs four deuterostome species: *Strongylocentrotus purpuratus, Paracentrotus lividus, Patiria miniata* and *Branchiostoma lanceolatum*. In *S. purpuratus* and *P. lividus,* which are closely related sea urchin species, the clustering of ParaHox genes is absent. On the contrary, the sea star *P. miniata* and amphioxus *B. lanceolatum* genes *Lox* and *Cdx* are in a cluster along with *Gsx,* the third ParaHox gene, highlighting the importance of clustering for control of ParaHox genes. This thesis attempts to untangle the various factors controlling expression of the ParaHox genes and place them in the evolutionary context. The newly assembled genome for *S. purpuratus* has allowed to confirm that the cluster is, indeed, broken up in this species. HiC interaction data showed that the loci occupied by ParaHox genes in the sea urchin are not spatially close to each other even in the three dimensional organization of chromatin, unlike in the sea star or amphioxus that both have a tight cluster of ParaHox genes. In addition, chromatin accessibility assays, such as ATAC-seq, allowed to assess the open DNA regions and gain insight into their role in the expression of *Lox* and *Cdx,* which control development

of the sea urchin embryonic gut, suggesting that some of these open regions function as cis-regulatory modules (CRMs). Differential ATAC-seq and RNA-seq data at 48 and 66 hours post fertilization (hpf) for *S. purpuratus* revealed, which of the predicted cis-regulatory regions control gut development in the sea urchin, while single cell RNA-seq datasets for *S. purpuratus* 72 hpf pluteus allowed to filter out predicted transcription factors (TFs) and draft a gene regulatory network (GRN) for the three regions of the developing sea urchin hindgut at this time point. *In vivo* transgenic experiments, using reporter constructs, resulted in validating some of the predicted *S. purpuratus* CRMs and TFs, in particular showing a positive effect of SpHox11/13b on a *SpLox* CRM that overlaps *SpLox* transcription start site. This work also allowed to gain insight into the evolution of ParaHox gene control in closely related sea urchin species, through sequence comparisons and use of *S. purpuratus Lox* gene cis-regulatory modules in reporter constructs in *P. lividus*. The results of this analysis suggested CRM conservation and presence of the same transcription factor repertoire in homologous parts of the embryo in both species. Transcriptomic datasets, obtained for *S. purpuratus* embryos and tissues, highlighted the need for similar datasets from other species, in order to confidently untangle evolution of ParaHox gene control. Generated datasets allow for assessment of importance of chromatin organization for collinearity and set the foundation for future studies pertaining to evolution of the gut gene regulatory network in deuterostomes.