## *cis*-Regulation and chromatin dynamics of the *hbox12* gene during the embryogenesis of *Paracentrotus lividus*.

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The GRN specifying the dorsal-ventral (D-V) axis of the sea urchin embryo is currently under investigation. An early input for D-V polarity is given by a redox gradient probably generated by an asymmetrical distribution of maternal mitochondria (1). Only on the future ventral side, the oxidizing environment induces the expression of the *nodal* gene, an essential regulator of D-V polarization (2). By contrast, on the future dorsal side, a reducing environment activates the hypoxia inducible factor (HIF-1α) (3). The *hbox12* transcription repressor is an early marker of the dorsal side of the embryo, in which it negatively regulates the expression of *nodal* (4, 5). Interestingly, by *in silico* analysis we identified an evolutionarily conserved HIF-1α binding element (HRE). Gene transfer assays also suggested that HIF1α stimulates *hbox12* expression. To map the physical interaction of HIF1α with HRE, a region of the *hbox12* promoter containing the HRE was cloned in a *Gaussia princeps* luciferase reporter system and the resulting construct was trasfected in HEK293 cells. Moreover, we cultured *P. lividus* embryos with two different HDAC inhibitors, VPA and TSA, and observed perturbation of the spatial-temporal expression profile of *hbox12*. Finally, by chromatin immunoprecipitation assays we determined an increase of the acetylation of the lysine 9 of the histone H3 and the failure of the HDAC-1 enzyme on the *hbox12* chromatin.

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## Toll-Like 3 Receptor In The Immune Response of *Paracentrotus lividus Sea Urchin*

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Sea urchin innate immune response involves important proteins such as membrane receptors which trigger different intracellular signaling pathways. The discovery of the Toll-like receptors (TLRs) in immune cells of invertebrates such as the sea urchin, has renewed the interest in innate immunity and its potentiality in invertebrate models. The sea urchin coelomocytes are considered a good model to study the immune response (1, 2, 3, 4). In the sea urchin *Strongylocentrotus purpuratus*, these receptors are encoded by a large multigenic family which comprises 253 genes (5). Here we report the isolation, the protein analysis and the phylogeny of a partial cDNA encoding for Pl-tlr3 belonging to the family of Toll-like receptor from the coelomocytes of sea urchin *Paracentrotus lividus*, and its expression in response to LPS and poly-IC treatments. The analysis of the protein has allowed to understand that this receptor, localized into the endosome, is small in size and contains a small number of Leucine Rich Repeats. It is not involved in the immune response bacterial, given that its transcription, analyzed by QPCR, is not affected by the injection of LPS. On the contrary, poly-IC treatment, a chemical compound that mimes dsRNA, causes Pl-tlr3 overexpression. The phylogeny of the partial protein Pl-tlr3, compared with Tlr3 proteins from different classes of animals, give a vision of the evolutionary path that, most likely, this receptor has made in the course of millions of years, starting from simple organisms up to man. The study of the functions of Tlr3