P22 - EFFECT OF Γ-AMINOBUTYRRIC ACID (GABA) EXPOSURE ON EMBRYOGENESIS OF *PARACENTROTUS LIVIDUS* AND IDENTIFICATION OF GABA-RECEPTOR GENES IN SEA URCHINS

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Developmental processes are controlled by regulatory genes encoding for transcription factors and signaling molecules. Functional relationships between these genes are described by gene regulatory networks (GRN), models which allow integration of various levels of information [1]. The sea urchin embryo is an experimental model system which offers many advantages for the analysis of GRN [2]. Recently, the GRN that governs the biomineralization of the sea urchin embryonic skeleton has begun to be deciphered [3-5]. Preliminary evidence suggest that the γ-aminobutyric acid (GABA) signaling pathway is involved in skeletal morphogenesis during development of the sea urchin. GABA is a molecule synthesized by nearly all organism, from bacteria to humans, and it acts through ionotropic and metabotropic receptors ($GABA_A$-Rs and $GABA_B$-Rs, respectively) [6].

We report that *Paracentrotus lividus* embryos exposed to GABA at concentrations ranging from 0.01 to 1.0 mM showed aberrations in axial patterning, with a dose dependent effect. In particular, at 24 hours post-fertilization (hpf) control embryos displayed two bilateral clusters of Primary Mesenchyme Cells (PMCs, Fig. 1Aa), which hold biomineralizing activity. By contrast, treated embryos contained a population of PMCs that was quite homogeneously distributed within the blastocoele (Fig. 1Ab). Moreover, at 48 hpf, when control embryos were normal angular-shaped plutei with the characteristic bilateral symmetry (Fig. 1Ac), GABA-treated embryos appeared spherical and contained supernumerary spicules (Fig. 1Ad).

Washout experiments allowed to determine that the period of sensitivity is restricted from the blastula to the gastrula stage.

In order to identify GABA-R genes we performed a comprehensive *in silico* analysis in selected sea urchin species (*P. lividus, Strongylocentrotus purpuratus,* and *Lytechinus variegatus),* and in phylogenetically related organisms, such as the hemichordate *Saccoglossus kowalevskii*, the chordate *Ciona intestinalis*, and the nematode *Caenorhabditis elegans*.

By combining iteration of *ab initio* predictions and pairwise comparative methods, we identified the orthologous genes encoding for $GABA_{B1}$ and $GABA_{B2}$, the two subunits which assemble $GABA_B$-R, and we confirmed that all of these organisms possess a unique α/β $GABA_A$-R gene pair clustered in the genome. Furthermore, we have observed that the reciprocal disposition of $GABA_A$-R genes is also evolutionarily conserved (Fig 1B).

Interestingly, in adjacent position to these genes, we have identified an additional gene, which shows significant sequence similarity to a invertebrate-specific $GABA_A$-R gene. Indeed, such a gene has been only identified in *C. elegans*, *Drosophila melanogaster*, and *Nematostella vectensis* [7-8].

We also retrieved several cDNA sequences from staged EST databases of the three sea urchin species inspected, indicating that these genes are actively transcribed during development. Some selected cDNA plasmids were also isolated from *P. lividus* total RNA samples and fully sequenced.

Hypothetical proteins were deduced and used for phylogenetic analysis, including a selection of vertebrate and invertebrate $GABA_A$-R subunit sequences. The resulting phylogenetic tree (Fig. 1C) strongly support the hypothesis that the sea urchins contain genes encoding for both canonical and invertebrate-specific $GABA_A$-R subunits. Such a collection of data should provide

a support to better understand the involvement of GABA-signalling pathway in the skeletal GRN.

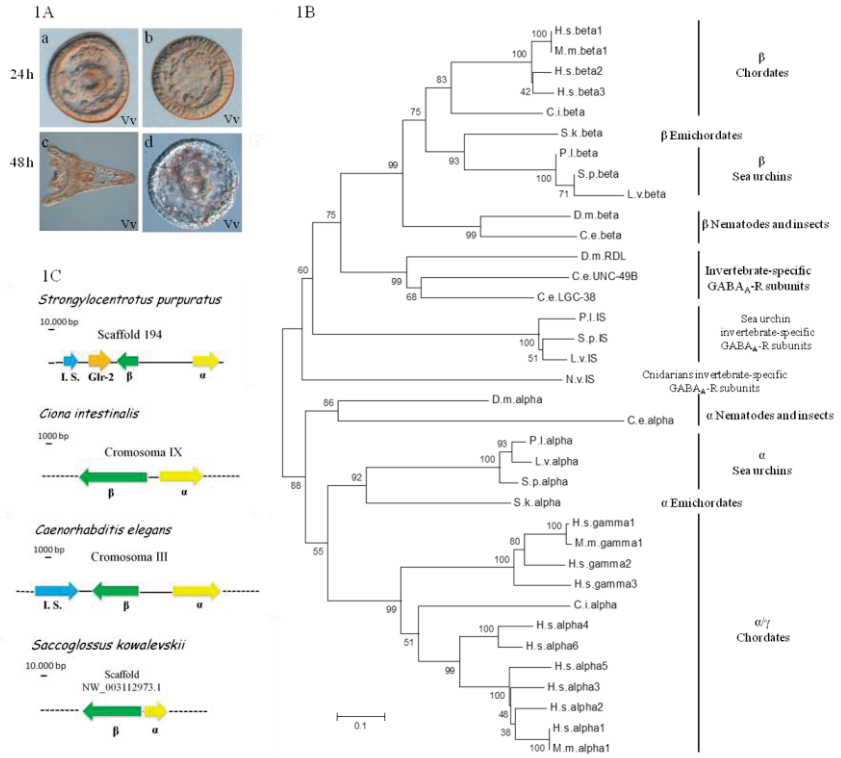

Figure 1. (A), Effect of GABA on embryogenesis of *P. lividus*. (a, c), control and (b, d) GABA-treated embryos observed at 24- (a, b) and 48- (c, d) hours post fertilization. Vv: vegetal view.
(B), Genomic configuration of $GABA_A$-R locus in different species of invertebrates. Glr-2: Glutammate receptor-2; I.S.: Invertebrate-specific $GABA_A$-R subunit gene.
(C), Neighbor-joining tree constructed with protein sequences of representative $GABA_A$-R subunits. Number above nodes indicate bootstrap values (1000 replicates). M.m., *Mus musculus*; H. s., *Homo sapiens*; P.l., *Paracentrotus lividus*; S. p., *Strongylocentrotus purpuratus*; L.v., *Lytechinus variegatus*; C.i., *Ciona intestinalis*; S. k, *Saccoglossus kowalevskii*; D.m, *Drosophila melanogaster*; C.e., *Caenorhabditis elegans;* N. v., *Nematostella vectensis*.

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