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

S. Casamirra, G. Spinelli, and V. Cavalieri*

Dept. of Biological, Chemical and Pharmacological Science and Technology (STEBICEF), University of Palermo, viale delle Scienze Edificio 16, 90128 Palermo, Italy
*Corresponding author: vincenzo.cavalieri@unipa.it

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 $\gamma$-aminobutyric acid (GABA) signaling pathway is involved in skeletal morphogenesis during development of the sea urchin. GABA is a molecule synthesized by nearly all organisms, 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 variegiatus), 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 GABAB-R, and we confirmed that all of these organisms possess a unique $\alpha/\beta$ 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: Glutamate receptor-2; I.S.: Invertebrate-specific GABA_A-R subunit gene.


REFERENCES