Volume 88(1) - 2015

Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale

86th SIBS National Congress

Palermo, Italy, 24-25 October 2013

Botanical Garden, Lanza Hall

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Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale

eISSN 2284-0230

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PAGEPress Publications
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27100 Pavia, Italy
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Effect of γ-aminobutyrric acid exposure on embryogenesis of *Paracentrotus lividus* and identification of γ-aminobutyrric acid-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; Figure 1Aa), which hold biomineralizing activity. By contrast, treated embryos contained a population of PMCs that was quite homogeneously distributed within the blastocoele (Figure 1Ab). Moreover, at 48 hpf, when control embryos were normal angular-shaped plutei with the characteristic bilateral symmetry (Figure 1Ac), GABA-treated embryos appeared spherical and contained supernumerary spicules (Figure 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 (Figure 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

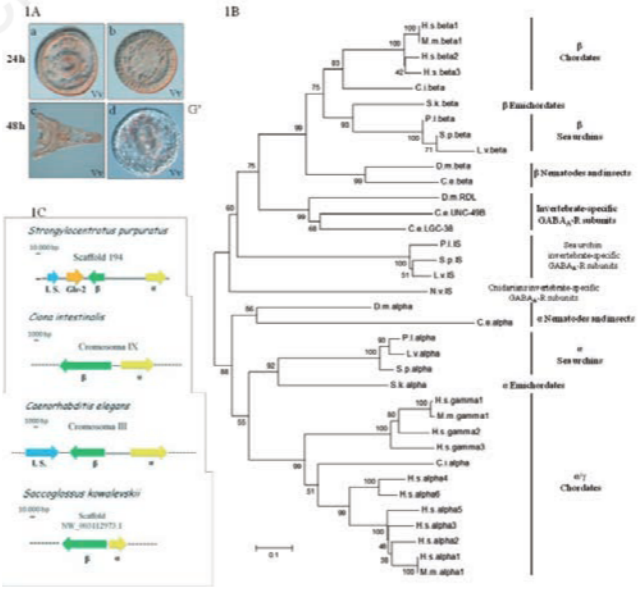

Figure 1. A) Effect of GABA on embryogenesis of *P. lividus*. (a, c), control and (b, d) GABA-treated embryos observed at 24- (a, b) and 48- (c, d) hours post fertilization. Vv: vegetal view. B), Genomic configuration of $GABA_A$-R locus in different species of invertebrates. Glr-2: Glutammate receptor-2; 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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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 GABAA-R subunit sequences. The resulting phylogenetic tree (Figure 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.

References

1. Levine M, Davidson EH. Gene regulatory networks for development. Annu Rev Biophys Biomol Struct 2005;102:4936-42.
2. McClay DR. Evolutionary crossroads in developmental biology: sea urchins. Development 2011;138:2639-48.
3. Duloquin L, Lhomond G, Gache C. Localized VEGF signaling from ectoderm to mesenchyme cells controls morphogenesis of the sea urchin embryo skeleton. Development 2007;134:2293-302.
4. Röttinger E, Saudemont A, Duboc V, et al. FGF signals guide migration of mesenchymal cells, control skeletal morphogenesis and regulate gastrulation during sea urchin development. Development 2008;135:353-36.
5. Cavalieri V, Guarcello R, Spinelli G (2011). Specific expression of a TRIM-containing factor in ectoderm cells affects the skeletal morphogenetic program of the sea urchin embryo. Development 138:4279-90.
6. Watanabe M, Maemura K, Kanbara K, et al. GABA and GABA receptors in the central nervous system and other organs. Int Rev Cytol 2002;213:1-47.
7. Tsang SY, Ng SK, Xu Z, Xue H. The evolution of GABAA receptor-like genes. Mol Bio Evol 2007;24:599-610.
8. Anctil M. Chemical transmission in the sea anemone Nematostella vectensis: a genomic perspective. Comp Biochem Physiol Part D Genomics Proteomics 2009;4:268-89.