

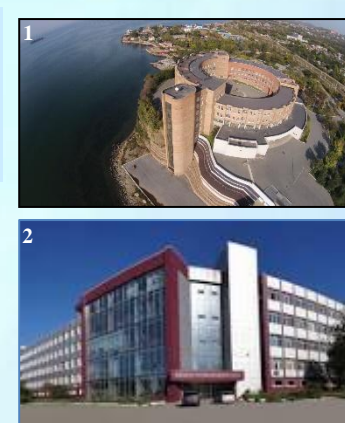
# Pigmentation and enzymes expressed in pigment cells throughout development and in cell cultures of embryos of the sand dollar *Scaphechinus mirabilis*

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*Scaphechinus mirabilis*

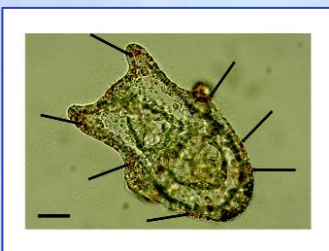


Fig. 1. A larva of the sand dollar *S. mirabilis* at the pluteus stage. Arrows show the pigment cells. Bar 20  $\mu$ m.

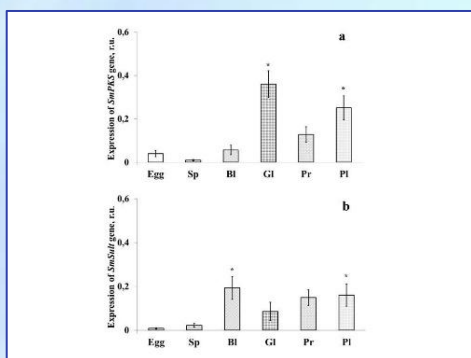


Fig. 2. The *pks* (A) and the *sult* (B) expression in vivo: in unfertilized eggs (Egg), spermatozooids (Sp), embryos, and larvae of the sand dollar *S. mirabilis* at various stages of development: blastula, 12 h post fertilization (BI), gastrula, 24 hpf (GI), prism, 34 hpf (Pr) and pluteus, 72 hpf (PI). \*P < 0.05; \*\*P < 0.01.

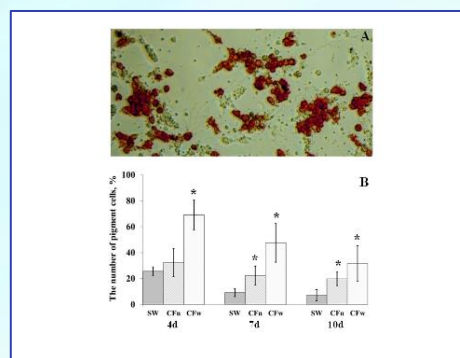


Fig. 3. Pigmentation in a blastula-derived culture from the sand dollar *S. mirabilis*. A. The appearance of sand dollar cells cultivated in the coelomic fluid of normal sea urchins for 4 days (Bar 10  $\mu$ m); B. Cellular dynamics of the sand dollar pigment cells cultivated in culture media - SW, CF<sub>n</sub> and CF<sub>w</sub> during 4–10 days.

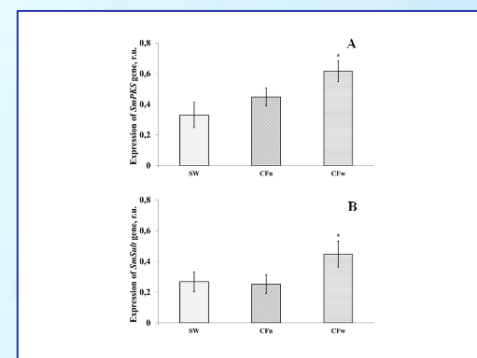


Fig. 4. Expression of two genes associated with biosynthesis of naphthoquinone during four days: A. The *pks* expression level; B. The *sult* expression level. Each bar represents the mean  $\pm$  SD of five biological replicates, each with three technical ones.

***In vivo*, the highest level of pigment expression in sand dollar embryos (the Sea of Japan, Russia) was observed at the blastula and gastrula stages. *In vitro*, genes of interest are also expressed significantly in blastula-derived cell cultures, confirming that primary embryonic cell cultures are suitable models for *in vitro* investigation of pigment differentiation. This assay is a useful tool for assessing the production of naphthoquinone pigments throughout development and in cell cultures of these sand dollars. The findings contribute to the understanding of pigment biology of Echinoid cells and create opportunities for commercial production of natural antioxidants of marine origin.**