Cadmium insult and defence mechanisms in *Paracentrotus lividus* embryos and larvae

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Marine invertebrate embryos represent a suitable model system where to investigate on the effects of many stressors, like heavy metals, on development and cell viability. Here we studied toxic effects and defense response by cadmium in *Paracentrotus lividus* sea urchin embryos and larvae, cultured with $CdCl_2$ since fertilization. We demonstrated that the metal is accumulated by the embryos depending both on concentration used and on time of exposure. In addiction we showed the possible competition between cadmium and calcium ions for using the same ionic channels. Our previous works described morphological abnormalities and apoptotic events induced by cadmium for both concentrations of 10^{-3} M and 10^{-4} M (Agnello et al, 2006; 2007). In this study we reduced noticeably the concentration of cadmium and prolonged the exposure time of *Paracentrotus lividus* zygotes until the feeding larval stages up to the metamorphosis, in order to test the toxic effects of low (10^{-12}M), medium (10^{-9}M) and high (10^{-6}M) cadmium chloride concentrations, mimicking environmental natural or polluted sea waters. We demonstrated that prolonged exposure to lower cadmium concentrations causes similar defects to those observed for short treatments with higher concentration of the metal. Moreover, we showed DNA fragmentation and activation of caspase-3, following $CdCl_2$ exposure. Thus, we can hypothesize that in sea urchin embryos/larvae apoptosis can be considered as part of a defence strategy in response to cadmium.
In addiction, we investigated the expression of caspase-3 protein and the transcription of relative mRNA during first stage o development of *P. lividus*. Immunocytochemical experiments demonstrated that caspase-3 protein is expressed in stage-dependent manner, during early development and it is quite represented since mesenchime blastula stage, 17h after fertilization. RT-PCR assays showed a modulation of transcription of mRNA of caspase-3 during first cellular divisions: caspase-3 mRNA goes to accumulation until mesenchime blastula stage; it is partially degradated during gastrulation and newly accumulated at pluteus stage. Other caspases seem to have a similar behaviour of transcription, suggesting that this could be a general synthesis strategy. In conclusion, our data suggest that sea urchin embryos would not be competent to activate defensive apoptotic mechanisms during stage preceding the mesenchime blastula stage.
Poster presentation

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Interference of metals in sea urchin embryo development

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Continuous exposure to all organisms to environmental stressors leads to greater risk of harmful health outcomes. Among compounds metals are a major contaminants generated by human activities and represent an actual hazard for aquatic ecosystems. Marine organisms can take up metals from solution and diet, which may consist of particles in suspension or deposited in the sediment. A great number of factors may influence dose-effect and dose-response relationships between metals and organisms.
Life began in the sea, and its tolerance to and use of trace metals reflect seawater concentrations. Trace metals are of environmental interest both as limiting nutrients (Fe, Zn, Mn, Cu, Co, Mo and Ni), playing important roles in metal-requiring and metal-activated enzyme systems, as toxicants. Toxic metals include heavy metals as Cd, Hg, Ag, Pb, Sn and Cr, but several nutrients metals can act as toxicants at elevated concentrations. Responses to toxicants are developmental stage specific, and for many echinoids, pluteus and larva stages are more sensitive to toxicants than earlier developmental stages (Gopalakrishnan et al, 2007). The stressors can trigger biological responses at the organisms only after initiating biochemical and cellular events. In the present study we investigated the distinct interference of cadmium and manganese in sea urchin embryo development and the capacity of these to withstand to each single insult activating different mechanisms.
We studied the effects of cadmium on sea urchin embryos and larvae (*Paracentrotus. lividus*), cultured in the presence of $CdCl_2$ since fertilization. We demonstrated that cadmium is accumulated during treatment depending both on metal concentration and on exposure time (AAS analysis). The accumulation leads to developmental delay and morphological abnormalities. In addiction, we found a probably relationship between cadmium and calcium bivalent ions for using the same ionic channels. We showed that cadmium exposure triggers: decrese of pattern proteins, HSPs synthesis, and/or DNA fragmentation, which is, almost partially, caused by apoptotic events, linked by the activation of caspase-3. Moreover, production of reactive oxygen species (ROS) was explained following cadmium treatment (Fig. 1).
We also investigated on effects of manganese on sea urchin embryos continuously cultured in the presence of the $MnCl_2$ from fertilization. manganese showed inhibitory effects on embryo development, producing specific malformation in a time- and dose-dependent manner (Fig. 2). More then 80% of the embryos treated with the highest