Poster n. 11

## Autophagy as defence strategy against cadmium stress in Paracentotus lividus embryos.

*Roberto Chiarelli, Maria Agnello and Maria C. Roccheri*

Sea urchin embryo is a developmental model that offers an excellent opportunity to investigate the possible adaptive response of cells exposed to different stress during differentiation. These embryos are able to respond to many stress by synthesizing a set of highly conserved proteins, the hsps and/or by activation of apoptosis. The exposure to cadmium trigger the accumulation of metal in embryo cells and the activation of both defence mechanisms depending on concentration and exposure time [1-4].

Recent experimental evidences demonstrate that by autophagy, a highly regulated mechanism that enhances cell survival under various environmental and cellular stress, the breakdown and recycling of macromolecules and organelles, in different cell types is possible [5].

Here we report that also in *P. lividus* embryos autophagic process occur, at lesser extent during physiological development and at greater levels after cadmium treatment.

By Acridine Orange staining, we found that embryonic cells exposed to cadmium display green fluorescence in cytoplasm and nucleus, and show considerable red fluorescent dots in cytoplasm. This evidence suggests formation of acidic auto-phagolysosomal vacuoles. By Neutral Red vital staining, specific for acid compartments, including lysosomes, we obtained analogous results (see figure). These data have been sustained by anti- LC3 antibody detection, a specific marker of autophagy.

Our results show the existence of autophagy in *P. lividus* embryos, suggesting that this process could be an additional defence strategy activated against cadmium stress.

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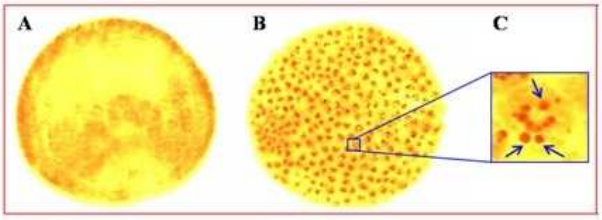


**Neutral Red vital staining** of embryos, after 18 hours of development. Images captured by light microscopy: Control **(A)**, 1mM $CdCl_2$ **(B)**, enlargement of B particular **(C)**. The arrows indicate some auto-phagolysosomes. Bar = 40µm.

Poster n. 12

## A new member of the discoidin family: in silico analysis of *Paracentrotus lividus* nectin based on its cDNA sequence

*Caterina Costa, Francesca Zito and Valeria Matranga*
Istituto di Biomedicina e Immunologia Molecolare "Alberto Monroy", Consiglio Nazionale delle Ricerche, Via La Malfa 153, 90146 Palermo, Italy

The *Paracentrotus lividus* nectin (*Pl*-nectin) is an extracellular matrix protein of the sea urchin embryo, composed of two 105-kDa equivalent subunits and shown to be involved in cell adhesion and embryonic morphogenesis (1, 2). We isolated the full length 3593bp cDNA coding for the 984aa precursor containing a 23aa signal peptide and six 151-156aa long, tandemly-repeated Discoidin (DS) domains, also known as F5/8 Type C domains of the Discoidin family (3, 4). Phylogenetic analysis with DS domains from other known proteins confirmed their biological role in cell adhesion and signaling mediated by protein-protein, protein-carbohydrate, or protein-lipid interactions. We identified a LDT motif, also found in the mammalian mucosal addressin molecule and known to bind the integrin α4β7 receptor (5). We obtained three-dimensional models of the six *Pl*-nectin domains by homology modelling based on known crystal structures of the C2 domains of the human coagulation factor V and VIII, and the bovine factor Va. Intra-domain S-S bridges were predicted by comparison and superimposition of the *Pl*-nectin domain models with the galactose oxidase crystal structure. One 105-kDa subunit model was obtained by the association of three dimers models, each consisting of two *Pl*-nectin domains. In conclusion, the whole quaternary structure proposed here, consists of two 105-kDa C-shaped subunits linked by a S-S bridge; the model is consistent with earlier biochemical reports and extends previous conclusions concerning its function.

This project has been partially funded by the Biomintec Project of the EU 7th FP Marie Curie ITN

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