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BIOMONITORING OF COASTAL AREAS: CADMIUM EFFECT ON CYTOSKELETON OF THE CALCISPONGE *CLATHRINA CLATHRUS*

MONITORAGGIO COSTIERO: EFFETTO DEL CADMIO SUL CITOSCHELETRO DELLA CALCISPONGIA CLATHRINA CLATHRUS

Abstract *- We detected the effect of Cadmium on the cytoskeleton of* Clathrina clathrus *by immunocytochemistry, confocal microscopy and immunoblotting. Results highlighted the potential of the sponge to resist to the action of heavy metals through the reorganisation of the tubulins and suggest the utilization of sponges for biomonitoring of environmental pollution.*

Key-words: *sponges, bioindicator, immunofluorescence, cytology, heavy metals.*

Introduction - As sessile filter feeders, sponges are highly exposed to environmental stress by pollutants of both anthropogenic and natural origin and are able to accumulate harmful substances (Verdenal *et al.,* 1990). Thus sponges are considered as experimental model for biomonitoring of coastal areas (Carballo *et al.*, 1996; Perez *et al.*, 2005). In this work we studied the effect of Cadmium on the cytoskeleton, focusing on tubulins, of the calcisponge *Clathrina clathrus* (Schmidt, 1864). Microtubules are elements of the cytoskeleton necessary for cell division, intracellular trafficking of macromolecules and organelles, beating of cilia and flagella, and the dynamic organisation of the cell morphology. Several studies have demonstrated that post-translational acetylation and detyrosination of α-tubulin occur on stable microtubules (Piperno *et al.,* 1987; Webster *et al.*, 1987) whereas tyrosination of α-tubulin is linked to relatively dynamic or labile microtubules (Webster *et al.*, 1987). We detected here the reaction of *C. clathrus* to heavy metal exposure as a potential indicator of contamination.

Materials and methods - Specimens of the marine sponge *Clathrina clathrus* were collected by SCUBA at the Portofino Promontory (Ligurian Sea, NW-Mediterranean) at a depth of 15-25 m. Sponges were cut into cubes (5 g each) and put into flasks containing 200 ml of filtered and oxygenated seawater supplemented with or without different concentrations of Cadmium chloride (0.2 or 1 mg/L) for 24 h. Thereafter, part of the sponge cubes were immediately fixed in 4% paraformaldehyde and paraplast embedded. Serial sections (5 μm thick) were incubated with a primary antibody (overnight at 4 °C) after pre-treatment with 3% bovine serum albumin in PBS plus 0.1% Triton X-100, and then with the appropriate secondary antibody (2 h at room temperature). The following antibodies were used: monoclonal antibodies specific for acetylated or tyrosinated α-tubulin, polyclonal antibody specific for detyrosinated α-tubulin. Images were acquired by a confocal laser-scanning microscope (Leica Microsystems Mannheim, Germany). Part of sponge cubes was utilized for cell isolation for immunoblotting and cell viability determination.

Results - Microtubules in control sponge cells in absence of Cd^{2+} were not labelled by the antibodies specific for acetylated or detyrosinated α-tubulin except for the choanocyte flagella. In contrast, the formation of microtubule clusters in the choanocyte cytoplasm was observed in cells treated with Cadmium chloride. Consistent with the immunofluorescence staining, predominant protein bands, representing acetylated and detyrosinated α-tubulin, appeared in the immunoblotting of Cd^{2+} treated cells in a dose-dependent manner whereas the corresponding labelled protein bands were not detectable in control cells. Furthermore a reduction of tyrosinated α-tubulin was evidenced. Flagella and large microtubule clusters were intensely labelled in choanocytes of both controls and 0.2 mg/L Cd^{2+} treated sponges, whereas only flagella and small microtubule clusters were stained in choanocytes of 1 mg/L Cd^{2+} treated sponges.

Conclusions - In this study we have documented increased levels of acetylated and detyrosinated α-tubulin as well as a reduced level of tyrosinated α-tubulin after Cadmium exposure in *Clathrina clathrus* cells. Since α-tubulin acetylation and detyrosination are convenient markers for the presence of stable microtubules (Piperno *et al.*, 1987; Webster *et al.*, 1987), the marked enhancement of α-tubulin acetylation and detyrosination in Cd^{2+}-treated cells indicates that divalent Cd ions stabilize microtubules, which in turn should favour the formation of microtubule bundles. Subpopulations of relatively dynamic or labile microtubules, enriched in tyrosinated α-tubulin, are instead disassembled in presence of Cd^{2+}. In conclusion, the results from this work indicate that a reduced α-tubulin tyrosination and an enhanced α-tubulin detyrosination and acetylation represent early sponge-specific markers of Cd^{2+} toxicity. This evidence suggest the ability of *C. clathrus* cells to tolerate heavy metal effect by reorganizing its microtubule network, and the potentiality to act as a sentinel of pollutants in coastal areas.

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