

KEY MOLECULES IMPLICATED IN APOPTOTIC PATHWAYS OF SEA URCHIN EMBRYOS.

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Previous studies on cell death showed that early pluteus and larvae of *Paracentrotus lividus* undergo spontaneous apoptosis, probably to shape the final structure of the adult (Roccheri et al., 2002, Int. J. Dev. Biol. 46: 801). Furthermore, treatment of gastrula and pluteus with TPA (apoptosis inducer) followed by a temperature increase to 31°C induces apoptosis, as judged by DNA fragmentation and supported by the appearance of known apoptotic signs (Roccheri et al., 1997, Biochem. Biophys. Res. Commun. 240: 359).

TUNEL assay on embryos treated with TPA showed a severe fragmentation of DNA, which announced apoptotic events.

In order to identify some key molecules involved in the apoptotic cascade, we analyzed the presence of cleaved-caspase-3 and pro-caspase-3, by immunocytochemical experiments. The results showed an increase of caspase-3 cleaved and a decrease of zymogen in treated embryos. Whether caspase-3 had an important role only in the stress induced apoptosis, was further supported by studies with Z-DEVD-FMK, a potent caspase-3 inhibitor. The embryos treated both with Z-DEVD and “TPA+31°C” showed inhibition of cleaved caspase-3 (figure). Conversely, embryos treated only with the caspase-3 inhibitor showed a regular morphology. Experiments with specific antibodies anti-PARP and anti- α -Fodrin, substrates of cleaved caspase-3, showed the cleavage of these death substrates in the treated embryos.

Besides, we observed the presence of cleaved-caspase-7 above all in plutei physiological growth, suggesting an involvement of this caspase in spontaneous apoptosis.

Key Words: Apoptosis, TPA, sea urchin embryos.

“Immunoreaction with anti-caspase-3”

