THE USE OF SEA URCHIN EGG AS A MODEL TO INVESTIGATE THE CELLULAR TARGETS OF MARINE NATURAL PRODUCTS

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Sea urchin gametes are, among many invertebrate gametes, one of the most sensitive cells to environment stress. Since 1980, many authors have widely described the properties of cultured sea urchin eggs and embryos resulting in several advantages over conventional mammalian cultured cells (Bougis 1981, Hose 1985). Cultured in simple sea water, sea urchins provide millions of eggs which can be successfully fertilized. Cell divisions remain synchronous at least until the third cleavage, which occurs within a few hours. Thus, it is easy to rapidly know, under a light microscope, if a drug affects the fertilization ratio or the cell cycle, which can be delayed or blocked (Jacobs and Wilson 1986, Fusetani 1987).

Among drug cellular targets, the role played by the plasma membrane and the membranes of intracellular organelles has been widely studied 1) maintenance of ionic balance of monovalents cations (Payan *et al.*, 1981, Girard *et al.*, 1982), intracellular pH (Payan *et al.*, 1983); 2) ionic signals following activation by sperm: cytosolic alkalinisation (Epel 1978, Shen and Steinhardt 1978), peak of cytosolic free calcium (Gillot *et al.*, 1990); enzymes activation (Heinecke and Shapiro 1989) and 3) stimulation of metabolic processes resulting in the first cleavage and embryogenesis: proteins and nucleic acids sythesis (Sluder *et al.*, 1990). Sperm triggers resumption of the egg activity, the first round of DNA synthesis being achieved 30 min. later. Like in other animal cells, activation starts by rapid hydrolysis of phosphatidylinositol-biphosphate generating the phosphoinositide messengers inositol trisphosphate (IP3) and diacylglycerol (DAG) Swann and Whitaker (1986) . Inositol trisphosphate triggers an explosive wave of cytosolic calcium (Oberdorf *et al.* 1986, Payan *et al.*, 1986) and DAG provokes a further sustained increase in intracellular pH (Payan *et al.*, 1987). The calcium increase during fertilization is the necessary and sufficient signal that induces egg development (Gillot *et al.*, 1990). In addition, other calcium transient changes occur throughout the first cell cycle particularly at anaphase and before cleavage (Suprynowicz and Mazia 1985, Whitaker and Patel 1990).

As previously proposed by us, drug inhibition of sea urchin egg fertilization and first cleavage can be explained in many cases by changes of ion transport through the plasma and subcellular membranes (Biyiti *et al.*, 1990, Pesando *et al.*, 1991, Pesando *et al.*, 1992). Many types of drugs can be characterized depending on their cellular effects and their targets. Inhibition of the Na^+/H^+ exchange alters the sustained alkalinization of egg after fertilization and the mechanism by which the egg maintains an intracellular pH below that of sea water. After fertilization, inhibition of the Na^+/K^+ exchange increases cell sodium content (Ciapa *et al.*, 1984a et b), inhibits amino acids uptake and leads to animalized embryos (Girard *et al.*, 1982). Calcium permeability and calcium sequestration by intracellular organelles such as endoplasmic reticulum or mitochondria can also be modified by drugs or heavy metals (Allemand *et al.*, 1988, Walter *et al.*, 1989). A rise in calcium permeability of the plasma

membrane can result in a calcium increase which in turn may uncouple mitochondrial metabolism, both events leading to cell death (Nicotera *et al.*, 1990, Reed 1990). Drug-induced calcium leakage from intracellular stores, mainly endoplasmic reticulum, elevates cytosolic calcium concentration and can prevent egg from undergoing later calcium transient changes (Biyiti *et al.*, 1990). In addition, sea urchin eggs offer the possibility to easely examine drug effects on the cytoskeleton by observing evolution of the mitotic spindle and the cleavage furrow which could be affected by toxins, resulting in abnormal cytokynesis or cleavage (Wagenaar 1983, Schatten *et al.*, 1986, Pesando *et al.*, 1992). With sea urchin eggs, it is therefore possible to consider the relations between intracellular calcium, pH and the behaviour of the cytoskeleton.

Many recent experiments have emphasize the role of sea urchin as a model in the assessment of the cellular targets of marine natural products. One of the first approach consists to determine in which interval of time, following fertilization, the toxin modify the kinetics of egg cleavage. For example, a methylene-chloride extract of the marine sponge Crambe crambe inhibits the first cleavage of eggs only when added 10 to 30 sec. following insemination (P. Amade, pers. comm. Fig 1). Ilimaquinone (MLK 201F) (Fig 2) is extracted from a desmosponge which present anti-microbial and cytotoxic activities (Kondracki and Guyot 1989). This toxin inhibits, in a dose- dependent manner the first cleavage of sea urchin eggs whether it is added early (30 sec.) or later (10 min.) after fertilization (Fig 3). Nevertheless, it is possible to do further concerning the characterization of the cellular targets of such a molecule. We show that MLK 201F does not modify the permeabilities to cations (Na^+, K , Ca^{++}) of the plasma membrane of both unfertilized and fertilized eggs. On the oposite, it is likely that the toxin affect the ability of intracellular compartments of egg to sequester calcium. Calcium storage by the endoplasmic reticulum, studied by the mean of isolated cortices preparation, is dramatically inhibited by MLK 201F (Fig 4). We show that this results from a ionophore-like effect which render the reticulum leaky to calcium (Fig 5). The succinate-stimulated influx of ^{45}Ca into mitochondria is also inhibited by MLK 201F which reduces the uptake to the level measured in presence of cyanide (Fig 6).

Our results show that the use of sea urchin egg permits to detect, among the cellular mechanism participating to cell activation and proliferation, which are affected by natural products extracted from the sea.

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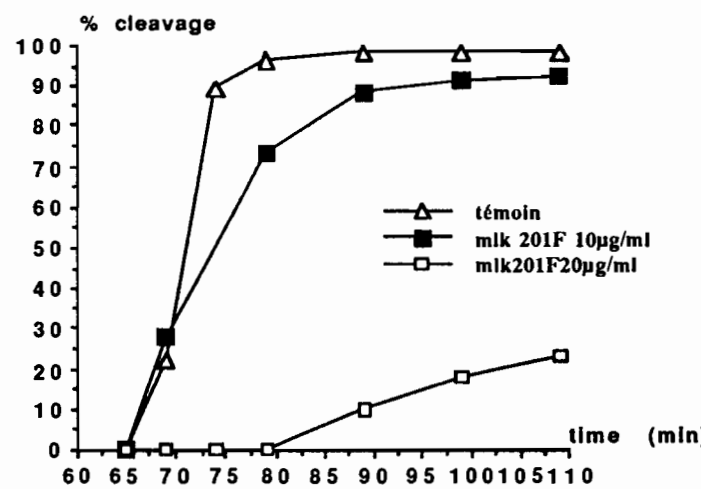

Figure 1: Extract of *Crambe crambe* inhibits the first cleavage of sea urchin egg when added within 30 seconds following insemination.

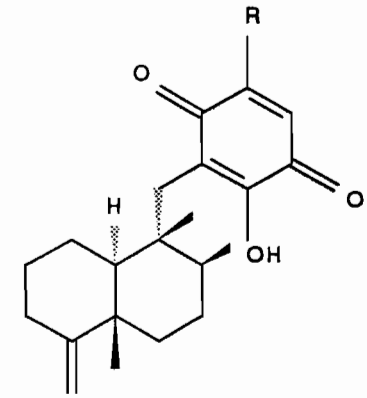

Figure 2 : Structure of ilimaquinone.

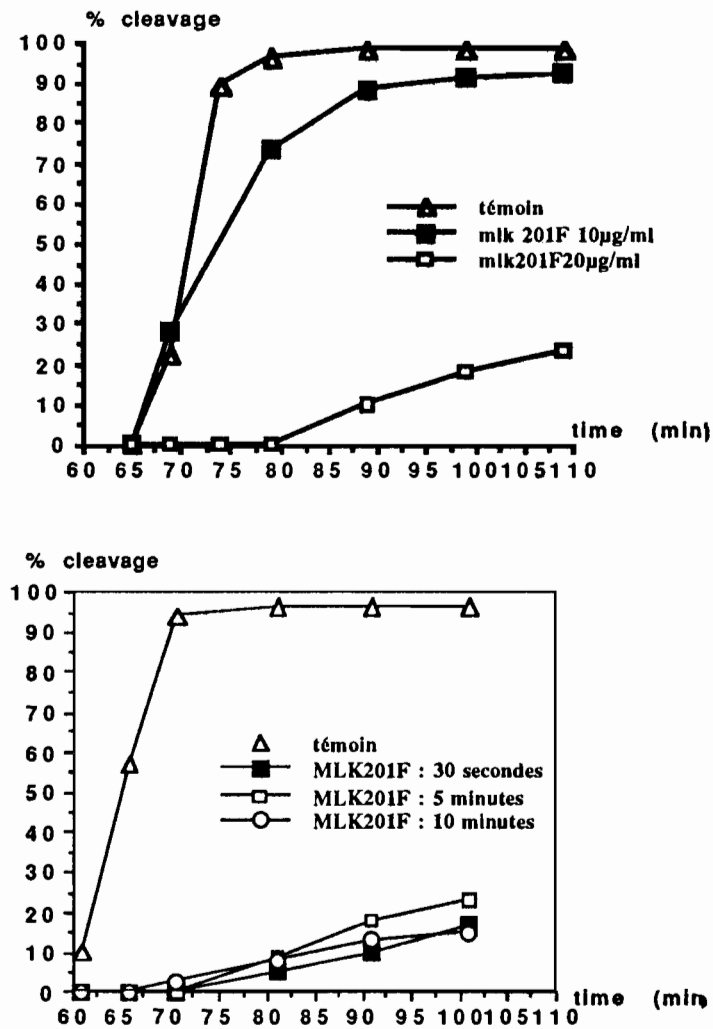

Figure 3 : Effect of illimaquinone on the first cleavage of sea urchin egg.

Up: toxin was added 30 sec. after fertilization.
Down: effect of adding toxin (20 µg/ml) 30 sec. after insemination.
Control (open triangle) was runned simultaneously.

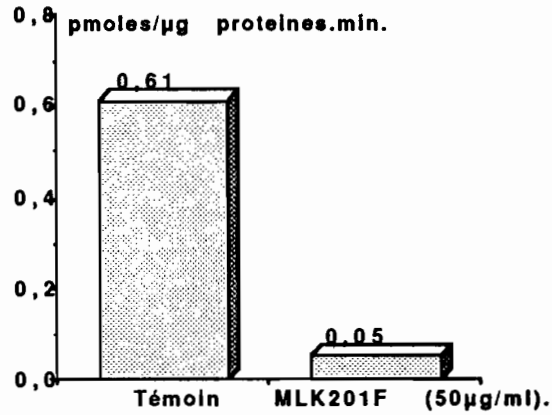

Figure 4 : Inhibition by MLK 201F (50 µg/ml) of the AATP-dependent uptake of ^{45}Ca by a preparation of isolated cortices from sea urchin eggs.

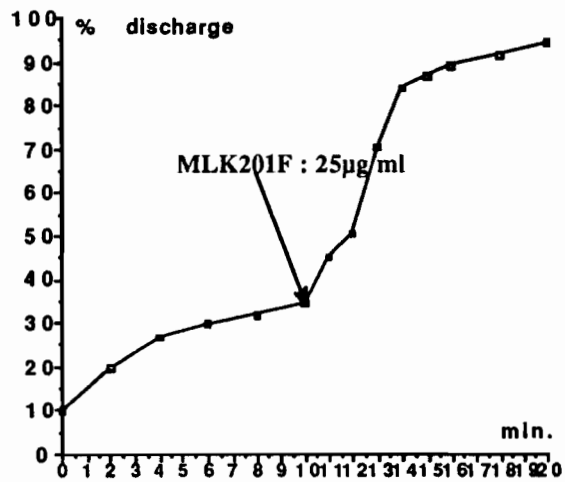

Figure 5 : Effect of MLK 201F on the radioactive discharge from a preparation of isolated cortices pre-loaded with ^{45}Ca.
Dashed line: discharge provoked after addition of calcium ionophore A.23187 (20 µM).

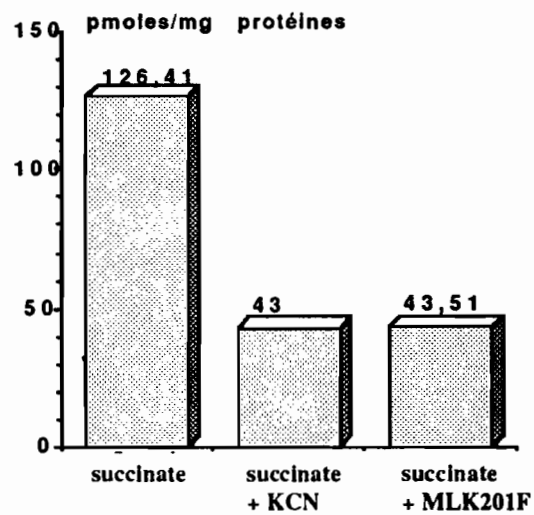

Figure 6 : Effect of MLK 201F (50 µg/ml) on the succinate-dependent uptake of ^{45}Ca by digitonin-permeabilized sea urchin eggs

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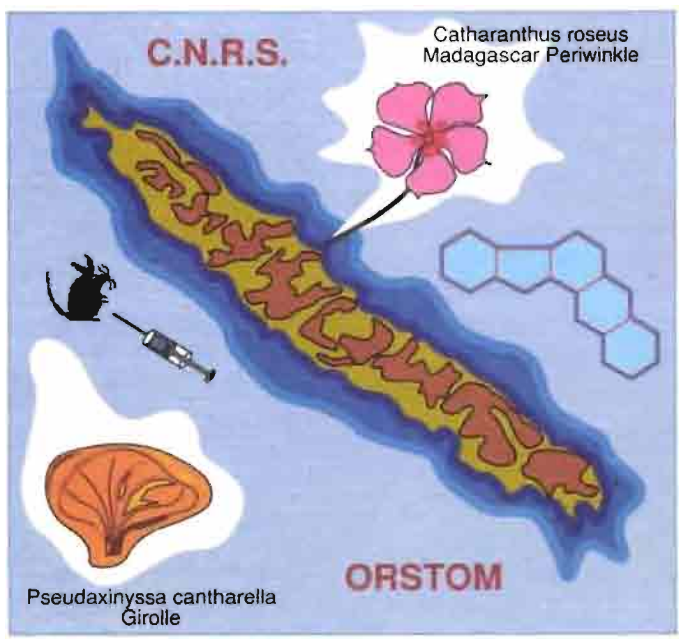

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