Apoptosis in Sea Urchin Oocytes, Eggs, and Early Embryos

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ABSTRACT Certain experimental manipulations with sea urchin oocytes, eggs, and early embryos result in induction of cell death. We were interested in whether or not these cells possessed functional apoptotic machinery, and whether cellular demise under certain experimental conditions is due to activation of a programmed cell death pathway. Therefore, we evaluated a number of apoptosis assays in sea urchin oocytes, eggs, and early embryos experimentally induced to apoptose with staurosporine. Our results indicate that these cells each possess and activate necessary apoptotic machinery that leads to characteristic apoptotic phenotypes. The eggs of this animal have completed meiosis, and are quiescent transcriptionally, translationally, and metabolically. Surprisingly, they still undergo apoptosis. The progression through apoptosis of treated specimens could be followed by morphological changes of the cells, by chromatin condensation and degradation, and by activation of caspases. The similarities and differences in the execution of apoptosis between the cell types studied are discussed. Results of this study will be useful for interpreting experiments in these model systems in which different molecules are targeted for interference and which brings about cell death.

INTRODUCTION

Apoptosis, or programmed cell death, plays a prominent role in many biological processes such as tissue formation and homeostasis, responses to environmental stimuli or damage, and elimination of abnormal cells. It is an evolutionarily conserved process, present in both animal and plant kingdoms. Apoptosis in animal cells is characterized by distinct morphological and biochemical hallmarks, including cell shrinkage and disintegration through blebbing, condensation and degradation of the chromatin, increase in plasma membrane permeability, and activation of specific cysteine proteases-caspases (Lockshin et al., 1998). Even though apoptosis can be induced by activation of multiple independent upstream pathways in different scenarios of programmed cell death, effector caspases are invariant downstream players that degrade nuclear matrix proteins, transcription factors and DNA repair enzymes, and activate apoptosis-specific deoxyribonucleases. Mitochondrial dysfunction, loss of mitochondrial membrane potential, and production of reactive oxygen species are also usually observed during apoptosis. Release of cytochrome C from mitochondria in many systems serves as a link between upstream and downstream events of apoptosis (Budihardjo et al., 1999; Strasser et al., 2000).

Programmed cell death of actively dividing cells is widely described during development and is important for tissue morphogenesis. Experimental induction of apoptosis in growing cell cultures can be achieved by treatment with a number of reagents that block key cellular enzyme activities such as kinases or topoisomerases (Lockshin et al., 1998). However, apoptosis is not confined to actively dividing cells. Mitotically quiescent cells such as neurons are well-known to undergo apoptosis during development or as a result of neurodegenerative disorders. The presence of functional apoptotic machinery has also been well documented for the germ cells in both vertebrates and invertebrate species. In females, apoptosis of the oocytes and mature eggs is wide-spread, and reinforces the notion that nondividing, but still metabolically active cells are capable of executing regulated cell death (reviewed in: Morita and Tilly, 1999).

Sea urchin embryos are one of the widely used systems for investigation of molecular mechanisms of development, mainly because large number of eggs can easily be obtained, fertilized externally, and both eggs and embryos are readily manipulated. Studies of various regulatory molecules in sea urchin embryogenesis showed that interference with their function, such as inhibition of SNARE proteins syntaxin and Rab3 (S. Conner, G. Wessel, personal communication), prevention of cyclin B synthesis (E. Voronina and G. Wessel, in preparation), or inappropriate retention of cyclin B activity (Hinchcliffe et al., 1998) each results in cellular death with a characteristic phenotype of blebbing. However, oocytes, eggs, and early embryos of sea

Abbreviations: ASW, artificial sea water; 3-AT, 3-amino triasol; FVf, FITC-VAD-fmk; MLCK, myosin light chain kinase; PKA, protein kinase A; PKC, protein kinase C; STS, staurosporine.
Grant sponsor: NIH.
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Received 12 April 2001; Accepted 22 June 2001

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urchin have not been evaluated for their ability to undergo apoptosis, for the characteristic changes that occur during this process, and for ways of detecting these changes experimentally. To determine whether these cells possess functional apoptotic machinery necessary for programmed cell death, we evaluated a variety of available methods of apoptosis detection for their efficacy in sea urchin oocytes, eggs, and early embryos. Our results show that these cells do possess functional apoptotic machinery, and we have established apoptotic hallmarks in these cells that can be used to ascertain apoptosis resulting from experimental manipulations.

MATERIALS AND METHODS

Chemicals were purchased from Sigma (St. Louis, MO) unless otherwise noted.

Animals. Adult Lytechinus variegatus were obtained from Susan Decker (Miami, FL). Females were shed by KCl (0.5 M) injection and ovaries were then removed and minced in artificial sea water (ASW) (Instant Ocean; Aquarium Systems, Mentor, OH). Oocytes and eggs were isolated and cultured in ASW at 22°C with or without addition of apoptosis-inducing reagent. To obtain 2-cell embryos, the eggs were fertilized and cultured for 50 min at 22°C.

Staurosporine treatment. Staurosporine (Kamiya Biomedical Company, Seattle, WA) was resuspended in DMSO at 1 mg/ml, and added to ASW at final concentrations of 50 nM, 2 and 5 μM. Cells were continuously exposed to the drug or to control conditions (DMSO only) for the times indicated.

Apoptotic Assays

Morphology changes. Cells were observed at the indicated times (30 min, 1, 2, and 4 hr), and assayed for the blebbing phenotype. A cell was scored as blebbing when it exhibited two or more well-defined blebs.

Caspase activation. Caspase activation was followed by FITC-VAD-fmk (FVf) fluorescence (Promega, Madison, WI) according to the manufacturer’s protocol. Briefly, after the treatment indicated, the cells were transferred into a 10 μM solution of FVf in ASW in a well of a microtiter plate, and incubated for 20 min. The cells were then washed twice with ASW, mounted on a slide, and fluorescence was observed at 530 nm following excitation at 470 nm for TUNEL labeling, and at 420 nm following excitation at 385 nm for Hoechst staining. The epifluorescent images were recorded by on Zeiss LSM 410 confocal microscope with a Spotcam.

RESULTS

To induce apoptosis, we exposed L. variegatus oocytes excised from the ovary, spawned eggs, or two-cell embryos to either staurosporine or camptothecin for different lengths of time. Staurosporine is a protein kinase inhibitor, which acts on a variety of kinases, such as PKC, PKA, and MLCK. It induces apoptotic death in many cell types, and is widely used as an inducer of apoptosis in cell culture studies. In contrast, camptothecin is an inhibitor of topoisomerase II that induces apoptosis in actively dividing cells following DNA damage during replication and activation of cell cycle checkpoint machinery. We found that camptothecin replicated the staurosporine induced apoptotic phenotype in cleaving embryos, so in this study we focus on the use of staurosporine for assaying apoptosis. We employed staurosporine at concentrations from 50 nM to 5 μM: at 50 nM staurosporine was found to have little to no effect as judged by cell morphology, in agreement with an earlier report (Roccheri et al., 1997) of apoptosis in gastrulae and plutei of another sea urchin Paracentrotus lividus. However, in our study of oocytes, eggs, and embryos, 2 and 5 μM staurosporine treatment resulted in a rapid induction of apoptotic phenotypes. These concentrations of staurosporine are similar to ones used in mammalian cell culture studies of apoptosis (see for example: Han et al., 1999).

Morphological Analysis

The morphology of treated specimens indicated that these cells underwent membrane blebbing and fragmentation as would be expected of the apoptotic cells (Fig. 1). In addition, staurosporine treated embryos stopped cleaving, and were arrested at interphase. This could be caused by general inhibition of cellular kinases.
by the staurosporine, or by induction of the apoptotic machinery. Control cells treated with DMSO only (the vehicle) exhibited no blebbing (Fig. 1), and embryos proceeded normally through cleavage.

Concentration dependence of the blebbing induction by staurosporine becomes evident in embryos and eggs by 1 hr of treatment (see Fig. 2). Oocytes appeared to be most sensitive to staurosporine: it was the only group where nearly 90% of cells blebbed by 4 hr in 2 mM staurosporine treatment. By this morphological criteria, the eggs appeared least sensitive to staurosporine: even the highest concentration of reagent used (5 mM) caused only 70% of eggs to bleb by 4 hr of treatment.

Caspase Activation During Cell Death

The central event in the execution of programmed cell death is activation of cysteine proteases-caspases, and it is a specific and reliable indicator of apoptosis. To determine whether caspases are activated in individual treated cells, we employed the in situ caspase activation marker FVf. This fluorescent marker diffuses into live cells and irreversibly binds to activated caspases, resulting in the inhibition of caspase activity and increased intracellular fluorescence. Therefore, FVf fluorescence retained in the cell represents a snapshot of caspase activity at a given time point. It should be noted that FVf does not discriminate between caspase types, and instead is used as an indicator of general caspase activity in the cell.

We find functional caspases in treated oocytes, eggs, and early embryos (Fig. 3) and absence of signal in controls. In eggs, fluorescence indicating caspase activity appears to be enriched at the cell surface (Fig. 3E). This could reflect the intracellular localization of activated caspases, or be a result of limited diffusion of the fluorescent marker across a cell 120 microns in diameter, and not significantly active in endocytosis. The more wide-spread cytoplasmic distribution of FVf in oocytes and embryos (Fig. 3D,F) could reflect a facilitated delivery of FVf into the cytoplasm via endocytosis in addition to passive diffusion through the plasma membrane.

The time course of caspase activation after initiation of staurosporine treatment was determined by calculation of the percentage of FVf-positive cells in treated groups (Fig. 4). Embryos are the first group to reach 100% activation of caspases when treated with either 2 or 5 mM staurosporine for 1 hr (Fig. 4C). By 4 hr of staurosporine treatment (both 2 and 5 mM), 100% of oocytes show caspase activation (Fig. 4A). Eggs reach the 100% mark by 1 hr of 5 mM treatment, however, the percentage of eggs displaying detectable caspase activity actually decreases to 50–70% by 2 hr of treatment (Fig. 4B). This could signify the predominance of initiator caspases over effector caspases in the sea urchin egg, since VAD is a pan-caspase substrate that binds to all caspase types with the same efficiency. Alternatively this could be a reflection of a biphasic activation pattern of caspases in staurosporine-treated eggs under our experimental conditions. In agreement of previous reports (Perez et al., 1999), caspase...
activation is an earlier indication of induction of apoptosis than cell blebbing, as it is apparent in most cases at 30 min after beginning staurosporine treatment, whereas blebbing is first significant within 1 hr of treatment.

Oocyte labeling after 30 min of staurosporine treatment was not consistent between groups of cells obtained from different sea urchins, such that one group of oocytes had only 10% positively-stained cells, while two other groups were 100% FVf-positive. Therefore, the plotted data represent percentages calculated after pooling the results from three groups of treated oocytes at 30 min, and pooling the results from two groups of all the other treated cells at all the other time points.

**Nuclear Changes**

Another prominent hallmark of regulated cell death is the condensation and fragmentation of DNA in apoptotic cells. Nuclear changes in staurosporine treated sea urchin cells were followed in situ with the vital nucleic acid stain SYTO11 (Fig. 5). The DNA in oocytes is dispersed over a large volume of the nucleus, which makes it harder to detect (see for example thin chromosomal fibers in Fig. 5D) whereas the DNA in eggs and embryos is easily detectable throughout the nucleus in controls and at the beginning of staurosporine treatment (Fig. 5J,P).

During the course of staurosporine treatment in embryos and eggs, the first change in the appearance of DNA was a condensation of chromatin along the nuclear envelope in a ring or in a single “speck” (“margination,” Fig. 5K), followed by dispersion of chromatin (nothing seen in the eggs, Fig. 5L, “empty nucleus” seen in embryos, Fig. 5R). A peculiar form of chromatin condensation in streaks could be seen in embryos in which staurosporine treatment was initiated too late to prevent mitosis (Fig. 5Q). Oocytes exhibited surprisingly minimal changes in DNA and nuclear morphology over the course of staurosporine treatment (Fig. 5E,F). Even by 4 hr of treatment, the changes of chromatin were not significant. Fig. 5F shows a blebbing cell, with still detectable DNA fibers in the nucleus. In addition, cytoplasmic granularity after staurosporine treatment could be seen in oocytes (Fig. 5E) and embryos (Fig. 5R), and to a lesser extent in eggs (Fig. 5L). This phenomenon could arise from degradation of cellular RNAs that could occur during apoptosis (for a discussion of RNA degradation and concomitant change in SYTO dyes staining during apoptosis see Poot et al., 1997).

Time course of the progression of observed apoptotic events (in embryos and eggs only) is represented in Fig. 6. As can be seen, by 4 hr of treatment nearly 100% of embryos and eggs display either chromatin condensation or degradation, changes that could not be detected in comparably treated oocytes. In eggs and embryos, DNA condensation is not the earliest feature of programmed cell death onset; it is evident after caspase activation, with about the same dynamics as cell blebbing. However, unlike blebbing, it appears in 100% of eggs. Thus, chromatin morphology is a more sensitive way of detecting programmed cell death (or blebbing underestimates the number of apoptotic eggs).

Embryonic cells appear to initiate DNA condensation earlier than eggs—at 30 minutes after initiation of staurosporine treatment, but still following caspase activation.

**TUNEL Assay**

We followed the morphological analysis of nuclear changes with an assessment of chromatin fragmentation by TUNEL assay, a way to detect DNA fragmentation. We were especially interested in the oocytes, since those cells did not exhibit chromatin dispersion as assessed by SYTO11 staining.

One hour staurosporine treatment was chosen for eggs and oocytes because at that point of time we saw all types of chromatin morphology represented in staurosporine treated eggs (normal, condensed, and dispersed, see also Fig. 6A). Our results indicated that a portion of mature eggs subjected to staurosporine treatment for 1 hr exhibited positive TUNEL labeling (see Fig. 7 and 8). Upon examination of chromatin morphology (assessed by Hoechst staining) in TUNEL-positive cells, we concluded that TUNEL-positive eggs represent a fraction of eggs exhibiting condensed and “marginized” chromatin (Fig. 7E,F insets), as compared with normal chromatin (Fig. 5J). Oocytes on the other hand were all negative for TUNEL staining after 1 hr of staurosporine treatment (Fig. 7), which is in agreement with the observation that chromatin morphology of these cells does not change at this time point. The untreated embryos, however, were very heterogeneous when labeled: about 25% of embryos were stained...
positive even though this staining depended upon addition of terminal transferase. Therefore, we concluded, that this is not the preferred method for assessing apoptosis of early blastomeres.

**DISCUSSION**

Investigation of various cellular processes often involves interference with the function of certain molecular targets. In some cases, the cell may respond by a phenotype reminiscent of apoptosis. We were interested in determining if apoptotic mechanisms were functional in oocytes, eggs, and early embryos of sea urchins. The relatively large size of these cells (compared either to the cell nucleus, or to other cells that are common apoptotic models) may introduce changes in apoptotic phenotypes or interfere with routine methods for detection. Furthermore, the low metabolic activity of eggs could conceivably negate the induction of apoptosis, which is an energy-dependent process, especially if cytochrome C efflux into the cytoplasm, a common apoptotic phenotype, is dependent on mitochondrial activity. We also wanted an ability to analyze a single cell or embryo, since most treatments of these specimens rely on microinjection procedures. Our results thus will be useful in the future to effectively detect and quantify apoptosis phenotypes induced in the cells analyzed by functional interference with various molecular targets.

Our results indicate that sea urchin oocytes, eggs, and early embryos do possess apoptotic machinery and are capable of executing programmed cell death upon staurosporine treatment. Dying cells exhibit morphological features characteristic of apoptosis, including blebbing, nuclear condensation, chromatin aggregation and degradation, and we thus conclude that the effects induced by staurosporine are truly apoptotic. The
blebbing phenotype of staurosporine treated cells appears to be very similar to that observed upon interference of SNARE proteins function in blastomeres (S. Conner, G. Wessel, personal communication) or cyclin B inhibition (E. Voronina and G. Wessel, in preparation). The dying cells could also be labeled with fluorescent marker of caspase activity, which specifically stains apoptotic cells. Caspase activation is the earliest marker of apoptosis initiation in these cells appearing at 30 min of staurosporine treatment. DNA condensation and cell blebbing follow simultaneously at 1 hr of treatment. TUNEL assay detecting DNA fragmentation in cells, labels a fraction of sea urchin eggs exhibiting abnormal chromatin morphology, and is not very efficient in detecting cells undergoing apoptosis. We suggest that chromatin fragmentation follows condensation of DNA in eggs, and relatively quickly results in complete degradation of chromatin, evident as an “empty nucleus” in SYTO11 stained cells. Therefore, we believe that at any given time point only a fraction of cells undergoing apoptosis are actively degrading DNA and thus could be labeled by TUNEL (detecting cells in a very narrow window of time after the chromatin has been condensed, but before it is cleaved beyond detection). This leads to an underestimation of the number of apoptotic cells by TUNEL staining.

This work is the first demonstration of caspase activity in sea urchins. Caspases have been found in many multicellular organisms, and their role in programmed cell death is highly conserved. Caspase 1 and 8 genes have been already identified in the sea urchin genome sequencing project at the California Institute of Technology,\(^1\) and although the expression data of these genes is yet unavailable, our data suggests that caspases are present in sea urchin oocytes, eggs, and early embryos. FVf does not discriminate between different caspase types, and more selective markers or assays of caspase activity can be used to further characterize which of these proteases are present in these cells. In addition to being an indicator of caspase activity, FVf is an irreversible caspase inhibitor, although we did not assess whether interference with caspase activity would prevent apoptosis in sea urchin cells.

We found several differences in apoptotic phenotypes between the cell types examined. For example, oocytes do not exhibit any apparent DNA condensation and degradation, eggs bleb less than other cell types, and embryos exhibit a high background when labeled with TUNEL. We can only speculate that these differences could be caused by a couple of reasons, such as (a) diverse targets of staurosporine (as the repertoire of active kinases present in these disparate cell types is probably different), (b) existence of multiple pathways of apoptosis and hence different downstream targets of the activated apoptotic machinery, and/or (c) physiological differences between the cells (i.e., low metabolic rate of eggs compared with oocytes and embryos).

Our failure to detect chromatin fragmentation in oocytes could be due either to the DNase activation pathway being nonfunctional in this particular cell type, or the fact that major DNA cleavage detected by

\(^1\) E. Davidson, R. A. Cameron. Sea Urchin Genome Project, http://sea-urchin.caltech.edu/genome/.
TUNEL is a late apoptotic event, in fact so late, that cells under our experimental conditions often disintegrate before starting to degrade chromatin. The latter suggestion is consistent with the recent report that DNA cleavage in oocytes of cultured fetal mouse ovaries is less pronounced and probably occurs after the appearance of the morphological features of apoptosis (Morita et al., 1999). Still another possibility is that the TUNEL signal in oocytes is dispersed over a large volume of the nucleus, and thus does not increase beyond the general background, preventing its detection. The significant percentage of untreated embryonic cells exhibiting positive TUNEL staining (25–30%), could be due to intensive DNA replication in these stages, which generates a lot of free 3'-ends detected by end-labeling. Alternatively, it might reflect a high sensitivity to chromatin fragmentation during processing of these cells for the TUNEL assay.

![Figure 6](image1.png)

**Fig. 6.** Dynamics of nuclear changes in eggs and embryos during staurosporine treatment. Shown is the percentage of cells exhibiting DNA condensation or DNA disintegration as assessed by SYTO11 labeling for each time point. White bars: 2 μM staurosporine; gray bars: 5 μM staurosporine. In the bar graphs, hatched and nonhatched portions represent contributions of DNA condensation and degradation respectively to the total percentage of cells demonstrating nuclear changes. A: Eggs treated with 2 or 5 μM staurosporine; B: embryos treated with 2 or 5 μM staurosporine. Data represents pooled results of two independent experiments. The group size varied from 20 to 73 per group. The values indicated by asterisks are significantly different (P < 0.01) than DMSO treated control by a two-sample t-test. STS, staurosporine.

![Figure 7](image2.png)

**Fig. 7.** TUNEL assay detects DNA fragmentation in eggs, but not in oocytes. Cells treated with staurosporine and DMSO-treated controls were processed as described in Materials and Methods, and visualized by epifluorescent microscopy. A–C: Control cells. D–F: staurosporine treated cells. A, DIC image of control egg (top) and two oocytes (bottom); B, Hoechst staining detects pronucleus in the egg, and chromatin in oocytes' nuclei. C, control cells are TUNEL-negative. D, DIC image of an egg (top) and an oocyte (bottom) treated with 5 μM staurosporine for 1 hr. E, Hoechst staining detects pronucleus in the egg, and chromatin in the oocyte's nucleus. F, the egg is TUNEL-positive, while the oocyte in TUNEL-negative. Insets in E and F represent enlarged boxed areas; the egg labeled with TUNEL exhibits condensed and marginized chromatin (compare with Fig. 5J,K).

![Figure 8](image3.png)

**Fig. 8.** TUNEL labeling in staurosporine treated eggs. The percentage of TUNEL positive cells was determined for the different treatments of sea urchin oocytes and eggs. Gray bars represent eggs; oocyte treatment resulted in no detectable TUNEL labeling. Data represents results of two independent experiments. Total number of cells analyzed was 110 per group. The values indicated by asterisks are significantly different (P < 0.01) than DMSO treated control by a two-sample t-test. STS, staurosporine treatment in concentrations noted.
Blebbing appears to underestimate the number of apoptotic eggs compared with caspase activation or changes in chromatin morphology (compare Figs. 2B, 4B, and 6A). Nevertheless, apoptotic eggs exhibit abnormal morphologies even when they don’t bleb (compare apoptotic egg in Fig. 5I to control egg in Fig. 5G). So, blebbing only underestimates the number of apoptotic eggs, but generally 100% of treated cells exhibit abnormal morphology by the end of treatment in one way or another. So why don’t all eggs bleb? Cell blebbing requires extensive reorganization of the actin microfilament network and is inhibited by cytochalasins or altered by microfilament regulatory proteins (Mills et al., 1999). The cortex of a mature, unfertilized sea urchin egg possesses a distinct layer of cortical actin, providing for stability of the cell surface (Spudich, 1992). Thus, eggs may be less efficient than other cell types in rearranging this layer of cortical actin, and hence in blebbing. In addition, blebbing is an energy-dependent process requiring ATP and mature sea urchin eggs are not very active in respiration.

Why is it important for oocytes to have functional apoptotic machinery? The female germ cells of vertebrate as well as invertebrate species are well-known to undergo apoptosis (reviewed in: Morita and Tilly, 1999). Gamete regression is documented to occur in response to starvation and/or seasonal changes in both vertebrates (Asotra and Malhotra, 1978) and invertebrates (Bell and Bohm, 1975; Nimitz, 1976) including sea urchins, and could employ apoptosis (which is compatible with the morphology observed upon gamete regression). Apoptosis could also serve the purpose of elimination of imperfect gametes/embryos (“quality control”) and recent reports suggest that the amounts of mitochondria influence susceptibility of oocytes to undergo apoptosis (Krakauer and Mira, 1999; Perez et al., 2000).

Do sea urchin eggs apoptose if not fertilized? Apoptosis was documented in mouse eggs cultured for prolonged periods in vitro (Perez et al., 1999), and is suggested to occur in starfish eggs upon in vitro culture as well (K. Sasaki and K. Chiba, personal communication). Sea urchin eggs certainly are able to undergo programmed cell death judging from their possession of apoptotic machinery, but they can remain in culture much longer than mouse or starfish eggs, without significant loss of viability or developmental potential. This major difference may reflect differences in reproductive strategies between the animals, and the meiotic state of the cells—mouse and starfish eggs are in the process of meiosis, whereas sea urchin eggs have completed meiosis and are stored as haploid cells with a pronucleus. An interesting consequence of this is that in response to starvation adult sea urchin females must provide a “death signal” for the mature eggs to resorb them, otherwise these cells may not degenerate on their own.

In addition to staurosporine, we have used camptothecin, a chemical known to induce apoptosis by a mechanism distinct from staurosporine. Camptothecin induces apoptosis only in dividing cells by inhibiting topoisomerase II, resulting in DNA damage during replication, and activation of a DNA damage checkpoint. Application of 5 μM camptothecin to the early embryos of sea urchin induced the same apoptotic phenotypes as did staurosporine (data not shown), i.e., chromatin condensation and degradation, and cell blebbing with similar dynamics as did staurosporine. This is consistent with the presence of apoptotic machinery in this model system.

Roccheri et al. (1997) described apoptosis in later stage sea urchin embryos (gastrulae and plutei) and reported that staurosporine at concentrations higher than 50 nM induced necrotic death instead of apoptosis, as assessed by the absence of distinct DNA laddering in the treated cells. Nevertheless, our results, especially caspase activation in dying cells, and the ability to induce specific phenotypes in the early embryos by an alternative drug, are consistent with the apoptotic nature of the observed process. The differences in sensitivity to staurosporine may arise from differences in developmental stages studied (oocytes, eggs, and early embryos vs. gastrula, and plutei), and differences between species (L. variegatus vs. P. lividus). As we have observed differences in apoptotic responses between oocytes, eggs, and early embryos, it is not surprising that later embryonic stages composed of altogether different cell types would respond to staurosporine in different ways.

Presently, apoptosis can be detected in single cells in many different ways but not all of these methods are applicable to sea urchin cell studies. Of the membrane impermeable dyes that are used to assess plasma membrane integrity, we tested SYTOX dye (Molecular Probes), but it resulted in a high background staining in control oocytes and embryos, presumably due to the high endocytosis rate of these cells (we chose not to use trypan blue, as it was reported inefficient in other systems: Leite et al., 1999). Annexin V translocation to the outer leaflet of the membrane assay was not employed in this study either, as the assay buffer is iso-osmotic for PBS, but not to the sea water which is necessary for sea urchin cells.

In this report, we discussed advantages and disadvantages of the different methods of apoptosis detection employed in sea urchin oocytes, eggs, and early embryos. By a combination of simple morphological indicators such as blebbing and chromatin deformations and specific molecular markers (caspase activity) we can now examine death phenotypes in manipulated specimens with some confidence of assessing an apoptotic phenotype, were it to occur.

ACKNOWLEDGMENTS

The authors would like to thank members of the Providence Institute of Molecular Oogenesis for support and thoughtful discussions, and Dr. Zhiyong Han for advice during early stages of this project. We appreciate Dr. Kazuyoshi Chiba’s sharing of unpublished information with us.
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