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Biochemical and Biophysical Research Communications 328 (2005) 150–157
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# UVB radiation prevents skeleton growth and stimulates the expression of stress markers in sea urchin embryos

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Received 6 December 2004
Available online 6 January 2005

**Abstract**

Ozone depletion results in an increased flux of biologically damaging radiations reaching the earth. Although ultraviolet (UV) penetration is attenuated by the seawater, harmful effects can be still observed at low depths where sea urchin embryos are living. We have used *Paracentrotus lividus* embryos to study the impacts of UV radiation on their development. Blastula cultures were exposed to different doses of UVB (312 nm) radiations and the resulting endpoint effects were evaluated in terms of embryonic morphological abnormalities, variations in specific gene expression, and changes in the levels of stress proteins. We found that embryos were moderately sensitive to 50 J/m$^2$ UVB radiation; an increase in the number of developmentally delayed and malformed embryos was detected when increasing doses, up to 1000 J/m$^2$, were used. Major developmental defects, observed 24 and 48 h after exposure, consisted in the failure of skeleton elongation and patterning. Accordingly, we found a reduction in the number of primary mesenchyme cells that expressed *Pl*-SM30, a gene coding for one of the specific matrix proteins of the skeleton. The morphological effects observed 1, 24, and 48 h after exposure were correlated with a dose-dependent increase in the level and in the activation of two recognized stress markers, namely hsp70 and p38 MAPk, respectively, consistent with their role in mediating cellular response to stress and suggesting a function in embryo survival.


*Keywords:* Development; Skeletogenesis; SM30; hsp70; p38MAPk

One of the major concerns among physical impacts on the marine environment relates to ultraviolet rays trespassing the atmosphere. Indeed, accumulating evidence indicates that the ozone layer surrounding our globe is deteriorating, resulting in an increased flux of biologically damaging UVB radiation (280–320 nm) reaching the earth. The deleterious effects of solar UV radiation on aquatic ecosystems have been known since 1925, while in recent times it has been established that dangerous UVB radiation can penetrate, dependent on the optical properties of the water column, up to 20 m of depth [1,2]. As a result, UVB radiation may damage those marine organisms that live most part of their embryonic and larval lives in shallow waters, and perhaps it contributes to mass mortalities that lead to stocks' decline. One of the putative targets are sea urchin embryos, planktonic larvae especially prone to UVB radiation because of their small size, rapid rates of replication and morphogenesis, and lack of specialized protective tissues. Recently, sea urchin embryos have been proposed as a suitable model system for eco-toxicological and environmental studies aimed to the determination of the effects of chemical pollutants in the field or in laboratory experiments [3–8]. However, in most of the studies the investigated endpoint was the frequency of abnormalities; rarely focusing on early events occurring after the environmental insult, involving at first

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doi:10.1016/j.bbrc.2004.12.161

the expression of target genes. Attention to this aspect is particularly relevant for a growing embryo where the proper execution of a developmental program requires well-orchestrated molecular and cellular processes, as well as the correct activity of many different gene products. Among genes involved in the sea urchin embryo developmental program, SM30 is coding for one of the specific matrix proteins synthesized by the primary mesenchyme cells (PMCs) [9], the only cells in the embryo that are essential for the set up of a simple, although well-characterized, skeleton [10,11]. A number of laboratories, including ours, have shown that SM30 expression, while it contributes to the elongation of the growing tips of the embryonic skeleton [12,13], is dependent on environmental and cellular cues [14–16].

The great number of potentially dangerous environmental pollutants dispersed in the sea has called for rapid and sensitive biochemical tests that could reflect their hazardous biological effects. Generally, stress proteins have been recognized as one of the primary defence mechanisms that are activated by the occurrence of denatured proteins. Among these, hsp70 has been taken as a valid tool for the assessment of the health state of a cell. In fact, from lower organisms to human, its synthesis is rapidly up-regulated by a great number of metabolic insults and environmental stressors, including temperature, glucose deprivation, infections, and even cancer [17]. In marine invertebrates hsp70 expression has been taken as an indicator of exposure to pollutants [7,8,18–23], indicating a correlation between high contamination and hsp70 protein levels. Similarly, compelling evidence comes from many laboratories investigating on the activation of the p38 mitogen-activated protein kinase (p38MAPk) induced by various stress stimuli in many systems [24]. In the sea urchin, the activation of p38MAPk, induced by hyperosmotic deciliation of swimming blastulae, has been demonstrated for the first time [25], indicating that the p38MAPk signalling cascade is a common conserved pathway of sensing stress, widespread in the animal kingdom.

In this study, we investigated the putative malfunctions, at the morphological, molecular, and biochemical levels, in sea urchin embryos exposed in laboratory experiments to different doses of UVB radiation, with the aim of simulating embryonic vulnerability occurring in natural seawaters in real life. We found that sea urchin embryos exposed to UVB undergo a severe impairment of morphogenesis, with particular respect to skeleton elongation and patterning, which we showed to be dependent on an equivalent strong reduction in the expression of *Pl*-SM30. The effects observed were dose-dependent and partially reversible after two days. In addition, we showed an increase in the hsp70 protein levels and in the activation of the p38MAPk in UVB exposed embryos. These results confirm the importance of hsp70 and p38MAPk as markers of cell stress and suggest a role for them as protective agents in the acquisition of tolerance and resistance to apoptosis.

## Materials and methods

*Embryo cultures.* Sea urchin adults of the species *Paracentrotus lividus* were collected in the North-Western coast of Sicily and used to obtain gametes. After fertilization, embryos were reared at 16–18 °C in Millipore-filtered seawater in the presence of antibiotics (30 mg/L penicillin and 50 mg/L streptomycin sulfate) at the dilution of 4000/ml in glass beakers, with gentle stirring.

*UVB irradiation and morphological analysis.* Embryos were harvested at the mesenchyme blastula stage, dispensed in 90 mm Petri dishes (20 ml), and irradiated with a 312 nm UVB lamp (Labortechnik, model VL-6.M.) placed at a distance of 6 cm. UVB doses used were: 50, 200, 300, 400, 500, and 1000 J/m$^2$. After irradiation embryos were cultured at 16–18 °C in the dark, to prevent the activity of DNA repairing enzymes. Morphological analyses were performed according to the procedure previously reported [26]. Briefly, about 100 embryos in 100 μl were fixed in 0.1% formaldehyde (f.c.) to prevent swimming and pipetted into chamber slides. Embryos were observed using an inverted microscope (Zeiss Axioscop 2 plus) and images were recorded by a digital camera. To determine the significant effects of UVB exposure, univariate analyses on the percentage of normal or malformed embryos found per each experimental point were performed by ANOVA.

*Whole-mount in-situ hybridization.* Whole-mount in situ hybridizations were performed as previously described [16]. Briefly, all prehybridization and hybridization steps were carried out in 96-well microtiter plates, using 30–40 embryos per well. Fixed embryos were re-hydrated, digested with 10 μg/ml ProteinaseK (Roche) for 20 min at 37 °C, and hybridized to 20 ng/ml single strand sense or anti-sense *Pl*-SM30 DNA probes [16], overnight at 65 °C. After washings, embryos were mounted on glass slides and observed using a Zeiss Axioscop 2 plus inverted microscope; images were recorded by a digital camera. Hybridizations with sense probe showed no specific signal above background.

*SDS–PAGE and Western blot.* After irradiation embryos were harvested at different time intervals, centrifuged at 1200 rpm for 5 min, and the pellets were lysed at 4 °C in a buffer containing: 20 mM Tris–HCl, 2 mM EDTA, 1% NP-40, 15% glycerol, and 2 mM DTT, supplemented with a cocktail of protease inhibitors: 2 μg/μl antipain and leupeptin; 1 μg/μl aprotinin and pepstatin, 1 mM benzamidine, and 0.1 mM PMSF. Lysates were centrifuged at 12,000 rpm for 10 min, at 4 °C; supernatants were collected, dialyzed against 50 mM Tris, pH 7.5, and total protein concentrations were determined by the Lowry method [27]. Amounts of proteins corresponding to 20 μg were lyophilized by speed vacuum centrifugation, re-suspended in the Laemmli SDS–PAGE buffer [28], and denatured at 100 °C for 5 min. Proteins were then separated by 7.5% SDS–PAGE minigels. High and low molecular weight range markers (Bio-Rad) were used. After electrophoresis proteins were transferred to nitrocellulose membranes (Amersham) as reported [29]; blocking with nonfat dried milk in 20 mM Tris, pH 7.6, 137 mM NaCl, and 0.1% Tween 20 (TBS-T). An anti-bovine brain 70 kDa heat shock protein mouse monoclonal antibody (hsp70 McAb) (SIGMA Chemical Company, H-5147) and an anti-phospho-p38MAPkinase (Thr180/Tyr182) rabbit polyclonal antibody (Cell Signalling, 9211) were diluted 1:4000 in TBS-T and 1:1000 in 3%BSA-TBS-T, respectively. Second antibodies used were an anti-mouse peroxidase-conjugated (Amersham–Pharmacia, NA931) and an anti-rabbit peroxidase-conjugated (Cell Signalling, 7074), diluted 1:18000 and 1:2000, respectively, in TBS-T. Proteins were detected by chemiluminescence (Supersignal West Pico, PIERCE) according to manufacturer's instructions. Films were scanned and

band intensities were quantified by the ChemiDoc system (Bio-Rad) equipped with the software Quantity One, version 4.2.1.

## Results

### *Dose-dependent damaging effects of UVB radiation in sea urchin development*

In the present study, we investigated the effects of UV radiation on *P. lividus* embryo development and morphogenesis. In preliminary experiments, in order to define the UVB range of embryo sensitivity, doses lower than 50 J/m$^2$ were used and no morphological effects were observed, even if observation was performed after 66 h (not shown). Conversely, embryos exposed to doses higher than 1000 J/m$^2$ showed severe difficulties in continuing development after the blastula stage, became necrotic and eventually died (not shown). Then, we investigated the ability of mesenchyme blastula stage embryos to carry on normal development until the pluteus stage after exposures to 50, 300, 500 or 1000 J/m$^2$. Results of three independent experiments in which 100 embryos were scored for the presence of malformations 24 h after exposure are shown in Fig. 1. A significant effect ($P < 0.001$) on the percentage of normal embryos scored was found, i.e., the number of delayed and malformed embryos, expressed as one category, increased with the increase in UVB dose used. In particular, at 300 J/m$^2$ the number of embryos with severe malformations was about a 2-fold higher than those having the same developmental patterns as controls. At 500 J/m$^2$ the number of embryos which were not able to develop correctly was near to the total number of exposed embryos. At 1000 J/m$^2$ virtually no embryos were able to develop into well-shaped early plutei. In order to identify which embryonic structures (germ layers, territories) were specifically affected by UVB, embryos were classified under morphological categories on the basis of the developmental defects observed. Figs. 2A–L show different developmental patterns recorded in one representative experiment where embryos were scored at 42 h of development. Observed defects fell into three major categories, defined by perturbed morphologies with respect to controls. The first group consisted of embryos showing an overall delay in development with no aberrant morphologies; as a result, a typical pattern of normal late gastrulae was observed (Figs. 2B and H) when same age controls were found at the pluteus stage (Figs. 2A and G). The second group was composed of embryos which, in addition to a delayed development, showed gut defects; i.e., they failed to elongate an archenteron

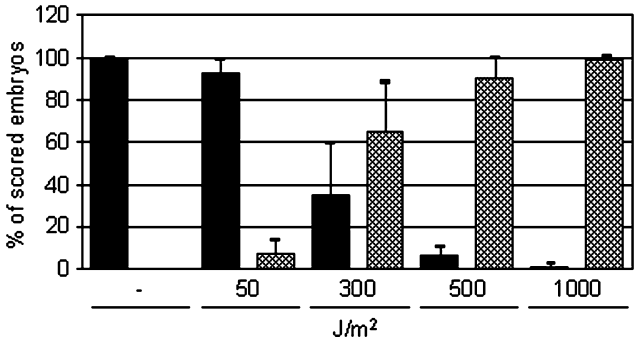


Fig. 1. Perturbation of embryo development correlates with the increase in UVB exposure. Histogram shows the percentage of normal (black bars) and malformed (hatched bars) embryos, exposed to the indicated UVB radiation at the mesenchyme blastula stage, cultured in the dark for 24 h, and microscopically inspected. Each bar represents the mean value ± SD of three independent experiments.

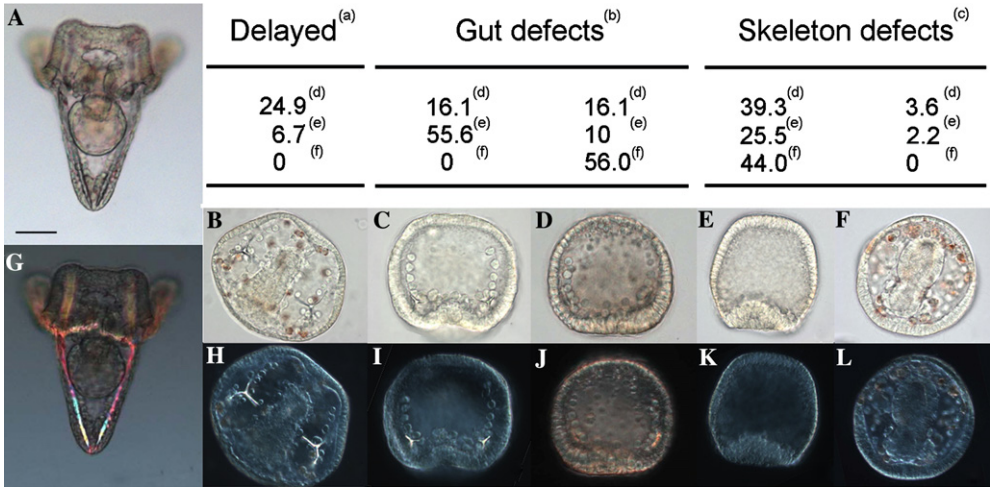


Fig. 2. Different developmental defects in embryos exposed to increasing UVB radiation. Embryos, observed 24 h after exposure, were classified into the three major categories: (a) delayed; (b) gut defects; and (c) skeleton defects. Numbers refer to the percentage of embryos belonging to the above mentioned categories, exposed to: (d) 300 J/m$^2$; (e) 500 J/m$^2$; and (f) 1000 J/m$^2$. Bright field (A–F) and interference contrast (G–L) most representative images for each category. Control embryos (A,G). Bar = 50 μm.

as it would be expected for late gastrula stage embryos. However, PMCs ingressed into the blastocoel at the right time and positioned at the appropriate sites; tri-radiate spicule rudiments raised in the correct positions at the two ventro-lateral sides of the embryo and spicules began to elongate (Figs. 2C,D and I,J). The third group was composed of embryos with major skeleton defects; most of them showed the beginning of archenteron invagination, but no initiation of the triradiate spicule rudiments was observed (Figs. 2E and K). In extreme cases, represented by a small proportion of embryos, a reasonably good differentiation of the gut, denoted by its partial constriction at the sphintere site, was found; but again no spicule rudiments were visible (Figs. 2F and L). The increase in UVB exposure paralleled a correspondent increase in the number of embryos with very aberrant morphologies. Quantitative data, expressed as percentage of the specified morphologically-defective categories observed at a given UVB dose (Fig. 2), indicate that embryos with skeleton defects were first detected at doses as low as 300 J/m$^2$ indicating the skeleton as a target structure damaged by UVB exposure.

We next asked whether the perturbations observed in endoderm and mesoderm derivatives would be permanently found at later stages or if a recovery of the developmental program could be detected. We found that the typical aberrant morphologies were maintained after 66 h of development; UVB exposed embryos were partially inhibited in the differentiation of their endoderm derivatives, as the three parts of the digestive apparatus failed to be properly organized (not shown). In addition, a moderate inhibition of skeleton development was observed in embryos showing a failure either in proper elongation or correct patterning of skeletal rods. As a consequence, UVB exposed embryos had poorly or asymmetrical developed arms (not shown). However, as reported in Table 1, which summarizes results obtained in six different experiments, a partial recovery of embryo development was found, indicating the ability of embryos to overcome the dangerous UVB effect observed after 24 h.

Table 1
Abnormalities in sea urchin embryos exposed to UVB radiation[a]

| | 0 J/m$^2$ | 50 J/m$^2$ | 300 J/m$^2$ | 500 J/m$^2$ | 1000 J/m$^2$ |
|---|---|---|---|---|---|
| N | | | | | |
| M | 91.6 | 68.6 | 75.1 | 62.4 | 53.1 |
| SE | 2.4 | 3.9 | 6.3 | 13.8 | 15.7 |
| G | | | | | |
| M | 0.7 | 1.1 | 5.5 | 6.4 | 24.7 |
| SE | 0.5 | 0.6 | 2.8 | 2.8 | 6.8 |
| S | | | | | |
| M | 7.6 | 14.7 | 19.3 | 31.0 | 21.8 |
| SE | 2.5 | 3.7 | 3.9 | 11.0 | 11.8 |

[a] Numbers refer to the percentage of 100 embryos, scored after 66 h of development, having the following morphologies: N, normal; G, gut defects; and S, skeleton defects. M, mean value; SE, standard error.

### *Expression of a skeleton specific gene is reduced in embryos exposed to UVB*

At the beginning of gastrulation PMCs, a subset of epithelial cells at the vegetal plate, elongate, lose connections with their neighboring cells, and move into the blastocoel. Later, they will become localized in two clusters within the prospective ventrolateral region of the blastocoel. Here PMCs fuse into syncytial cables, which will form the axis of the calcium carbonate spicules of the larval skeleton. Occluded within the spicule is a number of specific extracellular matrix proteins which are important for biomineralization [30] and whose differential expression has been extensively studied in normal and perturbed embryos [12,15,16]. To determine if UVB could affect the expression of skeleton-specific genes, we analyzed the expression of *Pl*-SM30, a gene coding for a spicule matrix protein and known to be active during the spicule elongation phase of skeletogenesis [12,13]. As shown in Fig. 3, which illustrates one of the in situ hybridization experiments performed on whole mount embryos, a significant reduction ($P < 0.001$) in the number of *Pl*-SM30 expressing cells is found in response to an increase in the UVB dose used. Specifically, about 30 positive cells were found in control embryos harvested 42 h after fertilization (Figs. 3A and B), in agreement with our previous study [16]; on the contrary, virtually no *Pl*-SM30-positive cells were found in embryos exposed to the highest dose (1000 J/m$^2$) (Figs. 3K and L). Quantitative information, expressed as the number of *Pl*-SM30-positive cells scored in 30–40 embryos per each experimental point (see Figs. 3C–J), is reported in Fig. 3M. This is consistent with the observed skeleton defective embryos found in the morphological analysis (see Fig. 2). However, a partial recovery of gene expression and subsequent rescue of skeleton development in embryos cultured for longer periods (66 h, see Table 1) is very likely.

### *Sea urchin embryos respond to UVB exposure by increasing the hsp70 protein levels and activating the p38MAPk*

Since the exposure of embryos to UVB radiation resulted in developmental delays and in severe morphogenetic abnormalities, it was interesting to measure the putative expression of recognized stress markers. To the best of our knowledge, no studies on the up-regulation of the constitutive and/or inducible hsp70 levels in sea urchin embryos exposed to UVB have been reported so far. To obtain some information on this issue, we analyzed embryos collected 1, 24, and 48 h after UVB exposure for their hsp70 expression by immunoblotting

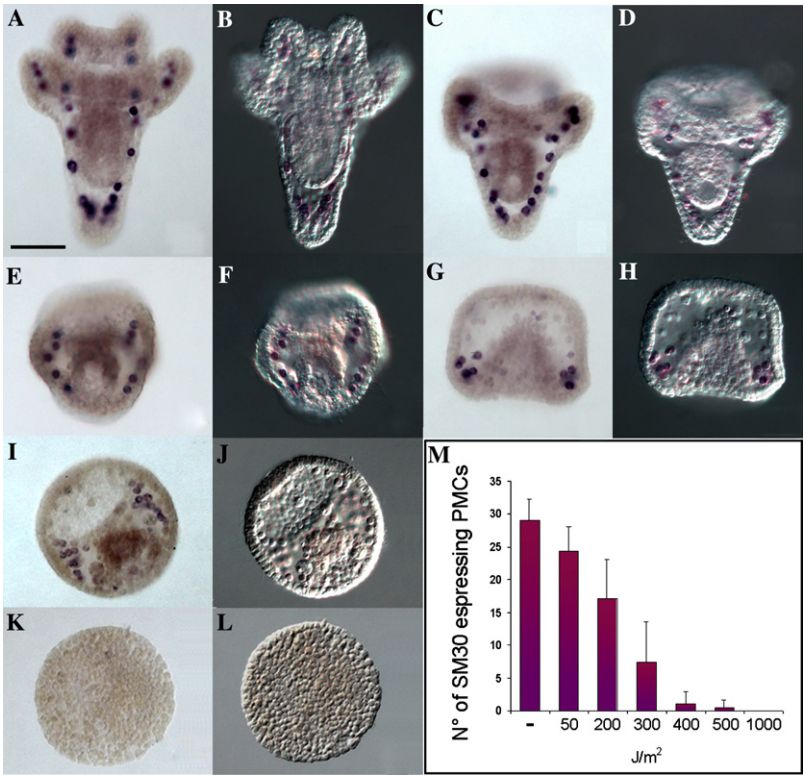


Fig. 3. Effects of UVB exposure on *Pl*-SM30gene expression. Whole mount in situ hybridization of control (A,B) and 50 J/m$^2$ (C,D), 200 J/m$^2$ (E,F), 300 J/m$^2$ (G,H), 400 J/m$^2$ (I,J), and 1000 J/m$^2$ (K,L) UVB exposed embryos. Bar = 50 μm. Histogram shows the number of *Pl*-SM30 expressing PMCs, reported as a mean value ± SD of two independent experiments (M).

on total cell lysates (Fig. 4). We found that embryos are able to promote hsp70 expression as early as 1 h after irradiation, with an apparent increase in the protein levels over control values which was measured as 2.8- and 3.8-fold for 300 or 500 J/m$^2$ exposures and 1000 J/m$^2$, respectively. A dose-dependent increase in hsp70 levels was found also in embryos collected after 24 h. Interestingly, 48 h after irradiation, exposed embryos continued to show high hsp70 levels at the higher doses tested (500 and 1000 J/m$^2$), but show no obvious increase over control values for the lower doses (50 and 300 J/m$^2$). It should be recalled here that commercially available McAbs to hsp70 used, previously shown to cross-react with other marine invertebrate cells [21,31], recognize both constitutive and inducible protein forms, known to be both up-regulated by other stresses [8].

In many organisms, in response to a variety of stresses, including ultraviolet radiation, the activation of the p38MAPk takes place by the phosphorylation of both threonine and tyrosine residues. To establish whether sea urchin embryos activate a p38MAPk signalling cascade in response to UVB, we performed Western blot analysis on exposed embryos using an antibody which specifically recognizes only the (Thr180/Tyr182) phosphorylated p38MAPk (Fig. 5). We found a dose dependent increase in the activation of the p38MAPk in

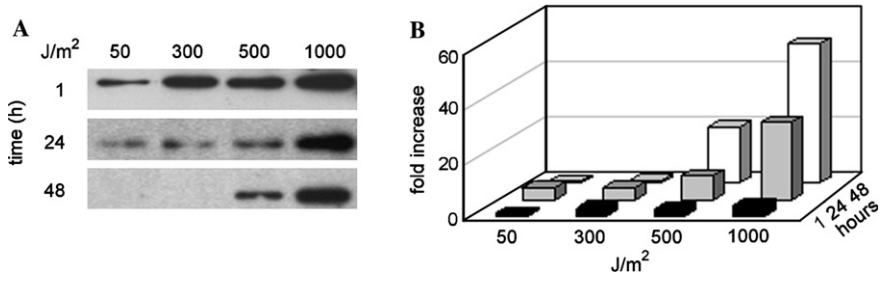


Fig. 4. Increase of hsp70 expression in UVB irradiated sea urchin embryos. Western blot of total lysates from embryos exposed to different doses of UVB, collected 1, 24, and 48 h after irradiation, and reacted with anti-hsp70 (A). Histogram shows the quantification, expressed as fold increase for each experimental point relative to unexposed control samples (B).

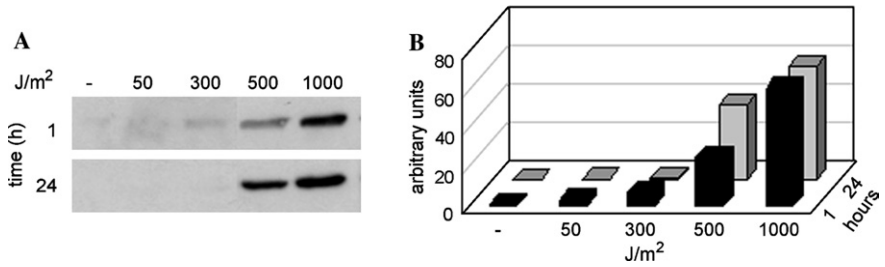


Fig. 5. Sea urchin embryos respond to UVB radiation by the activation of the p38MAPk. Western blot analysis of total lysates from embryos exposed to different doses of UVB, 1 and 24 h after irradiation, and reacted with anti-phospho-p38MAPk (Thr180-Tyr182) (A). The histogram shows results obtained by densitometric scannings of the filters, band intensities are reported in arbitrary units (B).

embryos analyzed 1 h after irradiation. The levels of phosphorylated p38MAPk remained especially high, when compared to control values, in embryos exposed to 500 and 1000 J/m$^2$ observed after 24 h. Western blots, performed in parallel on the same samples using an antibody which reacts specifically with the non-phosphorylated, non-activated form of p38MAPk, showed a band of the same intensity in any of the samples examined (not shown), indicating that p38MAPk protein levels were not changed upon UVB exposure.

## Discussion

In the present study, we analyzed the effects of UVB radiation, artificially produced in the laboratory, on the development of the sea urchin embryo *P. lividus*. In addition, we examined the correlation between the aberrant morphologies produced and the induction of specific responses at the biochemical and molecular levels. We demonstrated, for the first time in this Mediterranean species, that development is seriously impaired when embryos are exposed to UVB at the blastula stage: gastrulation is inhibited or delayed; and skeletogenesis has an abnormal pattern of development or is completely blocked. We found that the severity of malformations is dependent on the UVB dose used and it is partially reversible in the long periods. Our results are in agreement with those reported on the effects of UVB radiation on larvae and early juveniles of *Strongylocentrotus intermedius* [32], which suggest that reproduction of sea urchins might be greatly affected by sunlight in shallow water. Using UVC radiation on the Japanese sea urchin *Hemicentrotus pulcherrimus*, earlier reports described a biphasic inhibition of gastrulation [33] and a complete inhibition of pluteus larva formation [34]. However, although UVC has the highest energy among ultraviolet radiation, UVB is believed to be the most dangerous radiation since, through photochemical absorption, it causes damage to molecules of biological significance [2,35]. The above-mentioned studies paid attention to the toxic effects on development and only in one case correlated morphological abnormalities to the formation and repair of thymine dimers, without any consideration to changes in the expression of specific developmentally significant genes. In this report, we addressed this issue by measuring the expression of *Pl*-SM30, a gene coding for one of the specific matrix proteins of the sea urchin embryo skeleton. We found that *Pl*-SM30 expression is inhibited, in a dose-dependent manner, in embryos exposed to UVB, consistent with the observed reduction in skeleton elongation. In addition, we investigated the levels of hsp70, one of the proteins known to be up-regulated by a wide range of environmental stressors in many animal model systems, including human [36,37]. Originally discovered as a protein induced by heat treatment, its over-expression has been fully documented in the sea urchin embryo in response to a variety of different stimuli [38]. Recently, we demonstrated its increased synthesis in embryos continuously cultured in the presence of sub-lethal cadmium chloride concentrations, a treatment which also caused severe impairment of skeleton development [7]. It was later shown that also nickel and lead induced hsp70 up-regulation, eliciting different levels of the inducible or constitutive forms, according to the metal used [8]. In the present study, we demonstrated for the first time in the sea urchin embryo that the induction of hsp70 expression is triggered by UVB. This is in agreement with studies on hsps involvement in the cellular response to UV radiation [39]. Interestingly, it has been recently reported that hsp70 expression increased resistance to UVB of various cell lines [40], in accordance with emerging evidence claiming for a hsps role in resistance to apoptosis [41], defence mechanisms, and immunity [17]. Similarly, it has been recently shown that exposure of *Strongylocentrotus droebachiensis* embryos to UVB (290–320 nm) causes an increase in the protein content of the transcriptional activators *p*53 and *p*21, and a concomitant significantly lower protein concentration of the cell cycle downstream activator cdc2, which leads to apoptosis [42]. On the other hand, we have previously shown that 20% of early pluteus cells undergo apoptosis under physiological conditions [43], thus confirming the presence of a functional apoptotic machinery. Therefore, on the basis of previous

observations and present results we propose that one way the embryos have to cope with dangerous UVB radiation is to elevate the levels of hsp70 as protection to apoptosis.

Last, we present the first evidence that sea urchin embryos elevate the levels of the phosphorylated, activated form, of the p38MAPk in response to UVB. Our results are in agreement with what is known for keratinocytes where the activation of the p38MAPk has been proven to be protective against acute damaging effects caused by UVB [44].

In general, stress signalling triggered by acute UVB results in either cell cycle arrest or apoptosis. In the first case, affected cells have the time to repair the damaged genome before progressing through DNA replication and cell division [44]. This possibility could account for the delay in the developmental program observed in a certain proportion of UVB exposed sea urchin embryos described in this paper. Alternatively, severely damaged cells in the embryos might be eliminated by programmed cell death, as previously shown [42]. Interestingly, p53 has a role in both promotion of apoptosis and enhancement of DNA repair. Similarly, the p38MAPk, known to phosphorylate p53, thus increasing its stability and activity, has been reported to prevent [45] or induce [46] apoptosis of keratinocytes in response to UVB. As a consequence, we can assume that the UVB-mediated activation of the p38MAPk and the balance in its many downstream activated targets will determine whether sea urchin embryos exposed to UVB undergo to apoptosis or become stress resistant and survive. Since we detected persistently high hsp70 levels 24 h after UVB exposure at low doses (50 and 300 J/m$^2$), in contrast with a decline in the p38MAPk activation found after the same period of time, we can suppose that hsp70 is located downstream on a hypothetical linear cascade in the p38 signal transduction pathway, as suggested by other authors in cells over-expressing hsp70 in response to cadmium [47]. The fact that embryos exposed to low UVB doses (50, 300 J/m$^2$) showed low, if any, hsp70 protein levels and activated p38MAPk, after 48 or 24 h, respectively, can be explained either by a negative feedback on the p38MAPk itself or by the inactivation of any of the components of the UVB activated MAP kinase cascade. Further studies are needed to determine the precise role of each of the factors involved in the UVB stress activated pathways in the sea urchin embryogenesis.

In conclusion, we propose that increased hsp70 levels as well as p38MAPk activation might provide an adaptive cellular response of UVB exposed embryos and increase the probability of their survival. Finally, hsp70 and p38MAPk detection, together with that of UVB induced genes, could be used as a useful molecular biomarker to identify sunlight dangerous effects occurring in marine organisms living in shallow waters.

## Acknowledgments

This research has been partially supported by an EU program to V. Matranga, Contract No. EVK3-CT-1999-00005. Partial support was also obtained by the ASI Contract No. I/R/073/01.

## References

[1] R.C. Smith, B.B. Prezelin, K.S. Baker, R.R. Bidigare, N.P. Boucher, T. Coley, D. Karentz, S. MacIntyre, H.A. Matlick, D. Menzies, Ozone depletion: ultraviolet radiation and phytoplankton biology in antarctic waters, Science 255 (1992) 952–959.

[2] N.L. Adams, J.M. Shick, Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of green sea urchin *Strongylocentrotus droebachiensis*, Mar. Biol. 138 (2001) 267–280.

[3] G. Radenac, D. Fichet, P. Miramand, Bioaccumulation and toxicity of four dissolved metals in *Paracentrotus lividus* sea-urchin embryo, Mar. Environ. Res. 51 (2001) 151–166.

[4] R. Beiras, J. Bellas, N. Fernandez, J.I. Lorenzo, A. Cobelo-Garciam, Assessment of coastal marine pollution in Galicia (NW Iberian Peninsula); metal concentrations in seawater, sediments and mussels (*Mytilus galloprovincialis*) versus embryo-larval bioassays using *Paracentrotus lividus* and *Ciona intestinalis*, Mar. Environ. Res. 56 (2003) 531–533.

[5] R. Russo, R. Bonaventura, F. Zito, H.C. Schroder, I. Muller, W.E. Muller, V. Matranga, Stress to cadmium monitored by metallothionein gene induction in *Paracentrotus lividus* embryos, Cell Stress Chaperones 8 (2003) 232–241.

[6] D. Pesando, P. Huitorel, V. Dolcini, C. Angelini, P. Guidetti, C. Falugi, Biological targets of neurotoxic pesticides analysed by alteration of developmental events in the Mediterranean sea urchin, *Paracentrotus lividus*, Mar. Environ. Res. 55 (2003) 39–57.

[7] M.C. Roccheri, M. Agnello, R. Bonaventura, V. Matranga, Cadmium induces the expression of specific stress proteins in sea urchin embryos, Biochem. Biophys. Res. Commun. 321 (2004) 80–87.

[8] F. Geraci, A. Pinsino, G. Turturici, R. Savona, G. Giudice, G. Sconzo, Nickel, lead, and cadmium induce differential cellular responses in sea urchin embryos by activating the synthesis of different HSP70s, Biochem. Biophys. Res. Commun. 322 (2004) 873–877.

[9] N.C. George, C.E. Killian, F.H. Wilt, Characterization and expression of a gene encoding a 30.6-kDa *Strongylocentrotus purpuratus* spicule matrix protein, Dev. Biol. 147 (1991) 334–342.

[10] K. Okazaki, Spicule formation by isolated micromeres of the sea urchin embryo, Am. Zool. 15 (1975) 567–581.

[11] N. Armstrong, D.R. McClay, Skeletal pattern is specified autonomously by the primary mesenchyme cells in sea urchin embryos, Dev. Biol. 162 (1994) 329–338.

[12] K.A. Guss, C.A. Ettenshon, Skeletal morphogenesis in the sea urchin embryo: regulation of primary mesenchyme gene expression and skeletal rod growth by ectoderm-derived cues, Development 124 (1997) 1899–1908.

[13] T. Kitajima, H. Urakami, Differential distribution of spicule matrix proteins in the sea urchin embryo skeleton, Dev. Growth Differ. 42 (2000) 295–306.

[14] T. Kitajima, M. Tomita, C.E. Killian, K. Akasaka, F.H. Wilt, Expression of spicule matrix protein gene SM30 in embryonic and adult mineralized tissues of sea urchin *Hemicentrotus pulcherrimus*, Dev. Growth Differ. 38 (1996) 687–695.

[15] L.A. Urry, P.C. Hamilton, C.E. Killian, F.H. Wilt, Expression of spicule matrix proteins in the sea urchin embryo during normal and experimentally altered spiculogenesis, Dev. Biol. 225 (2000) 201–213.
[16] F. Zito, C. Costa, S. Sciarrino, V. Poma, R. Russo, L.M. Angerer, V. Matranga, Expression of univin, a TGF-beta growth factor, requires ectoderm–ECM interaction and promotes skeletal growth in the sea urchin embryo, Dev. Biol. 264 (2003) 217–227.
[17] J. Robert, Evolution of heat shock protein and immunity, Dev. Comp. Immunol. 27 (2003) 449–464.
[18] W.E.G. Müller, R. Batel, M. Lacorn, H. Steinhart, T. Simat, S. Lauenroth, H. Hassanein, H.C. Schröder, Accumulation of cadmium and zinc in the marine sponge *Suberites domuncula* and its potential consequences on single-strand breaks and on expression of heat-shock protein: a natural field study, Mar. Ecol. Prog. Ser. 167 (1998) 127–135.
[19] H.C. Schröder, H.M.A. Hassanein, S. Lauenroth, C. Koziol, T.A.-A.A. Mohamed, M. Lacorn, H. Steinhart, R. Batel, W.E.G. Müller, Induction of DNA strand breaks and expression of HSP70 and GRP78 homolog by cadmium in the marine sponge *Suberites domuncula*, Arch. Environ. Contam. Toxicol. 36 (1999) 47–55.
[20] H.C. Schröder, R. Batel, H.M.A. Hassanein, S. Lauenroth, H.S. Jenke, T. Simat, H. Steinhart, W.E.G. Müller, Correlation between the level of the potential biomarker, heat-shock protein, and the occurence of DNA damage in the dab, *Limanda limanda*: a field study in the Northern Sea and English Channel, Mar. Environ. Res. 40 (2000) 201–215.
[21] V. Matranga, G. Toia, R. Bonaventura, W.E.G. Muller, Cellular and biochemical responses to environmental and experimentally induced stress in sea urchin coelomocytes, Cell Stress Chaperones 5 (2000) 112–113.
[22] V. Matranga, R. Bonaventura, G. Di Bella, Hsp70 as a stress marker of sea urchin coelomocytes in short term cultures, Cell. Mol. Biol. 48 (2002) 345–349.
[23] V. Matranga, R. Bonaventura, Sea urchin coelomocytes the progenitors of vertebrate immune effectors, as bio-indicators of stress and pollution, in: Y. Yokota, V. Matranga, Z. Smolenicka (Eds.), The Sea Urchin: From Basic Biology to Aquaculture, Balkema Publishers, Lisse, The Netherlands, 2002, pp. 161–176.
[24] L. New, J. Han, The p38 MAP kinase pathway and its biological function, Trends Cardiovasc. Med. 8 (1998) 220–228.
[25] C. Casano, M.C. Roccheri, L. Maenza, S. Migliore, F. Gianguzza, Sea urchin deciliation induces thermoresistance and activates the p38 mitogen-activated protein kinase pathway, Cell Stress Chaperones 8 (2003) 70–75.
[26] F. Zito, V. Tesoro, D.R. McClay, E. Nakano, V. Matranga, Ectoderm cell–ECM interaction is essential for sea urchin embryo skeletogenesis, Dev. Biol. 196 (1998) 184–192.
[27] O.H. Lowry, N.Y. Rosebrough, L.A. Farr, R. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–276.
[28] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[29] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.
[30] F.H. Wilt, Biomineralization of the spicules of sea urchin embryos, Zool. Sci. 19 (2002) 253–261.
[31] C. Koziol, R. Batel, E. Arinc, H.C. Schröder, W.E.G. Müller, Expression of the potential biomarker heat shock protein70 and its regulator, the metazoan DnaJ homolog, by temperature stress in the sponge *Geodia cydonium*, MEPS 154 (1997) 261–268.
[32] K. Kagoshima, M. Makino, S. Onodera, M. Suzuki, K. Yabe, The effects of ultraviolet-irradiation on larvae and early juveniles of the sea-urchin, *Strongylocentrotus intermedius*, Nippon Suisan Gakkaishi 64 (1998) 606–609.
[33] S. Amemiya, S. Yonemura, S. Kinoshita, T. Shiroya, Biphasic stage sensitivity to UV suppression of gastrulation in sea urchin embryos, Cell Diff. 18 (1986) 45–49.
[34] Y. Akimoto, T. Shiroya, Photoreversibility of UV-induced thiamine dimmers and abnormal morphogenesis in sea urchin embryos, Photochem. Photobiol. 45 (1987) 403–406.
[35] D.P. Nigel, D. Gwynn-Jones, Ecological roles of solar UV radiation: towards an integrated approach, Trends Ecol. Evol. 18 (2003) 48–55.
[36] W.J. Welch, Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease, Physiol. Rev. 72 (1992) 1063–1081.
[37] I. Mukhopadhyay, A. Nazir, D.K. Saxena, D.K. Chowdhuri, Heat shock response: hsp70 in environmental monitoring, J. Biochem. Mol. Toxicol. 17 (2003) 249–254.
[38] G. Giudice, G. Sconzo, M.C. Roccheri, Studies on heat shock proteins in sea urchin development, Dev. Growth Differ. 41 (1999) 375–380.
[39] F. Trautinger, I. Kindas-Mugge, R.M. Knobler, H. Honigsmann, Stress proteins in the cellular response to ultraviolet radiation, J. Photochem. Photobiol. B 35 (1996) 141–148.
[40] S.B. Kwon, C. Young, D.S. Kim, H.O. Choi, K.H. Kim, J.H. Chung, H.C. Eun, K.C. Park, C.K. Oh, J.S. Seo, Impaired ability of *hsp70.1* KO mouse after UVB irradiation, J. Dermat. Sci. 28 (2002) 144–151.
[41] A. Samali, T.G. Cotter, Heat shock proteins increase resistance to apoptosis, Exp. Cell Res. 223 (1996) 163–170.
[42] M.P. Lesser, V.A. Kruse, T.M. Barry, Exposure to ultraviolet radiation causes apoptosis in developing sea urchin embryos, J. Exp. Biol. 206 (2003) 4097–4103.
[43] M.C. Roccheri, C. Tipa, R. Bonaventura, V. Matranga, Physiological and induced apoptosis in sea urchin larvae undergoing metamorphosis, Int. J. Dev. Biol. 46 (2002) 801–806.
[44] J. Hildesheim, A.J. Fornace Jr, The dark side of light: the damaging effects of UV rays and the protective efforts of MAP kinase signaling in the epidermis, DNA Repair (Amst). 3 (2004) 567–580.
[45] N. Chouinard, K. Valerie, M. Rouabhia, J. Huot, UVB-mediated activation of p38 mitogen-activated protein kinase enhances resistance of normal human keratinocytes to apoptosis by stabilizing cytoplasmic p53, Biochem. J. 365 (2002) 133–145.
[46] A. Van Laethem, S. Van Kelst, S. Lippens, W. Declercq, P. Vandenabeele, S. Janssens, J.R. Vandenheede, M. Garmyn, P. Agostinis, Activation of p38 MAPK is required for Bax translocation to mitochondria cytochrome c release and apoptosis induced by UVB irradiation in human keratinocytes, FASEB J. 23 (2004) (Epub ahead of print).
[47] J.J. Hung, T.J. Cheng, Y.K. Lai, M.D. Chang, Differential activation of p38 mitogen-activated protein kinase and extracellular signal-regulated protein kinases confers cadmium-induced HSP70 expression in 9L rat brain tumor cells, J. Biol. Chem. 273 (1998) 31924–31931.