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Overview of the molecular defense systems used by sea urchin embryos to cope with UV radiation

Rosa Bonaventura*, Valeria Matranga

Consiglio Nazionale delle Ricerche, Istituto di Biomedicina e Immunologia Molecolare "Alberto Monroy", Via Ugo La Malfa 153, 90146 Palermo, Italy

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This review is dedicated to the memory of Valeria Matranga who did not live enough to see this work published.

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ABSTRACT

The sea urchin embryo is a well-recognized developmental biology model and its use in toxicological studies has been widely appreciated. Many studies have focused on the evaluation of the effects of chemical stressors and their mixture in marine ecosystems using sea urchin embryos. These are well equipped with defense genes used to cope with chemical stressors. Recently, ultraviolet radiation (UVR), particularly UVB (280–315 nm), received more attention as a physical stressor. Mainly in the Polar Regions, but also at temperate latitudes, the penetration of UVB into the oceans increases as a consequence of the reduction of the Earth's ozone layer. In general, UVR induces oxidative stress in marine organisms affecting molecular targets such as DNA, proteins, and lipids. Depending on the UVR dose, developing sea urchin embryos show morphological perturbations affecting mainly the skeleton formation and patterning. Nevertheless, embryos are able to protect themselves against excessive UVR, using mechanisms acting at different levels: transcriptional, translational and post-translational. In this review, we recommend the sea urchin embryo as a suitable model for testing physical stressors such as UVR and summarize the mechanisms adopted to deal with UVR. Moreover, we review UV-induced apoptotic events and the combined effects of UVR and other stressors.

1. Introduction

In the last decade, the interest on the evaluation of UV radiation (UVR) effects on aquatic and terrestrial ecosystems was high considering the environmental increase in UV irradiance (Andrady et al., 2012; The EEAP Report, 2014). Since the early 1900s, many studies used Echinoderms as model systems to evaluate the biological effects of UVR and understand how marine organisms protect themselves, as recently reviewed by Lamare et al. (2011).

Among Echinoderms, the sea urchin embryo is beyond doubt an attractive model of study, which has played a key role in the fields of embryology, developmental and molecular biology, and has been unsurpassed for *in vivo* observations (Monroy, 1986; Ernst, 2011). Moreover, the sea urchin embryo is a widely used model in ecotoxicological studies to analyze the molecular defense systems to cope with: i) pollutants, as metals (Roccheri and Matranga, 2009; Pinsino et al., 2014); ii) climate change factors, as CO_2-increase, acidification and ocean warming (Byrne, 2011); iii) nanoparticles (NPs) (Corsi et al., 2014; Della Torre et al., 2014; Gambardella et al., 2015).

1.1. UVR in the marine environment

The wavelengths "beyond violet", i.e. the ultraviolet radiation (UVR), are a component of the electromagnetic radiation emitted by the Sun that are conventionally divided into UVC (200–280 nm), UVB (280–320 nm) and UVA (320–400 nm). The Earth's atmosphere strongly absorbs UVC and provides some shielding to UV-A and UV-B that reach the biosphere (Maverakis et al., 2010).

In the marine environment, the transmission of solar UVR depends on many variables, as for example the presence of dissolved materials and phytoplankton that in turn affect the amount and wavelength distribution of UVR (Häder et al., 2011). UVA can penetrate deeper than UVB, reaching depths between 40 and 60 m in clear ocean waters, affecting marine organisms differently distributed in the water column (Smith et al., 1992; Tedetti and Sempéré, 2006). Generally, UVR can ionize molecules and thereby induce chemical reactions that in turn can be harmful to organisms by affecting their DNA, proteins, and lipids (Dahms and Lee, 2010). In particular, UVB has negative effects on primary producers, as

* Corresponding author.
E-mail address: rosa.bonaventura@ibim.cnr.it (R. Bonaventura).

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cyanobacteria, phytoplankton, macroalgae and aquatic plants, as well as on many aquatic consumers, as zooplankton, crustaceans, amphibians, fishes and corals (Häder et al., 2011).

The ecological importance of UVR led many studies towards the need to understand their biological effects. In addition, the discovery of the ozone-hole in Antarctica in the 1980s has strongly increased the attention of scientists on the hazardous effects of UVR. The ozone-hole is now slowly recovering since 1989, when the Montreal Protocol entered into force, although an episodic decrease of ozone was observed in the Artic region in spring 2011 (The EEAP Report, 2014). This apparent contradiction is due to changes in factors other than ozone depletion, as clouds, air pollution (including aerosols) and surface albedo, as well as the interactions between ozone depletion and climate change (increasing greenhouse gases) (McKenzie et al., 2011; The EEAP Report, 2014). All these factors cause a large variability in UVB radiation, also outside the Polar Regions, and have important implications for both ecosystems and human health (McKenzie et al., 2011; The EEAP Report, 2014).

1.2. Sea urchin embryo as a model to study UVR

As many marine invertebrates, sea urchins release their gametes into the water column where fertilization occurs producing planktonic embryos and larvae that, depending on the species, need days or months to undergo metamorphosis and become juveniles (little sea urchins) (McEdward and Miner, 2001). During development, embryos have to cope with many adverse environmental conditions, including UVR, as well as many other pollutants that can affect their development, and consequently the success of their reproduction (Hamdoun and Epel, 2007).

Starting from the late 1980s, the sea urchin embryos have been exploited not only in laboratory but also in field studies to evaluate the UVR response, although the available data concerning the molecular mechanisms activated following UVR exposure are still fragmented (Dahms and Lee, 2010; Lamare et al., 2011; Adams et al., 2012). Nevertheless, many protection strategies have been highlighted, including the avoidance, i.e. negative phototaxis to UVR exposure, and other non-protein defensive strategies, i.e. sunscreen compounds as Mycosporine-like amino acids (MAAs) and carotenoids (Lamare et al., 2011; Lamare and Barker, 2013).

Here we gathered and elaborated all available information on the UVR molecular defense systems operating in the sea urchin embryo at: i) transcriptional, ii) translational and iii) post-translational levels, both in laboratory and field experiments. In addition, we correlated the activated molecular defense systems to the alternative morphologies induced by UVR, which mainly affected the embryonic skeleton. Moreover, we analyzed the embryonic defense response, which had been activated after the combined exposure of UVR and other stressors.

2. UVR effects on embryonic development

One of the advantages in the use of sea urchin embryos as a model is their transparency that easily allows the evaluations of the morphological effects caused by chemical and physical agents (Matranga et al., 2011). In many studies, gametes were irradiated before fertilization and the delay in cleavage analyzed afterwards. Table 1 summarizes data obtained in different studies, taking into account the i) emission properties of artificial lamps, ii) types of exposure, iii) stage chosen to irradiate gametes or embryos, both in laboratory and field experiments. For example, eggs from the European species *Sphaerechinus granularis* and *Paracentrotus lividus* have been irradiated with 65.6 kJ/m^2 UVA and 7.6 kJ/m^2 UVB, but following fertilization, only *S. granularis* embryos showed a delay or an inhibition in the first cell cleavage (Nahon et al., 2008). Later in development, abnormal plutei have been observed for both species with apically crossed body rods (percentage of normal plutei: 5.06% ± 1.07 SD for *S. granularis* and 38.46% ± 21.99 for *P. lividus* embryos) (Nahon et al., 2008). Exposure of *Echinometra lucunter* eggs to UVB with doses ranging from 0.9 to 7.2 kJ/m^2 inhibited the first and second cleavage in a dose-dependent manner (Leite et al., 2014). The characteristic dose-dependent delay in cleavage has also been observed in *Strongylocentrotus purpuratus* embryos irradiated at 30 and 90 min post fertilization with a total dose of 41.31 kJ/m^2 UVR (290–400 nm) and delivered over a period of 60 min (Campanale et al., 2011).

P. lividus embryos irradiated during cleavage using UVB doses ranging from 0.01 to 0.8 kJ/m^2 showed abnormal morphologies at 24 h post irradiation (hpi), starting from the dose of 0.15 kJ/m^2. At the highest doses used, i.e. 0.4 and 0.8 kJ/m^2 UVB, about 85.7% and 93.6% were abnormal embryos that lacked an organized epithelium and showed the blastocoelic cavity completely filled with cells (Bonaventura et al., 2006). This morphology was very similar to the so called "permanent" blastula, obtained irradiating *Hemicentrotus pulcherrimus* embryos with UVC at 0.038 and 0.45 kJ/m^2 (Amemiya et al., 1986), and to the so called "packed" blastula, obtained irradiating *S. purpuratus* embryos 20 min after fertilization onwards with PAR + UVA + UVB (with cycles of 12 h light/dark) (Adams and Shick, 2001). Observations performed later in development (48hpi) showed that 100% of 0.8 kJ/m^2 irradiated embryos were "packed blastula", while 80% of the 0.4 kJ/m^2 irradiated embryos were "packed blastula" with or without short skeleton elements (spicules), when control embryos were plutei (Bonaventura et al., 2006). Using the widely used skeletogenic cell marker, MSP130, in *P. lividus* embryos irradiated with 0.2 kJ/m^2 and analyzed after 24 h, we found that some of the cells inside the blastocoel of the abnormal blastula (Fig. 1B) were skeletogenic cells (Fig. 1D), even though they lacked the typical organization (Fig. 1C) of the normal embryo (Fig. 1A), see also Table 2.

P. lividus embryos exposed to UVB at the stage of mesenchyme blastula (Bonaventura et al., 2005) seemed to be more resistant to UVB if compared to embryos irradiated at early stages (Bonaventura et al., 2006), since they could tolerate the dose of 1.0 kJ/m^2. In particular, when control embryos were at the pluteus stage, 0.3 kJ/m^2 irradiated embryos showed developmental delays and/or absence of skeleton and gut at 24hpi. At the same time, embryos irradiated with 1.0 kJ/m^2 were nearly all blastulae- and early gastrulae-like embryos without skeleton and gut (Bonaventura et al., 2005). Whole mount *in situ* hybridization (WMIH) experiments using a *Pl-SM30* DNA probe showed a dose-dependent reduced number of the skeletogenic cells expressing SM30 mRNA, a gene coding for one of the skeleton matrix proteins of the sea urchin embryo. In UVB-embryos exposed to 1.0 kJ/m^2, *Pl-SM30*-positive cells were absent indicating that the skeleton was a target structure damaged by UVB (Bonaventura et al., 2005), see also Table 2. Similarly, X-rays caused a reduced expression of the skeleton markers SM30 and msp130 in irradiated embryos, as assessed by RT-PCR and by WMISH respectively (Matranga et al., 2010).

Using an appropriate floating devise for field experiments, *Sterechinus neumayeri* embryos have been suspended at 1, 3, and 5 m below the ice soon after fertilization and exposed for 5 days to ambient UVR (UVA and UVB) that was measured *in situ* (Lesser et al., 2004). Experiments carried out in spring 2002 and 2003 showed that UVR exposure determined significant mortality in embryos and caused abnormal development, i.e. "packed blastulae" among the survivor embryos (Lesser et al., 2004). Other field experiments showed different species-specific sensitivities, as indicated by the abnormal morphologies observed in exposed embryos from different geographic distributions: i) "packed blastulae" in