Proteomic Responses of Sea Urchin Embryos to Stressful Ultraviolet Radiation

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Synopsis Solar ultraviolet radiation (UVR, 290–400 nm) penetrates into seawater and can harm shallow-dwelling and planktonic marine organisms. Studies dating back to the 1930s revealed that echinoids, especially sea urchin embryos, are powerful models for deciphering the effects of UVR on embryonic development and how embryos defend themselves against UV-induced damage. In addition to providing a large number of synchronously developing embryos amenable to cellular, biochemical, molecular, and single-cell analyses, the purple sea urchin, Strongylocentrotus purpuratus, also offers an annotated genome. Together, these aspects allow for the in-depth study of molecular and biochemical signatures of UVR stress. Here, we review the effects of UVR on embryonic development, focusing on the early-cleavage stages, and begin to integrate data regarding single-protein responses with comprehensive proteomic assessments. Proteomic studies reveal changes in levels of post-translational modifications to proteins that respond to UVR, and identify proteins that can then be interrogated as putative targets or components of stress-response pathways. These responsive proteins are distributed among systems upon which targeted studies can now begin to be mapped. Post-transcriptional and translational controls may provide early embryos with a rapid, fine-tuned response to stress during early stages, especially during pre-blastula stages that rely primarily on maternally derived defenses rather than on responses through zygotic gene transcription.

Introduction

Solar ultraviolet radiation (UVR) has been known to negatively effect organisms since the late 19th century (Downes and Blunt 1877). Studies in the early 1900s revealed that exposure of echinoderm eggs and embryos to UVR resulted in dose-dependent delays in cell division (Giese 1938; Blum et al. 1954) and subsequently characterized the morphological abnormalities UVR caused in echinoderm larvae, including death (Giese 1964; Rustad 1964). Today, echinoderms remain the focus of many reports on the developmental and physiological effects of UVR (reviewed by Dahms and Lee 2010; Lamare et al. 2011). Many insights have been gained from the large body of knowledge relating to effects of UVR on proteins in mammalian cells, but a comprehensive examination of proteins affected by UVR in sea urchin embryos has been lacking. Recently, a comparative proteomic analysis of the embryos of Strongylocentrotus purpuratus (California purple sea urchin) revealed a number of proteins that respond to UVR during the first mitotic cleavage (Campanale et al. 2011). Here, we collectively evaluate these data to decipher clues about the molecular mechanisms of UVR-induced damage, defensive mechanisms, and the cellular basis controlling responses to UVR-stress.

While there have been reports evaluating effects of UVR on DNA, and the response of specific transcripts, only a few reports have identified UV-responsive proteins (Dahms and Lee 2010; Lamare et al. 2011).
Characterization of ultraviolet radiation in the marine environment: From molecules, to organisms, to ecosystems

Solar UVR reaching the Earth’s surface (UVR: 290–400 nm) significantly penetrates into seawater and can harm marine organisms (Bancroft et al. 2007; Häder et al. 2011). Ultraviolet A (UV-A) wavelengths (320–400 nm) are longer and penetrate deeper than those of ultraviolet B (UV-B; 290–320 nm; Smith and Baker 1979); however, UV-B is more biologically damaging than UV-A (Tevini 1993). Penetration of UV-B wavelengths has increased due to ozone depletion (Madronich 1998; WMO 2007) and despite global reductions in the release of ozone-depleting gases and stabilization of ozone levels, UV-B irradiance is predicted to increase by a few percent per decade due to global climatic change (McKenzie et al. 2003, 2007; WMO 2007). Similarly, penetration of UV in coastal oceans is expected to increase due to global warming and acidification of seawater. Increased UV-irradiation can photobleach UV-absorbing chromophoric dissolved organic matter (CDOM) and increase penetration of UV into the water column, leading to synergistic stresses in marine communities (Whitehead et al. 2000; Przeslawski 2005; UNEP 2005; Häder et al. 2011; Zepp et al. 2011).

UV-B causes direct damage to proteins, DNA, and membrane lipids. In addition to photochemical degradation of proteins, UV-B directly damages DNA through the production of cyclobutane pyrimidine dimers (CPDs) and other photoproducts (Vincent and Neale 2000; Sinha and Häder 2002). Reactive oxygen species (ROS) generated by UV-A, rapidly oxidize nucleic acids, proteins, and lipids (Tyrell 1991; Cadet et al. 2003; Lesser 2006). This UV-induced molecular damage is manifested as physiological stress, fundamentally affecting survivorship of embryonic, and larval stages that live at the ocean’s surface (Gleason and Wellington 1996; Przeslawski et al. 2005; Lamare et al. 2011; Häder et al. 2011). Eventually, these stressors delay development and increase mortality both directly and indirectly (Pechenik 1987; Worrest 1982; Karentz 1994; Whitehead et al. 2000; Häder et al. 2011), and ultimately alter trophic interactions and food webs (Bothwell et al. 1994; Caldwell et al. 1998; Mostajir et al. 2000; Häder and Sinha 2005).

Sea urchins as a model for studying effects of UVR

Echinoid embryos are well suited for quantitative studies of UV-induced damage. Their gametes are easily obtained, fertilized, and cultured and they have been used extensively as a model for elucidating the effects of environmental factors such as temperature, oxidative stress, chemical pollutants, and UVR since the early 1900s (Dahms and Lee 2010; Lamare et al. 2011). Large eggs (80–200 μm diameter) allow single-cell analyses, while abundant quantities of synchronously developing embryos facilitate molecular and biochemical analyses. Like many organisms, early sea urchin embryos rely on a robust pool of mRNA and proteins provided maternally and that control processes necessary for initiating and deploying the developmental program (Davidson et al. 1998; Davidson 2006a). Thus, the early sea urchin embryo develops and responds to stressors primarily through post-transcriptional and post-translational mechanisms (reviewed by Epel 2003).

Additionally, the sequencing of the S. purpuratus genome (Sea urchin Genome Consortium 2006; Sodergren et al. 2006) and description of the developmental transcriptome (Samanta et al. 2006) led to identification of the genes implicated in a variety of biological processes (Davidson 2006b). Significantly, the arsenal of gene products for chemical defense, including oxidative stress proteins, are now annotated (Goldstone et al. 2006). These surveys of the genome provide a platform for predicting and analyzing the proteins that are involved in responses to stresses such as UVR.

UV and sea urchin embryos

Developmental effects of UVR on sea urchin embryos

A large body of historical work documenting the effects of UVR on sea urchin embryos and larvae serves as an instructive base for molecular studies (reviewed by Häder et al. 2011; Lamare et al. 2011). The effects of UVR are both dose-dependent and wavelength-dependent. Sub-lethal doses lead to delays in development while higher doses or lower wavelengths arrest development or cause-death (Fig. 1; Rustad 1971; Adams and Shick 2001; Lamare et al. 2011). Experiments using controlled doses of environmentally-relevant UVR have allowed for quantitative assessments of developmental delays and qualitative morphological aberrations (reviewed by Dahms and Lee 2010; Lamare et al. 2011). Furthermore, in situ studies demonstrate that both solar UV-A and UV-B induce developmental abnormalities in both polar and temperate sea urchin species (Lamare et al. 2011). To assess the effects of UV-A and UV-B independently,
Fig. 2A shows the spectral irradiance of solar UVR that penetrates into surface waters in Central California through filters designed to expose or protect embryos from UVR. Using this experimental design, we have observed that embryos of *S. purpuratus* display the characteristic dose-dependent delay in cleavage when exposed to UVR *in situ* (Fig. 2B).

**Nonprotein defensive strategies**

Motile (later stage) sea urchin embryos have defensive strategies that guard against UVR damage, including avoiding light if held in static water (Miller and Emlet 1997). Nevertheless, these larvae may not be able to control their position in a mixed water column (Denny and Shibata 1989), possibly leaving them more vulnerable to sustained exposure to UVR. Sea urchin embryos also accumulate natural sunscreens, mycosporine-like amino acids (MAAs) that are provided maternally to eggs through the adult’s diet (Carroll and Shick 1996) and that protect embryos against UVR-induced cleavage delays in cleavage and against developmental abnormalities (Adams and Shick 1996, 2001; Lamare et al. 2011). However, adult *S. purpuratus* have relatively low and variable concentrations of MAAs in their gonads, epidermis, and gametes (Gravem 2009). Hoffman and Lamare (2004) observed similar results and emphasized that other modes of protection, such as carotenoids, may be more important. These and other nonprotein defenses were reviewed recently by Lamare et al. (2011), whereas the remainder of our review will focus on UV-induced changes in proteins.
Overview of cellular and molecular responses of early sea urchin embryos to UVR

The cellular stress response

Sea urchin embryos actively deploy a variety of defensive mechanisms that continue embryogenesis in stressful environments, including selective packaging of defensive proteins that combat toxic environmental chemicals, including heavy metals, and during periods of redox flux (Hamdoun and Epel 2007). Exposure to wavelengths between 320 and 500 nm also increases photolyase activity, allowing repair of CPDs (cf. Akimoto and Shiroya 1987a). Photolyase transcription can be upregulated in blastula-stage embryos after exposure to UVR (Isely et al. 2009), but studies of the production and regulation of photolyase protein in sea urchins are lacking.

In addition, cellular stress responses (CSRs) sense damage and often lead to delays in the cell cycle. All organisms studied to date appear to activate the CSR upon macromolecular damage during exposure to natural stressors, including UVR (Kültz 2005). The CSR mobilizes multiple signaling pathways that ameliorate molecular damage using primarily two mechanisms. First, cells activate defensive proteins, including heat-shock proteins (HSPs) that aid in folding and/or degrading proteins (Gething and Sambrook 1992; Feder and Hofmann 1999). Second, cells delay their developmental programs using kinases, such as check point kinases and cyclin-dependant kinases, that control passage through phases of the cell cycle and aid in providing time to respond to macromolecular damage before undergoing cell division (Kültz 2005).

Post-translational modifications and the responses to UV-stress

Responses to stress often involve coordinated cellular signaling pathways, including those that control the cell cycle, metabolism, transcription, development and differentiation, and apoptosis (Kültz 2005). Frequently, the proteins that control these processes are regulated by means of post-translational modifications (PTMs). Phosphorylation is a well-documented PTM (cf Cowan and Storey 2003), and only recently do we have the technology to both map and understand the importance of PTMs on a proteomic scale (Mann and Jensen 2003); this has not been broadly applied yet to the UVR stress responses such as delays in development.

The molecular pathways controlling cell division and the regulation of $\text{Ca}^{2+}$ in cells are highly conserved among organisms, including sea urchins (Fernandez-Guerra et al. 2006; Roux et al. 2006; Whitaker 2006). Dynamic protein phosphorylation is involved in the regulation of the signaling cascades for these processes (Kalume et al. 2003; Roux et al. 2006). Many directed studies, as well as the analysis of the S. purpuratus genome, have further characterized the rich array of protein kinases (Bradham et al. 2006) and phosphatases (Byrum et al. 2006) available during development. The egg and early embryo phosphoproteome is quite dynamic (Roux et al. 2008). Analysis of the sea urchin embryonic kinome revealed that 76% of kinases are expressed during embryogenesis (Bradham et al. 2006). Thus, it is plausible that in sea urchin eggs and early embryos, kinases (and attendant phosphatases) regulate pathways that are poised to respond to stressors such as UVR.

While phosphorylation is a well-studied PTM, the acetylation of proteins is also emerging as an important PTM, regulating many signaling mechanisms and affecting protein function (Polevoda and Sherman 2002). One example is the acetylation of amino-terminal peptide residues of proteins by sirtuins, which are NAD-dependent deacetylases. Sirtuins can be induced during hypoxia (Majmundar et al. 2010), are implicated in repair mechanisms, and appear to play a role in activation of the heat-shock response (Westerheide et al. 2009; Tomanek and Zuzow 2010). Further, microinjection of active sirtuins slows maturation and cell division in sea star eggs and embryos, indicating a possible regulatory role for acetylation in controlling timing of the cell cycle (Borra et al. 2002). At least two forms of sirtuins have been identified in the sea urchin genome, but no studies have yet examined protein acetylation in response to stress, such as UVR.

Ubiquitylation is another important regulatory PTM (Vucic et al. 2011). The ubiquitin/proteasome pathway is the major proteolytic system in eukaryotic cells for selective degradation of short-lived regulatory proteins (Ciechanover 1998; Ciechanover and Schwartz 1998). Ubiquitin has been identified in sea urchin embryos (Gong et al. 1991), but to our knowledge, there are no published reports documenting effects of UVR on ubiquitylation in sea urchins, although protein turnover and proteasome responses are evident (Campanale et al. 2011; see below). Along with phosphorylation and acetylation—as well as other PTMs not discussed here—ubiquitylation could serve as a rapid response to UVR damage.
The proteomic perspective

Proteomic approaches have the ability to objectively identify extensive responses by proteins to stress (Aiken 2011; Tomanek 2011). Some of the earliest studies of whole-embryo proteomes were conducted in sea urchins (Brandhorst 1976; Grainger et al. 1986) and there have also been proteomic studies probing the responses to and recovery from UVR (cf. Akimoto and Shiroya 1987a, 1987b). However, early studies were limited by the inability to identify proteins. With the annotation of the S. purpuratus genome and the power to identify proteins using mass spectrometry, this approach has gained new traction.

In sea urchin embryos, at least several thousand different proteins are represented in the unfertilized egg and a large percentage of these proteins turn over or exhibit dynamic phosphorylation in just the first few minutes post-fertilization (Roux et al. 2006, 2008; Campanale et al. 2011). Proteomic studies are also being employed to assess specific cell types and subcellular components (cf Mann et al. 2010), as well as the immune response of sea urchins to pathogenic bacterial infection (Dheilly et al. 2011). Furthermore, proteomic approaches evaluating redox responses to environmental pollutants have just been commissioned in a variety of model systems (Braconi et al. 2011). As technologies for exploring proteomes advance (cf Altelaar and Heck 2012), it is increasingly clear that this approach provides a powerful assessment of responses to stress.

Recently, a comparative proteomic assessment using 2DE followed by mass spectrometry identified UVR-responsive proteins in cleavage-stage embryos of S. purpuratus. A number of these proteins are members of the CSR, the kinome, and the proteasome, but many proteins had not been identified as responding to UVR, including the proteins controlling cellular metabolism (Campanale et al. 2011). Many of the differences between UV-protected and treated embryos observed in that study may be due to changes in protein levels or to PTMs (Fig. 3A), and represent particularly interesting proteins that we propose are part of the altered, or induced, cellular signaling pathways (Fig. 3B) responding to sub-lethal UVR stress.

Specific protein responses of early embryos to UVR

Changes in protein abundance or PTM serve as a signature of responsiveness to UVR treatment. Comparative proteomic surveys serve as screens for proteins that can then be investigated in more detail for roles in defense, recovery, or even as direct targets. UVR-responsive proteins identified in the proteomics screen (Campanale et al. 2011) were subjected to analysis through BioSystems (Geer et al. 2010) to begin mapping the UV-response pathways; then, targeted studies identifying individual proteins were added. Table 1 lists the biological pathways that are responsive to UVR and a subset of these are described in more detail below. Interestingly, assessing the collection of responsive proteins reveals critical proteins that intersect various pathways or systems, such as the cell cycle, translational control, and apoptosis, as well as conserved CSR pathways.

Cell division and apoptosis

In all eukaryotes, progression of the cell cycle is mediated by a series of phosphorylation and dephosphorylation events, as well as by formation and degradation of protein complexes. Entry into M-phase is regulated by mitosis-promoting factor (MPF), a complex of cyclin and a cyclin-dependent kinase, Cdc2. Activation of MPF by dephosphorylation of a regulatory tyrosine in Cdc2 allows entry into M-phase. Exit from M-phase requires the degradation of cyclin, mediated through ubiquitylation (Murray and Kirschner 1989). Vital checkpoints serve to ensure that replication of DNA is faithfully completed and that chromosomes are aligned and segregating properly. These checkpoints, often involving sensor and effector kinases, mediate cross talk between the mitotic spindle, pathways of DNA damage and repair, and apoptosis. Ultimately, they coordinate whether a cell will continue dividing or undergo cell death (Tanaka 2010). All of these pathways are UVR-responsive (Table 1).

Because UVR is known to cause damage to DNA and to arrest progression of the cell cycle in prophase (Rustad 1964; Adams and Shick 1996; Lesser 2010; Lamare et al. 2011; Fig. 2), it is not surprising that many cell-cycle proteins are responsive to UVR. For example, 14-3-3 proteins mediate progression of the cell cycle through interaction with Cdc25, the phosphatase that activates MPF by dephosphorylating Cdc2 (Borgne and Meijer 1996). The 14-3-3 proteins regulate pathways by binding to, and sequestering, client-signaling proteins such as kinases and phosphatases (Fu et al. 2000; Ferl et al. 2002). In mammalian cells, UVR treatment results in checkpoint kinase (p38)-dependent phosphorylation of Cdc25 as a response to DNA damage. This initiates the binding of Cdc25 to 14-3-3, sequestering it and preventing MPF activation and entry into M-phase (Bulavin et al. 2001).
Consistent with this, UV-irradiation of blastulae and later-stage sea urchin embryos causes an increase in, and anomalous patterns of, 14-3-3 transcription (Russo et al. 2010). Campanale et al. (2011) identified 14-3-3 proteins as UV-responsive during the first cell cycle in sea urchin embryos (Table 1). In particular, each of these proteins shifted in molecular weight after UV-treatments (Fig. 4), suggestive of a PTM.
The 14-3-3 proteins are regulated by phosphorylation and acetylation (van Heusden 2009), indicating this could partly provide a rapid response to UV by the early embryo.

If DNA damage checkpoints are acting upstream of 14-3-3 in early-cleavage sea urchin embryos, then delayed activation of Cdc25, and thus Cdc2, could partly explain the delay in the cell cycle. UVR causes delays in dephosphorylation of Cdc2 at Tyr 15 (maintaining it in its inactive form), which delays the first cell cycle (N. L. Adams, manuscript in preparation). Lesser et al. (2003) observed increases in levels of Cdc2 proteins in fertilized embryos with increasing UVR wavelengths that triggered apoptosis, suggesting that translation or turnover of Cdc2 may be responsive to UV as well.


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| Apoptotic signaling                      | p53, p38MAPK, cyclophilin D                                                   | Campanale et al. (2011), Bonaventura et al. (2005)^
| Cell cycle control                       | Cdc2, p53, p21, 14-3-3, pRB, Vasa                                              | Campanale et al. (2011), Bonaventura et al. (2005)^
| Cellular structure                       |                                                                                |                                                                           |
| Cytoskeleton                             | Actin, tubulin, tropomyosin, gelsolin                                          | Campanale et al. (2011), Akimoto and Shiroya (1987b) |
| CSR and macromolecular damage            |                                                                                |                                                                           |
| DNA damage response and repair           | Hhr23                                                                          | Campanale et al. (2011)                                                  |
| Stress response                          | p38 MAPK, 14-3-3 protein, carbonic anhydrase                                  | Campanale et al. (2011), Bonaventura et al. (2005)^
| ROS response                             | Superoxide dismutase, glutathione, catalase, thioredoxin, cyclophilin D, Glutathione peroxidase | Campanale et al. (2011), Lister et al. (2010)^
| Protein turnover, chaperones             | Hsp70, chaperonin, proteasome complex components                              | Campanale et al. (2011), Bonaventura et al. (2005, 2006)^
| Metabolism and transport                 |                                                                                |                                                                           |
| Amino acids                              | Glutamine synthetase                                                          | Campanale et al. (2011)                                                  |
| Carbohydrates                            | Adenosine kinase A, fructose-1,6-bisphosphatase, transaldolase                 | Campanale et al. (2011)                                                  |
| Coenzymes                                | Adenosylhomocysteinease                                                        | Campanale et al. (2011)                                                  |
| Energy production                        | Isocitrate dehydrogenase, malate dehydrogenase                                | Campanale et al. (2011)                                                  |
| Lipids                                   | Apolipoprotein B, hydroxymethylglutaryl-CoA synthase                           | Campanale et al. (2011)                                                  |
| Nucleotides                              | Bisphosphate nucleotidase, IMP cyclohydrolase, intermediate chain 1            | Campanale et al. (2011)                                                  |
| Transcription and translation            |                                                                                |                                                                           |
| Transcription, RNA processing            | Nucleolin, vasa, Ruvb-like protein                                            | Campanale et al. (2011)                                                  |
| Translation                              | Translation elongation factors, eIF2, eIF3, L10e/P0                            | Campanale et al. (2011)                                                  |

BioSystems are groups of proteins that interact as defined by NCBI BioSystems database (http://www.ncbi.nlm.nih.gov/biosystems/). ^Indicates protein response occurred any time from cleavage to post-blastula stage in these studies (whereas other studies specifically evaluated cleavage-stage embryos). References describing transcriptional responses in later stage embryos are not included on this list.
apoptosis is rare in early sea urchin embryos and more prevalent in post-blastula stages, in which transcription is occurring. It is possible that the differences among these studies are due to different exposures or to the detection techniques and species used. Campanale et al.’s (2011) proteomic screen of UV-stressed cleavage-stage embryos identified only proteins indirectly involved in apoptosis and in crossovers with other pathways (see “Cellular structure,” “CSR and macromolecular damage,” and “Translation” sections below).

Cellular structure
Studies in human skin cells indicate that natural UVR affects regulation of the cytoskeleton (Zamanski and Chou 1987). Campanale et al. (2011) identified potential UV-responsive cytoskeletal proteins in sea urchins, including cytoskeletal regulators as well as actins and tubulins (Table 1). UVR affected Rho-GDP dissociation inhibitor, which plays a role in cytokinesis by promoting the growth of microtubules, especially during interphase (Robinson and Spudich 2000). Tubulins are subject to a variety of PTMs, including acetylation and phosphorylation, and these also modulate assembly and stability of microtubules (Janke and Bulinski 2011). Actin dynamics may be similarly affected by PTMs (Dominguez and Holmes 2011). Gelsolin (regulated by tyrosine phosphorylation), a regulator of actin polymerization/severing (Sun et al. 1999), Arp3, an actin-related protein, and F-actin capping protein all exhibited UVR-responsiveness (Table 1). Interestingly, disruption of actin cytoskeleton by lethal doses of UVR or chemical disruptors can initiate apoptosis in mammalian cells (Kulms et al. 2002; Levee et al. 1996). Although these studies used UV-lamps that had peak excitations in the UV-B spectrum and used doses much higher than is observed in the environment, they set the stage for future investigations linking UVR treatment with the cytoskeletal dynamics that interface with regulation of the cell cycle and potentially control induction of apoptosis.

CSR and macromolecular damage
Chaperone function
Molecular chaperones, called HSPs, are necessary for protein folding and translocation (Gething and Sambrook 1992) as well as for control of the cell cycle (Helmbrecht et al. 2000; Tomanek 2010). Expression and function of HSP proteins can change during development and after macromolecular damage incurred by environmental stress, whereas interactions among HSPs and macromolecules sensitive to UVR result in upregulation of HSP expression (Feder and Hoffman 1999; Niu et al. 2006). HSP70 in the sea urchin *Paracentrotus lividus* localizes on the mitotic asters with Cdc2/Cyclin B in dividing embryos (Geraci et al. 2003) and is required for proper assembly of the mitotic asters during cell division (Sconzo et al. 1999). Agueli et al. (2001) proposed that HSP70 performs an essential chaperone role for the folding of tubulin during assembly of the embryo’s mitotic apparatus in sea urchins. Abiotic stressors induce HSP70 transcription in later-stage sea urchin embryos, but not in early-cleavage stages (which are relatively transcriptionally silent; Giudice et al. 1999) and UVR causes up regulation of HSP70 transcripts and protein in the blastula stage of *P. lividus* embryos (Bonaventura et al. 2005, 2006). HSPs were also captured in the UV-responsive proteomic screen of early embryos (Campanale et al. 2011; Table 1). UVR caused the pI of HSP70 to
become more acidic compared to protected embryos at 30 min post-fertilization, possibly due to PTM (Fig. 5A and B). In contrast to small increases in the concentration of HSP70 protein in early-cleavage stages observed by Bonaventura et al. (2006), our recent studies in early embryos of *S. purpuratus* reveal that the concentration of HSP is not significantly affected by sub-lethal doses of UVR (Fig. 5C and D). This difference may be due to differences in species or in UV-dose. In addition to measuring spot densities and migration (Fig. 5A and B), direct immuno-detection on 2D gels reveals that UVR treatment of early embryos causes changes in the HSP 70 pI, indicating a potential PTM of HSP 70 (Fig. 5D). Collectively, the directed and proteomic studies support a model in which HSPs are part of a UVR stress response in early sea urchin embryos, primarily through PTMs.

**Oxidative stress pathways**

Shortly after fertilization, a respiratory burst transitioning a metabolically quiescent egg to a rapidly dividing embryo results in the production of ROS (Shapiro 1991; Wong et al. 2004). Many studies have shown that oxidative pathways are affected by UVR in sea urchin embryos, indicating that superoxide dismutase (SOD) is upregulated in response to UVR (Lesser 2006, 2010). A recent study by Lister et al. (2010) showed that in addition to the SOD response, glutathione reductase, glutathione peroxidase, and catalase are also altered by UVR in sea urchin blastulae. In addition, the Campanale et al. (2011) proteomic screen of cleavage-stage embryos revealed UV-induced changes in a number of proteins that are hallmarks of oxidative stress, including glutathione peroxidase, thioredoxin, s-crystallin, and carbonic anhydrase (Table 1). Proteomic analyses also identified cyclophilin D as being UVR-responsive. This is an important mitochondrial chaperone that serves as an apoptosis sensor by regulating the permeability of the transition pore complex, essential to detecting the redox state of the mitochondria, and recognizing intracellular Ca$^{2+}$ flux (Green and Reed 1998; Lin and Lechleiter 2002; Baines et al. 2004). Therefore, cyclophilin D may be sensing cellular redox states and UV-induced changes in early sea urchin embryos after an assault by UV.

**Protein turnover**

Subunits of the major protein-degrading complex, the proteasome, appear to be affected by UVR.

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**Fig. 5** Response of a HSP70 to UVR. (A) Magnification of spots from composite proteomes (Fig. 3) that were identified as HSP70 isoform 3. (B) Comparisons of spot density shown in A indicate a UVR-dependent significant difference in spot density and a possible shift in pI (suggestive of a PTM, ANOVA, *P* < 0.05). (C) One-dimensional immunoblots of total TX-soluble protein lysates sea urchin embryos exposed to, or protected from, artificial UVR for 60 min from fertilization (QPanel UV340 lamps). (D) Immunoblots of 2DE-separated proteins of UV-irradiated or protected embryos of *Strongylocentrotus purpuratus* exposed from 0 min to 60 min post-fertilization. Samples were collected at 30 and 60 min. Gels in C and D were transferred to nitrocellulose, blocked, and probed with Anti-HSP70 (Sigma #H5147). Binding was detected using GAM-HRP (BD Transduction Laboratories), and blots were imaged with a Typhoon Trio Scanner (GE Healthcare).
More specifically, UVR caused changes in the abundance of 26S proteasome non-ATPase subunit, p44.5 subunit, and the proteasome C11 subunit in early sea urchin embryos. There is a natural increase in proteasome activity during activation of the egg and during the transition from egg to embryo (Horner and Wolfner 2008), but this appears heightened upon exposure to UV. The identification of a large number of proteasome subunits that showed changes in UV-treated embryos (Table 1) indicates this entire complex may be involved in an important response to UVR.

Consistent with this, is the above-mentioned link between ubiquitylation and stress responses (Vucic et al. 2011). As a result of UV-stress, the proteasome complex mediates the degradation of p53 in human cells (Glockzin et al. 2003). Sea urchin embryos also express p53 and it may play a role in the apoptotic pathway activated after UV-stress during later stages in development (Lesser et al. 2003). Not surprisingly, inhibition of the proteasome arrests mitosis in sea urchin embryos by delaying the degradation of cyclin and also regulates exit from the S-phase of the cell cycle (Kawahara et al. 2000). It seems reasonable that protein turnover would intimately control the molecular switches controlling the activity of CSR pathways.

Translation

There is some evidence that UVR may be interfering with the ability to translate maternally stored mRNA in sea urchin embryos. Iordanov et al. (1998) suggested that part of the cellular response to UV-stress in mammalian cells may be generated at the ribosome from damaged rRNA. Similarly, the proteomic screen (Campanale et al. 2011) revealed multiple proteins involved in translation that were responsive to UVR treatment, including L10e/P0 (an acidic ribosomal protein), eIF2α, and eIF4AII (proteins required for the initiation of translation), and nucleolin, a phosphoprotein needed for the synthesis and maturation of ribosomes. eIF2α kinases have been shown to play a role in UV-induced apoptosis in human cells, in part due to the phosphorylation of eIF2α (Parker et al. 2006). Overexpression of nucleolin inhibits the translation of p53 whereas down regulation of nucleolin promotes the expression of p53 along with ribosomal subunits in mammals after DNA damage (Takagi et al. 2005). Therefore, alteration of any of these proteins may affect translation of all, or a subset of, mRNAs during first cleavage, and may explain the observed delays in division.

Metabolism and transport

The proteomic screen also revealed UVR-responsiveness in a suite of proteins that fall under the BioSystems
metabolic pathway (Table 1). For example, levels of mitochondrial isocitrate dehydrogenase 2 (NADP+) decreased in response to UVR. This enzyme plays a role in recycling NADP+ within the mitochondria and overexpression of it results in protection from ROS-induced damage in mouse NIH3T3 cells (Jo et al. 2000). This observation suggests that sea urchin embryos may have an altered capacity to sense and deal with ROS that interfaces with metabolic pathways. As another example, the activity of isocitrate dehydrogenase is regulated by glutathionylation during periods of oxidative stress (Kil and Park 2005). To our knowledge, no studies have yet assessed global metabolic responses to UVR in embryos or how this interfaces with the other responsive pathways.

**Conclusions and future directions**

Proteome-wide and targeted studies have revealed key pathways affected by UVR in early sea urchin embryos (Table 1). How the components of these pathways interact and how the pathways themselves interface remain outstanding questions. We propose that sea urchin zygotes are poised to cope with stressors, such as UVR, using maternally derived toolkits. In Fig. 6, fertilized eggs subjected to UVR implement defenses using proteins that are already in use as mediators of early development. These include cell cycle regulators such as the 14-3-3 proteins and Cdc2, and modulators of metabolic homeostasis and protein turnover. Many of these proteins are involved in multiple systems; for example, the proteasome complex is crucial not only for protein turnover to regulate the cell cycle through cyclin degradation, but also to clear mis-folded proteins and remove maternal proteins no longer needed for the egg to make the transition to an embryo. We propose that UVR triggers these defensive proteins (mostly through PTMs) as a first-line response. In response to UVR, for example, proteasome activity increases and cell cycle checkpoints initiate pauses in response to DNA damage. However, if homeostasis and the progression of the cell cycle are affected, second-line CSRs deploy. Apoptotic sensors likely interface with these pathways. Collectively, one effect of UVR is manifested in a delay of cell cycle progression. When the defensive and stress-response systems are successful in responding to the damage (a sub-lethal dose of UVR), homeostasis is restored and progression of the cell cycle re-commences. However, if defenses and CSRs are unsuccessful (a lethal dose of UVR), cell-cycle halts, and death ensues (Fig. 6). The same scenarios may apply to embryos that are exposed to UVR at later stages in development, with lethal doses resulting in aberrant development (such as exogastrulation) or apoptosis, but the defenses and CSRs may also be able to employ robust transcriptional responses.

Functional studies to examine the proteins identified in proteomic studies are needed to resolve long-standing questions regarding the mechanisms by which UV-stress alters cellular physiology and delays development. Identification of specific PTMs of UVR-responsive proteins is essential for understanding regulation and activity. While protein identification after 2DE is powerful for tracking individual proteins, complementary, higher throughput proteomic technologies (c.f. Altelaar and Heck 2012) will result in a more complete collection of proteins and pathways that respond to UVR. Comprehensive and unbiased assessments of UV-induced changes in proteomes can provide a deeper understanding of how UVR affects overall cell physiology and the molecular regulation of stress, thereby generating rich data sets that can be mined for critical targets and regulators. Furthermore, advancement of proteomic approaches will help identify the proteins that are directly damaged by UVR, or are indirectly regulated after exposure to UVR. Importantly, these studies will begin to explain how protein abundance and PTMs affect protein–protein interactions and thus how the various pathways interact to coordinate a response to UVR.

Whether these same pathways and key proteins are signature responses in later stages, or how they will change with dose and wavelength of UVR also remain to be determined. For example, a cleavage-stage embryo may respond differently (with only maternally derived proteins) than will later stages that can use transcriptional responses. Functional studies to examine the proteins identified in proteomic studies are needed to resolve long-standing questions regarding the mechanisms by which UV-stress alters cellular physiology and delays development. Identification of specific PTMs of UVR-responsive proteins is essential for understanding regulation and activity. While protein identification after 2DE is powerful for tracking individual proteins, complementary, higher throughput proteomic technologies (c.f. Altelaar and Heck 2012) will result in a more complete collection of proteins and pathways that respond to UVR. Comprehensive and unbiased assessments of UV-induced changes in proteomes can provide a deeper understanding of how UVR affects overall cell physiology and the molecular regulation of stress, thereby generating rich data sets that can be mined for critical targets and regulators. Furthermore, advancement of proteomic approaches will help identify the proteins that are directly damaged by UVR, or are indirectly regulated after exposure to UVR. Importantly, these studies will begin to explain how protein abundance and PTMs affect protein–protein interactions and thus how the various pathways interact to coordinate a response to UVR.

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Proteomic responses of sea urchin embryos


