

# Autophagy as a defense strategy against stress: focus on *Paracentrotus lividus* sea urchin embryos exposed to cadmium

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**Abstract** Autophagy is used by organisms as a defense strategy to face environmental stress. This mechanism has been described as one of the most important intracellular pathways responsible for the degradation and recycling of proteins and organelles. It can act as a cell survival mechanism if the cellular damage is not too extensive or as a cell death mechanism if the damage/stress is irreversible; in the latter case, it can operate as an independent pathway or together with the apoptotic one. In this review, we discuss the autophagic process activated in several aquatic organisms exposed to different types of environmental stressors, focusing on the sea urchin embryo, a suitable system recently included into the guidelines for the use and interpretation of assays to monitor autophagy. After cadmium (Cd) exposure, a heavy metal recognized as an environmental toxicant, the sea urchin embryo is able to adopt different defense mechanisms, in a hierarchical way. Among these, autophagy is one of the main responses activated to preserve the developmental program. Finally, we discuss the interplay between autophagy and apoptosis in the sea urchin embryo, a temporal and functional choice that depends on the intensity of stress conditions.

**Keywords** Stress · Defense strategies · Autophagy · Apoptosis · Cadmium · Sea urchin embryos

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## Introduction

### Molecular and cellular defense strategies against stress

Aquatic organisms are exposed to adverse changes in their own environment and they are able to sense these deleterious changes as stressful insults. The source of the environmental stress can be natural or anthropogenic. During the last centuries, the quality of water resources has been further deteriorating because of the continuous addition of different types of pollutants. Several contaminants are non-degradable and so they persist in the environment for long periods, causing long-term effects in tissues and organs of aquatic organisms that can accumulate high levels of pollutants.

To face all these different types of stress, during evolution, aquatic organisms developed various defense mechanisms, such as the activation of cellular and molecular strategies (synthesis of heat shock proteins, metallothioneins, apoptosis, and autophagy) to survive in adverse conditions (Hamdoun and Epel 2007; Chiarelli and Roccheri 2012).

Heat shock proteins (HSPs) and metallothioneins are able to produce a detoxifying and antioxidant effect that is not always sufficient to block the toxic action of the pollutant, depending on the extent of cell damage (Samali and Cotter 1996; Hamada et al. 1997). In such circumstances, the mechanisms of programmed cell death, such as apoptosis and autophagy, may be triggered (Yuan and Kroemer 2010), contributing to remove the irreversibly damaged cells in order to maintain the integrity of the tissues.

Heavy metals represent one of the main agents of environmental stress. It is well known that the extent of metals bioaccumulation depends on their total amount in the environmental medium and on their uptake, storage, and excretion mechanisms: the metals can accumulate in animals and plants when

they are taken up and stored faster than they are broken down (metabolized) or excreted (Chapman et al. 1996).

Recently, it was shown that autophagy is one of the most useful pathways activated against cadmium (Cd) stress, a non-essential element commonly detected in aquatic and terrestrial environments, released from both natural sources (volcanic activities or leaching of Cd-rich soils), and anthropogenic activities (e.g., mining, smelting, electroplating, production and use of pigments, plastic stabilizers and nickel–cadmium batteries) (Bargagli 2000). This pollutant is an environmental stressor due to its toxicity, persistence, and accumulation in biota and it is widespread in the aquatic environment (Waisberg et al. 2003; Chora et al. 2009). Cd is present at low concentrations in open seawater, while in coastal and estuarine areas, its concentration may increase even more than twenty times (Pigeot et al. 2006). In some cases, this toxicant can induce both apoptosis and autophagy (Dong et al. 2009; Templeton and Liu 2010). Cd toxic effects have been well documented, especially in molluscs, crustaceans, echinoderms, and fishes (Roccheri and Matranga 2010).

Although the toxicity of many marine contaminants has already been highlighted, scientific research needs to be further developed in order to obtain a complete profile of the chemicals effects on living beings, including the molecular and cellular aspects governed by specific pathways.

In this review, we will analyze the role of autophagy as a protective system against the consequences of stress, discussing its significance in tolerance development of aquatic organisms. We will consider physical stress, such as light, hyperthermia, and starvation, as well as chemical stress, such as metals, chemical compounds, and nanoparticles.

In particular, among invertebrate organisms, we will focus on the sea urchin embryo, which represents a suitable model system to investigate the adaptive response of cells exposed to stress during development and differentiation. Due to its sensitivity to several aquatic contaminants, the sea urchin embryo adopts different defense mechanisms to preserve the developmental program (Chiarelli et al. 2011). We will discuss the relationship between the activation of autophagy and/or apoptosis: a temporal and functional choice that depends on the intensity of the stress conditions, suggesting a choice made in a hierarchical way. Therefore, we hypothesize that the autophagic and apoptotic processes may be used as an alternative and/or as a combined defense strategy in embryos exposed to several kind of stress.

### Autophagy as a stress defense mechanism

Autophagy is the most important intracellular process by which eukaryotic cells sequester and degrade cytoplasm portions and organelles via the lysosomal pathway (Klionsky and Emr 2000). Autophagy, encompassing at least three related

processes (i.e., macro- and micro-autophagy and chaperone-mediated autophagy), is essential for development, growth, and maintenance of cellular homeostasis in multicellular organisms and is able to prevent the accumulation of malfunctioning cellular structures. Increased autophagy is usually induced by environmental conditions such as starvation, hyperthermia, hypoxia, salinity increase, bacterial or viral infections, accumulation of misfolded proteins and damaged organelles, toxic stimuli, radiation, and many other stress agents (Cuervo 2004; Moore and Allen 2006a; Tasdemir et al. 2008).

Excessive levels of autophagy can lead to autophagic programmed cell death, whose features differ from those of the apoptotic process. Since autophagy plays a key role under both physiological and pathological conditions in several animal species, the components of the autophagic machinery are evolutionarily conserved (Di Bartolomeo et al. 2010).

The lysosomal-autophagic system appears to be a common target for many environmental pollutants as lysosomes are able to accumulate many toxic metals and organic xenobiotics (Moore et al. 2008).

Prognostic use of adverse lysosomal and autophagic reactions to environmental pollutants can be used to predict cellular dysfunction and the health status of aquatic animals, such as shellfish and fishes, which are extensively used as sensitive bioindicators to monitor the ecosystem health (Moore et al. 2006a, b, c).

### Autophagy induced by environmental stress on aquatic invertebrates and vertebrates

Many studies have been carried out to assess the effects of chemical pollutants on different bioindicator organisms and to evaluate the possible risks on human health.

Living organisms are naturally exposed to many kinds of pollutants and use various mechanisms to counteract their potential toxicity. Some toxicants can be sequestered in cellular vesicles and granules, activating the autophagic process. Actually, lysosomal membrane integrity or stability appears to be an effective generic indicator of cellular well-being: in bivalve mollusks and fish, lysosomal stability is correlated with many toxicological responses and pathological reactions (Moore et al. 2006a).

At present, the role of autophagy in aquatic invertebrates was investigated in bivalves, corals, and in the sea urchin embryos. Bivalves, representing a significant food resource for at least 20 % of the global human population, are extensively used as sensitive bioindicators to monitor the health of the ecosystem. One of the most sensitive organs for lysosomal detection is the digestive gland, or hepatopancreas, of bivalve shellfish such as the blue mussel *Mytilus edulis*. The lysosomal system plays a central and crucial role in cellular food degradation (intracellular digestion), toxic response, and internal

turnover (autophagy) of the digestive cells (McVeigh et al. 2006).

The marine mussel cells of the digestive gland are the major environmental interface for the uptake of contaminants, particularly those associated with natural particulates filtered from seawater. Digestive cells showed well-characterized reactions in response to the exposure to lipophilic xenobiotics, such as oil-derived aromatic hydrocarbons (AHs), which accumulate in these cells with minimal biotransformation. It was developed a simulation model, based on processes associated with the flux of carbon through these cells, including the following physiological parameters: fluctuating food concentration, cell volume, respiration, secretion/excretion, storage of glycogen and lipids, protein/organelle turnover (autophagy/resynthesis), and carbon export to other mussel tissues. The major response to AHs was the induction of autophagy in these cells. The simulations indicated that the reactions to AHs and food deprivation represent a good model for the *in vivo* measured responses (Moore and Allen 2002; Moore 2002; Allen and McVeigh 2004). On the basis of a hypothesis formulated by Moore, the autophagic removal of oxidatively damaged organelles and proteins provides a second tier of defense against oxidative stress (Moore et al. 2006a). It has been further hypothesized that repeated triggering of autophagy can protectively minimize the generation of lipofuscin, a product of the oxidative attack on proteins and lipids that can accumulate in lysosomes, where it can generate reactive oxygen species (ROS) and inhibit the lysosomal function, resulting in autophagic failure. Consequently, animals living in fluctuating environments, in which autophagy is repeatedly stimulated by natural stressors, seem to be generically more tolerant to pollutant stress. Therefore, the authors speculate that organisms making up functional ecological assemblages in fluctuating environments, where upregulation of autophagy should provide a selective advantage, may be preselected to be tolerant of pollutant-induced oxidative stress (Moore et al. 2006a, b, c, 2007, 2008).

The filter feeding blue mussel has been used to analyze the uptake and the effects of nanoparticles from glass wool, a new absorbent material employed in floating oil spill barriers. Using both gills and hepatopancreas of *M. edulis*, it analyzed the uptake of nanomaterials by transmission electron microscopy and their accumulation in endocytotic vesicles, lysosomes, mitochondria, and nuclei. Dramatic decrease of lysosomal membrane stability occurred. Furthermore, lysosomal damage was followed by excessive lipofuscin accumulation, indicative of severe oxidative stress. Several events indicated progressive cell death: increased phagocytosis by granulocytes, autophagy, and apoptosis in epithelial cells of gills and in primary and secondary digestive tubules epithelial cells. Final evidence of cause-effect relationships was showed by the combinational use of biomarkers and the ultrastructural localization of nanoparticle deposition (Koehler et al. 2008).

The effects of Cd have been investigated, by proteomics, in the gill and digestive gland of the bivalve sentinel species

*Ruditapes decussatus*. Protein expression profiles in Cd-exposed tissues were compared to unexposed ones, showing a tissue-dependent response that depends mainly on differences in Cd accumulation, protein inhibition and/or autophagy. An overall decrease of protein spots was detected in both treated tissues, being higher in gill. Some of the spots more drastically altered after pollutants exposure were excised and nine were identified, including three proteins upregulated, one downregulated, four suppressed, and one induced. In particular, Cd induces major changes in proteins involved in cytoskeletal structure maintenance, cell maintenance, and metabolism, suggesting potential energetic change. An overall decrease of protein spots was detected in both treated tissues. Lysosomal autophagy could explain this decrease since it is considered a survival strategy. Autophagy confers advantages to organisms, especially molluscs exposed to pollutants, by protecting the cells against the harmful effects of damaged and malfunctioning proteins (Chora et al. 2009).

Studies on cnidarians showed that the autophagic process is implicated in the phenomenon called coral bleaching. This process, primarily caused by the increased temperatures of seawater, is characterized by the loss of symbiotic algae, their pigments, or both, and it is a major contributor to the global decline of coral reefs. The ecological and socioeconomic implications of bleaching are immense (Hoegh-Guldberg et al. 2007). Autophagy, one of the mechanisms involved in the bleaching process, has been identified using specific autophagic markers (Rab7, a key GTPase to lysosome biogenesis, and LAS, a lysosomal acid phosphatase). Results showed that during light and temperature stress, the symbiont vacuolar membrane is transformed from a conduit of nutrient exchange to a digestive organelle, resulting in the consumption of the symbiont, a process termed symbiophagy by the authors. It has been hypothesized that, during a stress event, the mechanism maintaining symbiosis is destabilized and symbiophagy is activated, ultimately resulting in the phenomenon of bleaching. This work was focused on the *Symbiodinium* cell as the initiator of the bleaching cascade (Downs et al. 2009).

Two other studies examined the role of host cell stress and death in the bleaching response, considering the symbiotic anemone *Aiptasia pallida*, a useful laboratory model for the study of corals. The studies suggested that host cell death can contribute to subsequent symbiont cell mortality, a process that appears similar to a host innate immune response. The results indicated that no single pathway is responsible for symbiont release during bleaching, allowing to formulate a model for cellular processes involved in the control of cnidarian bleaching where apoptosis and autophagy act together in a see-saw mechanism such that if one is inhibited the other is induced (Dunn et al. 2007; Weis 2008; Paxton et al. 2013).

Among aquatic vertebrates, two fishes fulfill a primary role in the study of autophagy: the rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*). As it belongs to

salmonids, the rainbow trout migrate long distances to the spawning sites, experiencing long fasting periods during which it degrades a major part of its lipids and about half of its white muscle mass for recycling cytoplasmic components to provide nutrients to other tissues (Mommsen et al. 1980; Mizushima et al. 2004). Several studies indicate that autophagy plays a key role in controlling muscle mass by protein degradation (Mommsen 2004; Salem et al. 2006; Seiliez et al. 2012). Most components of autophagy and its associated signaling pathways (AKT1, TOR, AMPK, FOXO) are evolutionarily conserved in the rainbow trout (Polakof et al. 2011; Seiliez et al. 2008; Seiliez et al. 2012). Using qRT-PCR and Western blot analysis, Seiliez and colleagues were able to demonstrate that fasting fish or serum depletion of trout myocytes strongly induces the expression of several major genes involved in autophagy (LC3B, gabarap11, atg12l, atg4b), suggesting that autophagy is acting as a cell survival mechanism to provide the energy and the nutrients essential for allowing fish to survive during nutritional starvation (Seiliez et al. 2010). More recent results demonstrated that rainbow trout myoblasts can trigger the activation of both short- and long-term autophagy-lysosomal degradative programs during nutritional starvation: the first is a rapid and transient transcription-independent induction, while the second is a slower mechanism requiring gene expression (Seiliez et al. 2012).

Zebrafish is an exceptional model for developmental research, but it also has many features that make it a valuable vertebrate model organism for the analysis of autophagy (Klionsky et al. 2012). One of these is the transparency of the embryo, thanks to which autophagosome formation can be observed by the use of transgenic GFP-LC3. This method was used to study the induction of autophagy in cultured zebrafish cells under starvation conditions. Subcellular localization of endogenous MAP1-LC3B (microtubule-associated protein 1-light chain 3B) protein in zebrafish embryonic cells was examined by ultracentrifugal fractionation and Western blot analysis, and the results indicated that MAP1-LC3B translocates from the cytosol to membranes, including lysosomes and the endoplasmic reticulum, during amino acid starvation. The induction of the autophagy-lysosome pathway during starvation was also confirmed by Western blot analysis, showing the conversion of endogenous MAP1-LC3B-I to MAP1-LC3B-II in the skeletal muscle and hepatopancreas (Yabu et al. 2012).

Two papers reported the activation of the autophagy-lysosome pathway in zebrafish embryos exposed to nanoparticles. Cheng and colleagues observed the appearance of lysosome-like vesicles after multi-walled carbon nanotubes exposure at single-cell stage of zebrafish embryos (Cheng et al. 2009). Another paper reported that the simultaneous treatment of embryos with S-doped TiO<sub>2</sub> nanoparticles and simulated sunlight irradiation caused receptor-mediated

autophagy and vacuolization, indicating entrance of the nanoparticles via endocytosis rather than diffusion (He et al. 2014).

The exposure of the red common carp (*Cyprinus carpio*) to Cd significantly increased the mRNA and protein levels of Beclin 1 (a key gene in the cell autophagic process), indicating that the common carp Beclin 1 gene may play a regulatory role against Cd toxicity, suggesting that autophagy may have a role in the regulation of cellular adaptive response to Cd-induced toxicity (Gao et al. 2014).

Among the environmental stressors that aquatic vertebrates have to face, temperature changes have a major role for the growth and survival of ectothermic animals living in variable thermal environments (Iwama et al. 1998; Mosser et al. 1986). HSPs participate in chaperone-mediated autophagy (CMA), a chaperone-dependent selection of oxidized and abnormal proteins produced under stressed conditions and targeted to lysosomes for degradation. Using a cell line derived from the tailfin of the marine teleost yellowtail fish *Seriola quinqueradiata*, Yabu and colleagues showed that CMA was induced in heat-shocked cells, suggesting that chaperone-mediated autophagic protein degradation may play a key role in stimulating catabolism in heat-shocked fish cells. Furthermore, using wortmannin, which acts as a PI3 protein kinase inhibitor against other autophagic signaling pathway via IGF-1 and TOR kinase, the authors were able to show that macroautophagy, as well as CMA, were enhanced in fish cells under stress conditions (Yabu et al. 2011).

### Autophagy as a defense strategy in sea urchin embryo

The sea urchin embryo is an exceptional model organism for developmental research mainly because of its rapid development, transparency, and easy manipulation, as well as thanks to the large number of gametes that can be easily obtained from adult urchins and the external fertilization (Chiarelli and Roccheri 2013; Walker et al. 2013). Sea urchin embryos are also ideal organisms for in vivo toxicity test, because of their ability to modulate different defense strategies, depending on the nature of the physical or chemical stress.

Autophagy has been reported for the first time in *Paracentrotus lividus* embryos in 2011, and it is now known as one of the most important cellular/molecular pathways triggered by sea urchin embryos both in physiological and stress conditions (Chiarelli et al. 2011). Recently, some genes involved in the regulation of autophagy were identified in *Strongylocentrotus purpuratus* (KEGG PATHWAY: spu04140). Even if there is now a large number of model systems used to study autophagy, *P. lividus* sea urchin embryo still represents one of the most suitable systems for both in vivo and in vitro analysis (Klionsky et al. 2012). The advantages of studying the induction of autophagy in this model

system (or other defense strategies) under stress conditions depend on the possibility of adding many chemicals to the media (filtered sea water) that will subsequently be directly absorbed through the membrane of embryo cells (Chiarelli and Roccheri 2012). Another advantage is the possibility to detect LC3A/B by means of both protein gel blot and immunofluorescence in situ analysis. Furthermore, in vivo staining of autolysosomes with acidotropic dyes can also be performed. Studies on whole embryos provide qualitative and quantitative data about autophagy induction and about the spatial localization of autophagic markers in the cells. It is worthy to note that the application of the techniques on whole organisms gives the remarkable opportunity to investigate the autophagic phenomenon in multipotent cells interacting among themselves in their natural localization and environment, bypassing the disadvantages of studying isolated cells that are deprived of their normal network of interactions (Klionsky et al. 2012).

Among the chemical stressors, Cd has been particularly studied because of its ability to interact with cellular and molecular components that makes it an activator/modulator of several cellular/molecular pathways of defense mechanisms (Roccheri and Matrangola 2010). The first study about Cd exposure in *P. lividus* sea urchin was carried out in 1982, showing dose- and time-dependent developmental defects (Pagano et al. 1982).

More recent studies demonstrated that sea urchin embryos are able to activate different defense strategies after Cd exposure, providing a clear structure of the defense strategies hierarchically triggered at the cellular and molecular levels. These

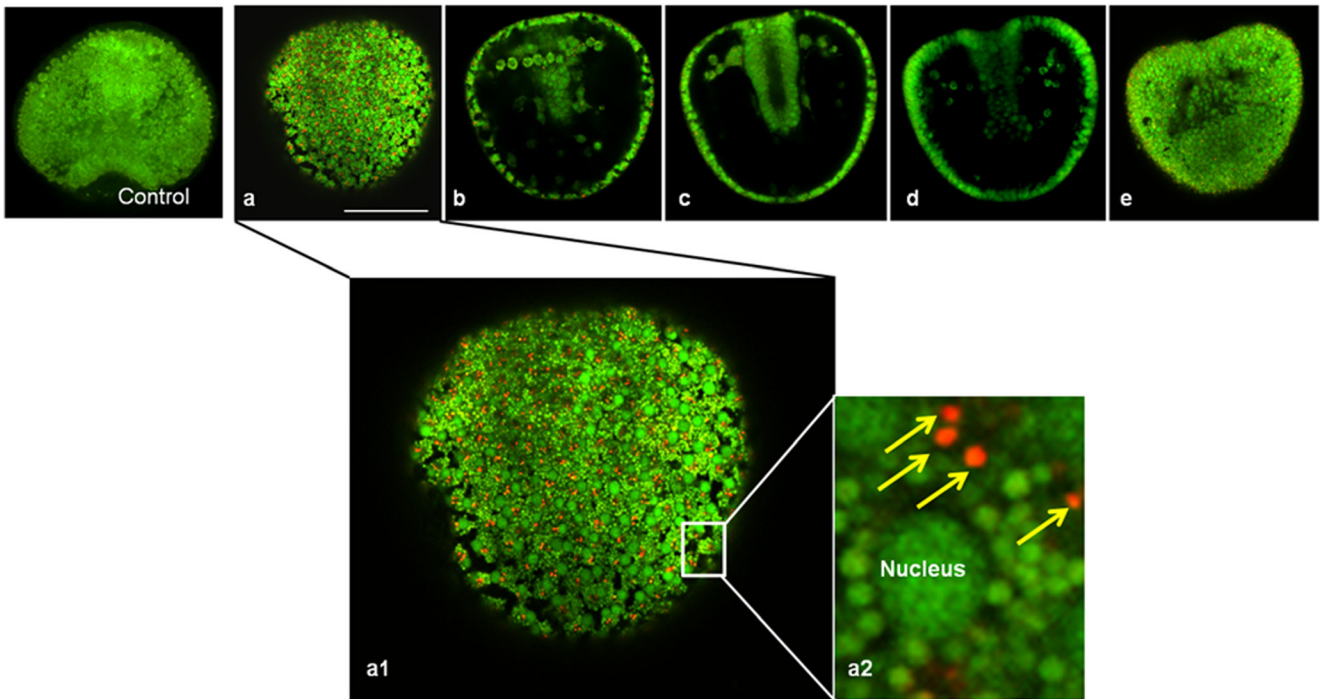
researches indicated that the exposure of the developing embryo to subacute/sublethal concentrations of Cd induce, firstly, the activation of the early defense mechanisms, such as the synthesis of HSPs and metallothioneins (Russo et al. 2003; Roccheri et al. 2004), but if this is not sufficient to safeguard the developmental program, embryos will trigger the autophagic and/or apoptotic processes (Chiarelli et al. 2011; Agnello et al. 2007). Some of the pathways activated in sea urchin *P. lividus* embryos, showed in Table 1, can be occur in tandem or in sequence.

In 2004, it was showed that, if the Cd (1 mM) insult lasts no more than 15 h, embryos are able to restore the normal development after the removal of the metal from the culture medium and further recovery in physiological conditions, probably because only a few cells are damaged and subsequently removed through apoptosis. In contrast, if the Cd insult is much higher (i.e., 1 mM lasting 24 h), the killed cells are more numerous and, after the Cd removal, the normal development cannot be restored (Roccheri et al. 2004; Agnello et al. 2007). A further study showed for the first time the induction of autophagy that displays a maximum peak after 18 h of Cd exposure, when apoptosis is just beginning.

Depending on their localization into the embryo, the cells differently activate the autophagic mechanism. This was demonstrated by acridine orange (AO) vital staining and confocal laser scanning microscopy (CLSM) on whole-mount embryos, finding a major localization of autolysosomes in the ectoderm, probably due to the major exposure of this section of cells to Cd insult (Fig. 1).

**Table 1** Cellular/molecular defense strategies induced by Cd stress in *P. lividus* embryos and larvae

CdCl <sub>2</sub> concentration	Developmental stage of <i>P. lividus</i>	Cellular/molecular defense strategy and markers	References
1 mM	Morula (6 h), hatching blastula (12 h), gastrula (24 h), pluteus (48 h)	Metallothionein expression (PI-MT gene)	Russo et al. (2003)
	Blastula (15 h), gastrula (24 h)	Synthesis of a specific set of HSPs	Roccheri et al. (2004)
	Blastula (15 h), gastrula (21 and 24 h)	Apoptosis (by TUNEL assay and by caspase-3, $\alpha$ -fodrin, lamin-A immunodetection)	Agnello et al. (2007)
	Hatching blastula (12 h), blastula (15 h), gastrula (18 h, 21 and 24 h)	Autophagy (by acridine orange (AO) and neutral red staining, LC3-II immunodetection)	Chiarelli et al. (2011)
	Gastrula (24 h)	Autophagy (by LC3-II immunodetection)	Chiarelli and Roccheri (2012)
	Gastrula (24 h)	Apoptosis-autophagy interplay (by TUNEL, caspase-3, and LC3-II immunodetection, AO staining)	Chiarelli et al. (2014)
100 $\mu$ M	Pluteus (38 h)	Apoptosis (by TUNEL assay and caspase-3 immunodetection)	Agnello et al. (2006)
	Pluteus (38 h)	Autophagy (by AO staining)	Chiarelli and Roccheri (2014)
1 $\mu$ M, 1 nM, 1 pM	Larva (10–30 days)	Apoptosis (by TUNEL assay)	Filosto et al. (2008)
0.1–100 $\mu$ M	Gastrula (24 h), prisma (30 h)	Metallothionein expression (PIMT4, PIMT5, PIMT6, PIMT7, and PIMT8 genes)	Ragusa et al. (2013)
0.5–30 $\mu$ M	2-cell, 8-cell, early blastula, swimming blastula, prisma, pluteus	Oxidative stress (nitric oxide production)	Migliaccio et al. (2014)



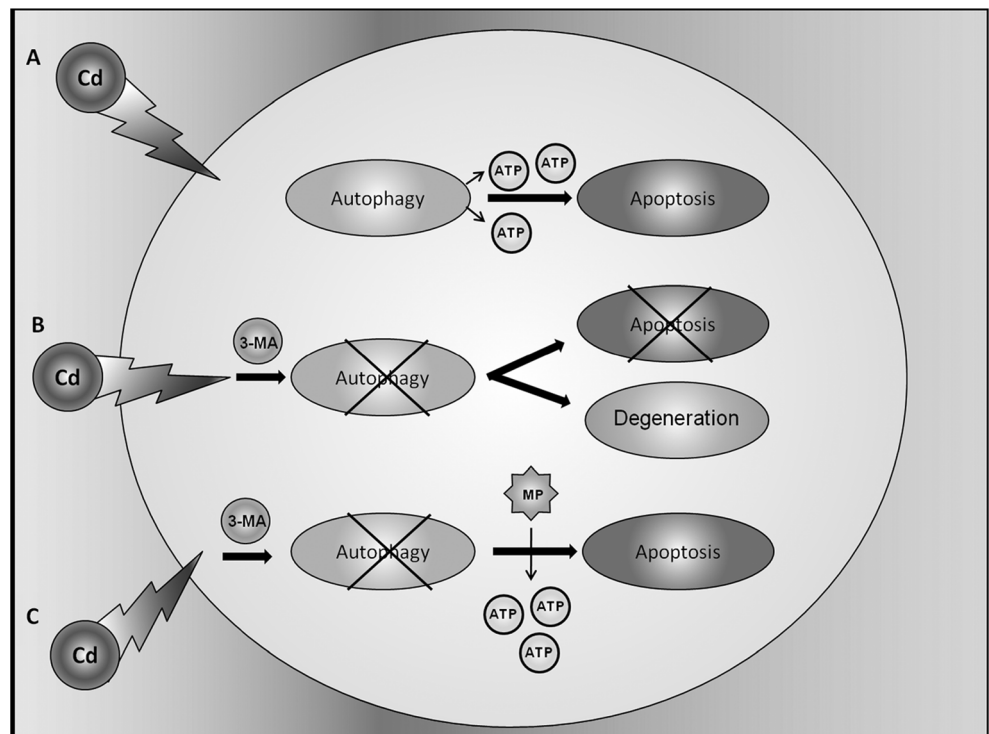
**Fig. 1** AO vital staining. Images of a representative embryo captured by CLSM. Cd-treated embryo after 24 h of development. Several optical sections of the same embryo: **a, e** externals, **b, d** intermediates, and **c**

equatorial, (**a1**) enlargement of an external (ectoderm) section of (**a**), (**a2**) enlargement of a section of (**a1**). Autophagolysosomes are indicated by *yellow arrows*. Bar=50  $\mu$ m

Taken together, these data lead to the conclusion that the first response to the Cd insult is the induction of HSPs and metallothioneins. Subsequently, the embryo activates the

autophagic process as a cell survival mechanism; finally, if the damage is too extensive, the apoptotic program is massively activated leading to the death of the embryo.

**Fig. 2** Temporal and functional relationship between autophagy and apoptosis in sea urchin embryos cells exposed to Cd. **a** Cd induces autophagy and then triggers apoptosis. **b** Cd-treated embryos, incubated with the autophagic inhibitor 3-MA, do not show autophagy or apoptosis activation and so embryos degenerate. **c** Cd-treated embryos incubated with the autophagic inhibitor 3-MA, but after incubation with the energetic substrate (MP), the apoptotic process starts again



## Relationship between autophagy and apoptosis in sea urchin embryos

The homeostatic relationship between autophagy and apoptosis during sea urchin development represents a very interesting chapter that is being deeply investigated in our laboratory.

On the basis of our data, we have proposed three different hypotheses about the role of autophagy during development of sea urchin embryos exposed to Cd: (1) hierarchical choice of defense mechanisms, (2) energetic hypothesis, (3) clearance of apoptotic bodies. According to the first hypothesis, we suggested that the temporal choice of the two mechanisms depends on the fact that the embryo tries to face the stress conditions using, initially, a defense strategy that is less deleterious, i.e., autophagy, to preserve the developmental program. If this process is not sufficient to offset the damage caused by a continuous stress, the mechanism of apoptosis is activated, probably as a last attempt to save the embryo by removing only some few damaged cells. If the insult persists, the whole embryo dies. In this case, second hypothesis, autophagy could provide the energy necessary for apoptosis through the supply of ATP molecules, by recycling of damaged cellular components. According to the third hypothesis, since the sea urchin embryo does not have specialized phagocytes, it could begin the clearance of apoptotic bodies through autophagy (Chiarelli et al. 2011).

To evaluate the relationship between autophagy and apoptosis induced by Cd, we blocked autophagy by the administration of the inhibitor 3-methyladenine, and so we analyzed the occurrence of apoptotic processes (by TUNEL assay and anti-cleaved caspase-3 immunofluorescence detection). In these conditions, apoptosis was not detected and the embryos degenerated. In parallel, we carried out the same assay providing an energetic substrate, the methyl pyruvate (MP). The results demonstrated that autophagy temporally anticipates the massive apoptotic response and the inhibition of autophagy produces a concomitant reduction of apoptosis (Chiarelli et al. 2014). The administration of MP restored the apoptotic signals, suggesting that the energetic hypothesis could be probable. Indeed, autophagy may operate to supply energy through the recycling of damaged cellular components, necessary to sustain the ATP-dependent apoptotic pathway, essential for the clearance of dying cells (Fig. 2). This would justify the temporal relationship between autophagy and apoptosis.

## Conclusions

Autophagy is essential in the turnover of cellular components, including proteins and organelles, and in the maintenance of tissue homeostasis in adult organisms. The

lysosomal system plays a central role in intracellular digestion of food, in internal turnover of the digestive cells, and in the response to environmental stress of some organisms, such as bivalve and fish (Moore 2004). In order to survive, aquatic organisms have evolved the capability to adapt, constantly and quickly, to environmental changes. In both invertebrate and vertebrate taxa, autophagy operates as a protective mechanism, playing a crucial role in the response to stress, sometimes in concert with the apoptotic machinery. Even if a wide variety of information is available about the role of autophagy and apoptosis in the response to environmental stressors, at present, the interplay between these two processes has been investigated only in some organisms, but not in the aquatic ones.

Aquatic organisms constantly have to face a wide variety of environmental stress, such as starvation, hyperthermia, and exposure to xenobiotics, nanoparticles, and different chemical compounds. The exposure to chemical substances, such as heavy metals, can lead to an increase of their concentration inside the cells of aquatic organisms. This event can be amplified as consequence of global warming and excessive evaporation.

It is known that sea urchin populations are subjected to the effects of ocean acidification and increased seawater temperature. Several studies investigating the impact of ocean acidification on echinoderms have demonstrated a wide range of effects, including reduced fertilization and larval development rates, survival rates, and altered body size (Byrne 2012; Dupont et al. 2010; Evans and Watson-Wynn 2014).

In this context, autophagy might serve as a survival mechanism when cellular damage is not too extensive or may lead to cell death when the damage/stress is persistent and/or irreversible; in the latter case, it can act as an independent pathway or in association with apoptosis (Agnello and Roccheri 2010).

The results of the recent transcriptomic analyses in some sea urchin species could provide useful information about the expression of genes involved in the autophagic process and their spatial-temporal expression. The entire expressed gene set during *S. purpuratus* sea urchin embryos development was measured, showing complex patterns of expression (Tu et al. 2014). The transcriptomic approach could also be very useful in environmental monitoring, as was reported on the Arctic green sea urchin (Rhee et al. 2014).

This review, although not comprehensive in its reporting, aims to provide an overview of current knowledge on the role of the autophagic program activated in several aquatic organisms, vertebrate and invertebrate, exposed to different types of stress conditions. Because of the proven key role of autophagy during embryonic development, we then focused our attention on the sea urchin embryo, a suitable bioindicator of the marine health,

considering that different markers have been evaluated in this organism to study the dangerous effects induced by chemical and physical stress.

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