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Effects of oxidative stress on *Ascidia interrupta* embryogenesis

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Introduction

Oxidative stress

All aerobic organisms naturally produce reactive oxygen species as part of respiration. These reactive oxygen species have a variety of deleterious effects (Marx, 1985), including DNA damage, protein modification, and lipid peroxidation. Thus, organisms have a variety of natural mechanisms to absorb reactive oxygen species, but if the amount exceeds an organism's ability to remove them, the organism is in a state of oxidative stress. Oxidative stress has been linked to many disorders, such as cancer and neurodegenerative diseases, as well as normal aging.

Protection from stressors during early development

Rapidly developing embryos often lack protective mechanisms against environmental stress that adults have, such as apoptosis or expression of heat shock proteins (Epel, 2003). The "be prepared" hypothesis suggests that embryos have static defences in place during early stages of development to prevent cellular damage from environmental stresses rather than repairing damage after the fact. This study tests the "be prepared" hypothesis by subjecting ascidian embryos to oxidative stress.

Methods

Adult *Ascidia interrupta* Heller, 1878 were collected from settlement slides from the waters around South Padre Island, Texas (Lambert et al., 2005), and housed in the Coastal Studies Laboratory of the University of Texas – Pan American. They were brought to the main campus of the University of Texas – Pan American as needed. Individuals were dissected, and gametes were fertilized *in vitro* (no self fertilization). After fertilization, embryos were challenged with the oxidant hydrogen peroxide (H_2O_2) in concentrations ranging from 0.02 µM to 2000 µM in ten-fold dilutions. The control group received no hydrogen peroxide.

Developmental stage was recorded for ten randomly selected individuals (sampled with replacement) at 2 hour intervals.

For settlement experiments, 20 embryos were sorted into individual dishes and given several days to settle. Replications were pooled.

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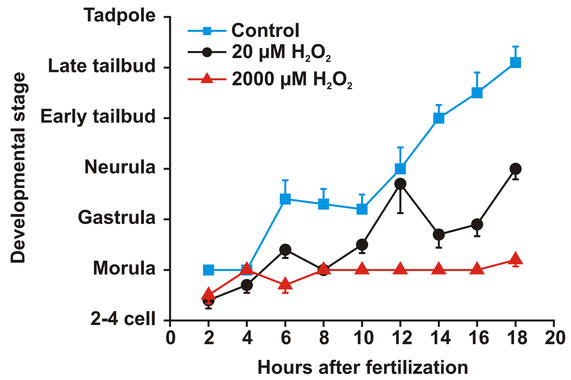
Results

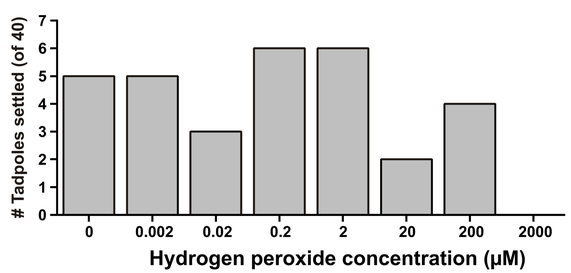
Oxidative stress impairs development

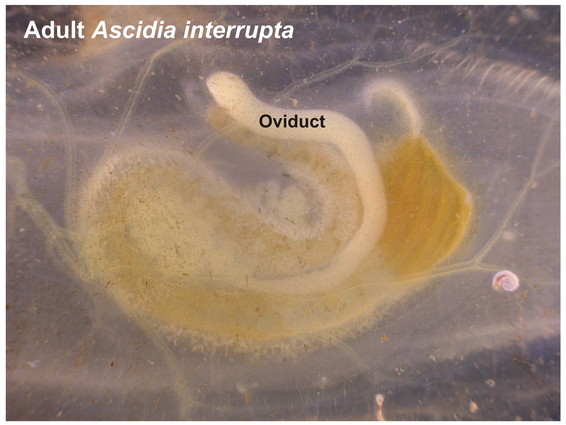
Oxidative stress significantly affects ascidian development (general linear model analysis, $F = 8.485$, $df = 56$, $p < 0.01$). Under high levels (200-2000 µM) of oxidative stress, fertilized eggs cleave but do not complete gastrulation. Lower levels of oxidative stress (< 20 µM) caused no gross defects.



Do low levels of oxidative stress affect tadpoles?

Oxidative stress may cause subtle defects that are not visible using light microscopy. Because settlement is a complex behavioural and physiological event, tadpoles' ability to settle arguably indicates that tadpoles subjected to low levels of stress have no significant defects. Preliminary data indicate that stressed embryos reaching the tadpole stage are fully normal (number settled out of 40 fertilized eggs in all groups). Note that in this experiment, hydrogen peroxide concentrations of 200 µM yielded tadpoles able to settle, whereas no tadpoles were seen at this concentration in the prior experiment; future experiments will clarify this discrepancy.

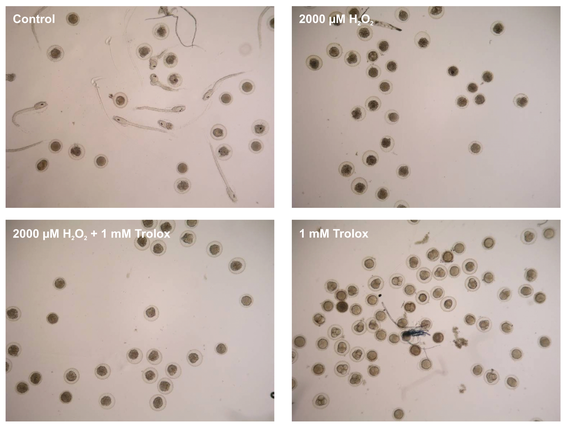




Does anti-oxidant rescue development?

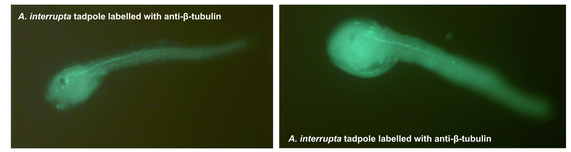
Although hydrogen peroxide is a potent oxidant, it is possible that it impairs development for some reason unrelated to oxidative stress. If oxidative stress is responsible for the toxicity, an anti-oxidant should rescue embryos exposed to hydrogen peroxide doses that would otherwise be lethal.

Preliminary data show that controls (no oxidative stress) developed into tadpoles, and high levels of hydrogen peroxide arrested development at the point of gastrulation. A saturated concentration (1 mM) of the anti-oxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) halted development, however, either alone in combination with hydrogen peroxide. This suggests that lower levels of Trolox should be used, or that an oxidant normally plays a role in ascidian embryogenesis.



Discussion

Oxidative stress does not affect very early development, which is consistent with the "be prepared" hypothesis (Epel, 2003). Two possible hypotheses could explain this result. The "be prepared" hypothesis suggests that very early embryos have static but short-lived anti-oxidant defensive mechanism in place, such as enzymes that neutralize hydrogen peroxide. An alternative hypothesis is that the mechanism whereby oxidative stress impairs development is irrelevant to early development. Future experiments will be aimed at distinguishing between these hypotheses. For example, gastrulation may be sensitive to oxidative stress because oxidative stress affects the embryonic cytoskeleton; because gastrulation is required the first major cell movement during development, cytoskeletal defects could prevent gastrulation. Cytoskeletal proteins will be visualized with immunocytochemistry and fluorescent microscopy. Tadpoles have been successfully labelled for ß-tubulin, which is abundantly expressed in neurons (Pennati et al., 2003), in preliminary studies.



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