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Regeneration in bipinnaria larvae of the bat star *Patiria miniata* induces rapid and broad new gene expression

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ABSTRACT

Background: Some metazoa have the capacity to regenerate lost body parts. This phenomenon in adults has been classically described in echinoderms, especially in sea stars (Asteroidea). Sea star bipinnaria larvae can also rapidly and effectively regenerate a complete larva after surgical bisection. Understanding the capacity to reverse cell fates in the larva is important from both a developmental and biomedical perspective; yet, the mechanisms underlying regeneration in echinoderms are poorly understood.
Results: Here, we describe the process of bipinnaria regeneration after bisection in the bat star *Patiria miniata*. We tested transcriptional, translational, and cell proliferation activity after bisection in anterior and posterior bipinnaria halves as well as expression of SRAP, reported as a sea star regeneration associated protease (Vickery et al., 2001b). Moreover, we found several genes whose transcripts increased in abundance following bisection, including: Vasa, dysferlin, vitellogenin 1 and vitellogenin 2.
Conclusion: These results show a transformation following bisection, especially in the anterior halves, of cell fate reassignment in all three germ layers, with clear and predictable changes. These results define molecular events that accompany the cell fate changes coincident to the regenerative response in echinoderm larvae.

"*If there were no regeneration there could be no life. If everything regenerated there would be no death. All organisms exist between these two extremes. All things being equal, they tend toward the latter end of the spectrum, never quite achieving immortality because this would be incompatible with reproduction.*"

[Richard Goss, *Principles of Regeneration* 1969.]

1. Introduction

Regeneration enables an organism to restore lost tissue and organ functions following damage or removal. This process first requires wound healing, which is then followed by cell fate changes, migration, cell proliferation, and differential signaling to compensate for the lost region(s) of the organism (Goss, 1969). The mechanisms underlying regeneration are predicted to be under strong selection, as they provide the organism with the capacity to return to its full reproductive and developmental potential after injury (Bely and Nyberg, 2010). Most organisms do not have an ability to regenerate significantly and a major question in biology is whether this phenomenon could be induced in an otherwise non-regenerative species. To answer this question, we require a deep understanding of the mechanisms underlying this phenomenon.

In both planarians and *Hydra*, a stem cell population in the adults is set aside for growth of adult tissue, as well as for replication and pluripotency of a regenerating individual. Planarians use neoblasts as their sole replicative cell type capable of replacing all other cells of the adult, even the germ line (Alvarado and Yamanaka, 2014). So too in *Hydra*: the stem cells of ectodermal and endodermal epithelium and the I-cells are the source for the replicative cells of the adult. One or another of their progeny contributes to all cell types of the adult, including the germ line (Technau and Steele, 2011; Tomczyk et al., 2015). These highly proliferative and pluripotent (or totipotent in the case of the planarian) stem cells are responsible for the vast regenerative capacity of

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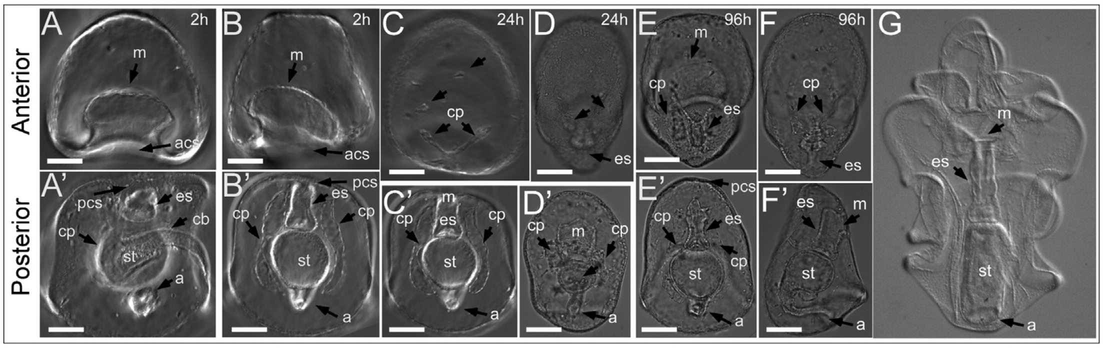

Fig. 1. Documentation of regenerative process over the course of the first 96 h after bisection. We bisected bipinnaria larvae (2 weeks old) and performed a detailed morphological analysis of anterior (A–F) and posterior (A′–F′) parts 2 h, 24 and 96 h post-cutting. Posterior parts clearly regenerate a gut morphologically within 96 h while anterior parts require more time (see text for details). (G) Uncut sibling larva. a: Anus; acs: anterior cut site; cp: coelomic pouches; es: esophagus; m: mouth; pcs: posterior cut site; st: stomach. Scale bars A, A′, B, B′, C, C′ (100 μm); D, D′, E, E′, F, F′ G (75 μm).

these adults. It is not clear though whether this reliance on specialized stem cells devoted to tasks of replenishment and regeneration is a shared strategy amongst metazoans.

Sea stars (Echinodermata: Asteroidea) have been used extensively as a classic example for regeneration studies in invertebrates (Anderson, 1962; Goss, 1965; Mladenov et al., 1989). Adults may lose an arm, or multiple arms, but are capable of regenerating all lost tissues and appendages as long as they still have a portion of the central body disk. The regenerated tissues include the many cell types of the dermis and epidermis, the gut, nerves, and even the gonads and germ cells (Huet, 1974). Significant efforts are being made to understand this complete replacement of each arm in a sea star, but this regeneration process may take several months, depending on the species.

Interest in experimental testing of regeneration in echinoderms was reinvigorated when larval budding was observed in four of the five echinoderm classes: ophiuroids (Balser, 1998), echinoids (Eaves and Palmer, 2003; Vaughn and Strathmann, 2008), asteroids (Rao et al., 1993) and holothuroids (Eaves and Palmer, 2003). In general, a significant percentage of larvae bud off clones from the parent larva, which serves to develop a new, fully functional lava. This cloning characteristic in echinoderm larvae led to experimentation in regeneration following bisection of sea star larvae that resulted in the observation of wound healing, and even complete regeneration of lost body parts. For example, the posterior halves would develop features of the anterior, including new coelomic pouches, foreguts, and mouth openings, and the anterior halves would develop posterior features, *e.g.* a new stomach, intestine, and anal openings (Vickery and McClintock, 1998; Vickery et al., 2002). In an attempt to uncover underlying mechanisms of sea star larval regeneration, SRAP (sea star regeneration associated protease) emerged as a potential candidate gene from a differential cDNA library screen by Vickery and colleagues (Vickery et al., 2001b). This protease was suggested to be functionally involved in modifications of the extracellular matrix needed for wound healing and regeneration.

Here we explore the wound healing and regenerative capacity of larvae from the bat star *Patiria miniata*, and document general mechanisms of the regeneration phenomenon based on spatial and temporal gene expression patterns, transcription and translation activity and general morphological changes after bisection. Enormous genomic, transcriptomic, antibody, and experimental resources are available for this species, thus making it an excellent model to explore regeneration using a molecular and cellular approach.

2. Results

2.1. Morphological changes during regeneration

We performed a detailed morphological analysis of regeneration in bisected bipinnaria larvae in this species by documenting anterior and posterior halves for 96 h post cutting (Fig. 1). Posterior fragments can regenerate the mouth within 96 h (Fig. 1E',F') while anterior pieces require more time to regenerate the digestive tract (up to ~15 days; see also Fig. 2, but this largely depends on the rearing conditions. Under

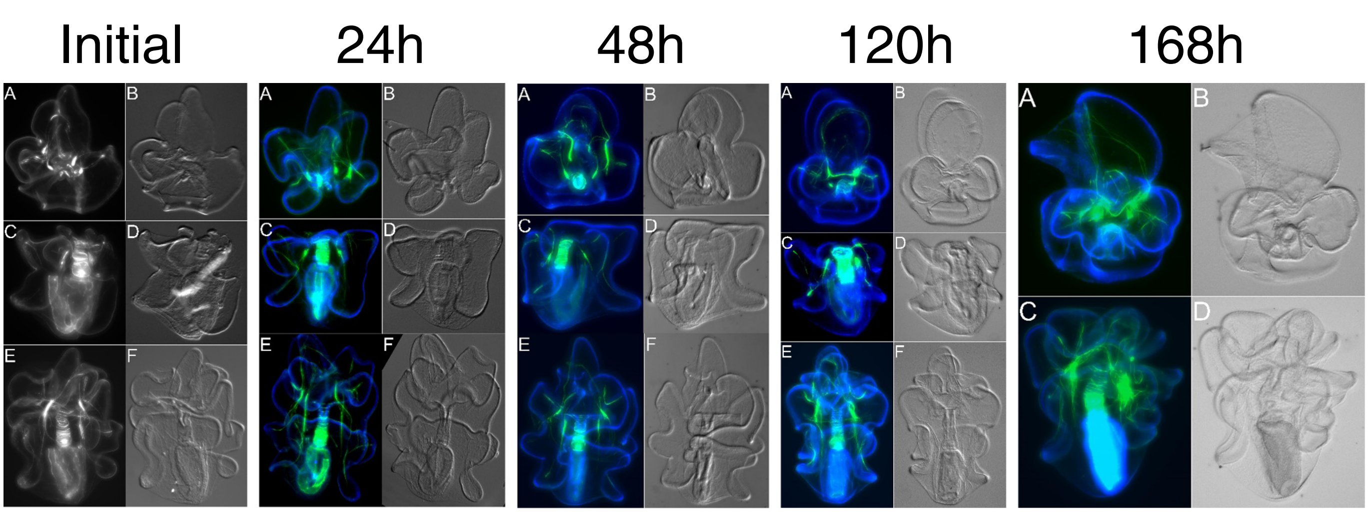

Fig. 2. Muscular regeneration of anterior and posterior pieces after bisection of two week old larvae. Bisected larvae were stained using phalloidin 24, 48, 120 and 168 h post bisection. We observed the generation of new muscle fibers post-bisection in the cut area. Blue: DRAQ-5 nuclear stain, green: phalloidin.