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The link between injury-induced stress and regenerative phenomena: A cellular and genetic synopsis ☆

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ABSTRACT

Injury is an inescapable phenomenon of life that affects animals at every physiological level. Yet, some animals respond to injury by rebuilding the damaged tissues whereas others are limited to scarring. Elucidating how a tissue insult from wounding leads to a regenerative response at the genetic level is essential to make regenerative advantages translational. It has become clear that animals with regenerative abilities recycle developmental programs after injury, reactivating genes that have lied dormant throughout adulthood. The question that is critical to our understanding of regeneration is how a specific set of developmentally important genes can be reactivated only after an acute tissue insult. Here, we review how injury-induced cellular stresses such as hypoxic, oxidative, and mechanical stress may contribute to the genomic and epigenetic changes that promote regeneration in animals. This article is part of a Special Issue entitled: Stress as a fundamental theme in cell plasticity.

1. Introduction

Regenerative abilities are varied among animals, yet all utilize regeneration to some extent for an ultimate end result: healing and functional restoration. Animals undergo a continuous bombardment of environmental stresses that cause nucleic acid, lipid and protein damage, as well as direct local tissue damage. Environmental stresses can come from an external source or may simply be generated by general wear and tear and inflammation. On the other hand, internal stresses arise within the tissue itself upon alteration of homeostasis. Various types of stress such as physical trauma, oxidative stress, hypoxia, and mechanical stress exerted on tissues result in cellular and tissue damage leading to localized apoptosis and debris removal. When the damage exceeds general wear and tear, there is inflammation and activation of cellular precursors of regeneration to further the repair. Even though stress has destructive effects on cellular homeostasis, in a regenerative context it can be an initiator.

The relationship between stress and regeneration, when investigated at a genomic level, provides an explanation to how a cell under stress can reactivate transcription of specific genes in order to increase plasticity and regulate the process of healing. It is crucial to identify these cellular pathways that reactivate developmental programs necessary for regeneration. It is also important to identify the upstream signals associated with injuries that trigger epigenetic and genetic changes, which may facilitate a regenerative response. For example, further to be discussed, oxidative stress produced by reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) are becoming appreciated as upstream signaling mechanisms for inducing cellular processes necessary for tissue regeneration. Yet, there is a clear missing link in our understanding of the injury response and the activation of developmental pathways that regulate regeneration. In this review, we will cover how animals that have remarkable regenerative abilities respond to injury in terms of stress and coordinate gene expression used to mount a regenerative response. We will also highlight how epigenetic modifications provide access to developmental networks during regeneration.

2. The cellular mechanisms of animal regeneration

Regeneration is a common strategy to maintain bodily homeostasis that is observed across plant and animal kingdoms [1]. Injury in principle is the initiator of regeneration. The challenge is that injury is complex since it activates a number of downstream processes. Both genomic and cellular adjustments are made to carry out a regenerative response to various types of injury-induced stresses. Yet, to appreciate the response to stress, we first need to understand possible types of repair through the process of regeneration.

The most basal form of regeneration is physiological regeneration, which entails continuous replacement of damaged and dead cells over time using adult stem cells (ASCs) including hematopoietic blood stem cells, mesenchymal stem cells, brain stem cells, epidermal stem cells, and muscle satellite cells. Since physiological regeneration in nature is a continuous process, it does not necessarily depend on an external/additional trigger for initiation. For example, the continuous renewal

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of the gut epithelial lining after shedding is controlled by physiological regeneration.

Reparative regeneration replaces missing or damaged tissue after traumatic injury. Encompassing numerous levels of tissue complexity, reparative regeneration may result in a repair process ranging from an entire animal to a single tissue type. Basal animals like planarian worms and hydra show the most extreme examples of reparative regeneration, where entirely new organisms can be generated from only a small fragment of the original organism (for reviews [2]). Furthermore, even within a given tissue, stem cell niches can undergo both physiological and reparative regeneration depending upon the different types of stimuli received from the surrounding tissues. For example, the epidermal stem cell niche undergoes continuous renewal to replace skin cells, but a local injury triggers a repair-specific response that signals local stem cells to migrate towards the epithelial defect and increase proliferation.

Reparative regeneration can be divided into two concepts: repair without blastema formation which is utilized in tissue and cell regeneration, and blastema-based regeneration. A blastema is a mass of highly proliferative cells located at the tip of an injury/amputation that will give rise to the missing structure. Blastema-based regeneration encompasses most examples of appendage regeneration including the salamander limb and tail, zebrafish fin, *Xenopus laevis* tadpole limb and tail, mouse digit tip, crustacean limb, planarian head, and to some extent hydra head. It proceeds by a conserved mechanism that includes the migration of epidermal cells to cover the wound, inflammation, reorganization of ECM, stimulation of cells to reenter the cell cycle, blastema formation, morphogenesis and growth. The idea that cells turn back time to a dedifferentiated/pluripotent state during blastema formation has dominated the scientific literature for decades, yet modern lineage labeling strategies show that the cells giving rise to a blastema retain the plasticity they had prior to amputation (for review [3]). For example, planarians use progenitor cells that show pluripotency throughout life, while crustaceans and vertebrates use progenitors that remain lineage-restricted during regeneration [4–7] Furthermore, lineage-restriction is also noted in zebra fish heart regeneration [8]. Even though there are some exceptions as seen in bone formation during digit tip regeneration in the mice model [9,10], regardless of the cell source, most blastema-based systems recapitulate development by reactivating dormant developmental mechanisms.

In contrast, tissue regeneration utilizes the following pathway: ischemia, inflammation, removal of dead cells, ECM reorganization, proliferation, differentiation and morphogenesis. Hence, between the two regenerative events, blastema formation is the major difference. This difference in the regenerative pathways should be considered when evaluating the regenerative capacities of different animals and tissue types. Here, we focus primarily on blastema-based regeneration in animals with extensive regenerative capacities following injury.

The commonality between all reparative regeneration events is that an initiating trigger kick-starts the process. Our knowledge of the link between injury and recruitment/activation of progenitor/stem cells is lacking, and this is a fundamental problem in understanding regeneration. Thus, we overview the present data which suggests that the damage response signals emanating from damaged and dying cells are utilized as upstream inducing signals of tissue regeneration.

3. Cell response to injury, stress, and the induction of regeneration

Regeneration is initiated locally and transiently in response to trauma. Local cell stress is a logical relay mechanism between cell damage and regeneration because it occurs rapidly and across all injury paradigms. Most of our knowledge on the molecular basis of regeneration has been limited to studying the signaling pathways that were originally used during embryonic development [11]. The problem is that developmentally important genes are often downstream consequences of the extracellular signals that initiate regeneration. It is of particular interest to understand the relay signals between extracellular stimuli and the activation of intracellular signaling networks. Fortunately, this gap is becoming smaller with recent work, suggesting that cells' response to stress may be an important component of regeneration and the activation of local stem cells [12–15].

3.1. Oxidative stress and damage response signals

Transcription independent damage signals emanate from injuries and activate signaling transduction pathways in neighboring cells to initiate transcription of developmental signaling and cell proliferation genes [16]. A key early damage signal that has come to be appreciated as necessary for regenerative processes is reactive oxygen species (ROS) (for review [17]). Cellular stress caused by ROS including hydrogen peroxide (H_2O_2), superoxide anion, and hydroxyl radicals often result in damaged proteins, lipids, and DNA breaks. ROS can be produced by mitochondrial electron reduction, lipoxygenase, peroxisome, NADPH oxidase, and Cytochrome P-450 to act as internal sources of oxidative damage [18]. The antioxidant defense system works against ROS, yet when the production and the removal is not in balance oxidative damage is inevitable, leading to severe dysfunction and apoptosis. Although cell stress and apoptosis logically seems detrimental to the well being of the cells, it has been shown to be a positive force in the context of regeneration. For example, regeneration is found to be defective when apoptosis is blocked with caspase inhibitors in planaria [12], hydra heads [14], *Drosophila* [15,19,20], zebrafish fins [21], *X. laevis* tails [13], and mouse livers [22]. Overall, this suggests that stressed-induced apoptosis, partially caused from oxidative stress, activates local cell proliferation to support regeneration (Fig. 1).

ROS molecules also have the capacity to act as cytokines as seen in their role in paracrine signaling in plant cell differentiation [18], and they have recently been identified as an upstream damage signal driving regeneration. After larval *Drosophila* epithelial wounding, an immediate calcium wave initiates DUOX-mediated H_2O_2 production, which acts as a cytokine to recruit macrophages to the injury site [23,24]. Zebrafish tail fin wound regeneration is also dependent upon epithelium-derived H_2O_2 production to recruit leukocytes [25] and peripheral axons [26] (Fig. 1). Interestingly, macrophages have also been shown to be necessary for salamander limb regeneration [27]. The early H_2O_2 generation is responsible for necessary downstream Src family kinase signaling [28], and sustainment of ROS production is necessary for fgf20 expression and blastema formation through JNK1 signaling [21]. After larval *X. laevis* tail amputation, H_2O_2 is also locally produced and required for Wnt/β-catenin signaling, *fgf20* expression, and proper regeneration [29]. Thus, across multiple regeneration paradigms injury-induced production of H_2O_2 1) plays a positive intracellular role of inducing oxidative stress and apoptosis to promote nearby cell proliferation or 2) acts as a cytokine that recruits leukocytes to the injury site and activate downstream developmental gene expression (Fig. 1).

3.2. Mechanical stress and regeneration

Tissue injury disrupts cell–cell and cell–extracellular matrix connections that are normally held constant in adult organisms. Wound healing across vertebrates cause epithelial cells at the wound margin to alter contact with their underlying extracellular matrix and migrate to close off the exposed tissue in conjunction with wound contraction [30–32]. Dermal cells such as fibroblasts, nerves, and blood vessels underneath the epithelium also undergo changes in mechanical force caused by injury [33]. Hence, mechanical forces are altered near injury sites and may activate signaling pathways that link tissue disruption to local cell proliferation utilizing mechanotransduction pathways such as integrins [34], delta-notch [35] and hippo/YAP [36]. Interestingly, each of these pathways is important in both blastema- and non blastema-based regeneration, although direct links are lacking between mechanical stress and the induction of these pathways [37–42].