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The early wound signals

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Wounding of tissue barriers, such as epithelia, disrupts homeostasis and allows infection. Within minutes, animals detect injury and respond to it by recruitment of phagocytes and barrier breach closure. The signals that activate these first events are scarcely known. Commonly considered are cytoplasmic factors released into the extracellular space by lysing cells (Damage Associated Molecular Patterns, DAMPs). DAMPs activate inflammatory gene transcription through pattern recognition receptors. But the promptness of wound responses is difficult to explain by transcriptional mechanisms alone. This review highlights the emerging role of nonlytic stress signals in the rapid detection of wounds.

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Introduction

With every minute that an open wound exposes the organism's interior to the outside environment, the likelihood of infection and secondary host damage rises. Owing to the frontline role of epithelia in pathogen protection, evolution of efficient wound detection mechanisms is driven by high selective pressure. This review briefly summarizes conserved damage and stress signals involved in early wound detection (Figure 1).

New approaches for studying early wound signaling *in vivo*

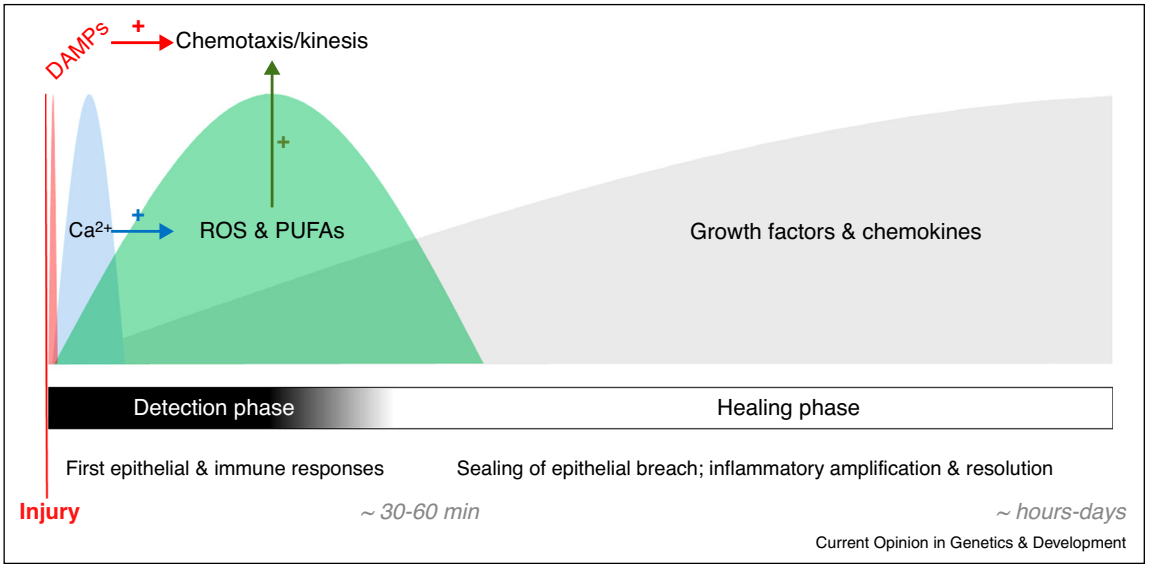
Systematic research into early wound signaling has been greatly facilitated by imaging approaches that allow measuring cellular responses to tissue injury in real-time within the intact organism. To this end, animal models such as *Caenorhabiditis elegans*, *Drosophila melanogaster* and zebrafish (*Danio rerio*) have become popular owing to their microscopic and genetic tractability. For example, the caudal tail fin of zebrafish larvae is a small, flat, non-vascularized, and transparent tissue that is well suited for live imaging of inflammatory and healing responses to wounding by a laser beam or a microneedle. The innate immune system of zebrafish closely reflects that of higher vertebrates. All major cell types, including neutrophils and macrophages, are present. The model provides a simple, yet powerful way to study how leukocytes, and other cell types, rapidly perceive information on tissue damage over distances within an intact tissue. Genetically encoded biosensors for wound signals, such as Ca^{2+} or hydrogen peroxide (H_2O_2) can be expressed in relevant cell types (e.g., immune/epithelial/endothelial cells, among others) using tissue-specific promotors. Imaging biochemical together with morphological responses reveals when and where a cell senses a wound signal, and whether or how it directly reacts to it. In combination with molecular pathway perturbation and *in vitro* reconstitution, this general approach has been applied to tease apart some of the early wound signaling circuits discussed below.

Cell lysis signaling by DAMPs

Even before biosensor imaging enabled direct peeks into the first, biochemical events following injury, it was clear that cell lysis acts as an important tissue damage cue in many situations [1]. Cell lysis at the wound site causes DAMP leakage into the extracellular space. Some DAMPs, such as formylated peptides and ATP, can act as chemoattractants by binding to G-protein coupled receptors on leukocytes [2–6]. Formylated peptides are released by damaged host cells or bacteria. Host derived formylated peptides attract interstitial neutrophils to sites of liver damage [3], but do not mediate early neutrophil recruitment to dermal injury in mouse [7••]. Also the chemotactic activity of ATP is context dependent [8,9,10••]. Most DAMPs are best known for activating proinflammatory gene transcription through Toll-like (TLR), NOD-like (NLR), advanced glycation endproduct (RAGE), Mincle, or interleukin 1 (IL1) receptors in tissue resident sentinel cells (for example, Kupffer cells in the liver). After DAMP exposure, these cells synthesize inflammatory mediators, including IL1. Pro-IL1 is processed by caspase-1 into the mature cytokine. Caspase-1 is activated by the inflammasome, a macromolecular complex of NLR subunits that senses molecular signs of host damage or infection. IL1 coordinates local and systemic inflammatory responses, such as vascular adhesion of leukocytes or fever. The above mechanisms are extensively reviewed elsewhere [1,11,12].

In the zebrafish tail fin wounding model, first motile responses of leukocytes and epithelial cells are observed

Figure 1



Idealized cartoon scheme of early wound signaling sequence. Epithelial injury triggers a momentary efflux of damage associated molecular patterns (DAMPs) from lysing cells at the onset of wounding. Seconds-to-minutes lasting cytoplasmic Ca^{2+} transients stimulate NADPH oxidases, mitochondria, and phospholipases of stressed (but intact) cells to release reactive oxygen species (ROS), and polyunsaturated free fatty acids (PUFAs). ROS regulate proteins involved in the wound response, for example, by oxidation of 'thiol switches', and possibly NADPH depletion (for explanation see body text). PUFAs, such as arachidonic acid and linolenic acid, are metabolized into eicosanoids and jasmonic acid, which are powerful, early wound mediators in animals and plants, respectively. All the above signals also modulate wound-induced gene transcription to influence later stages of healing.

within 5 minutes after injury [10••,13,14•], that is, too fast to be regulated by *de novo* gene expression [15]. Aside from DAMP-induced chemotaxis (see above), other immediate wound signaling functions of DAMPs are conceivable, but have been little described. Interestingly, morpholino-mediated knockdown of Myd88, an essential adaptor protein for DAMP signaling through TLR and IL1-receptors, suppresses immediate leukocyte recruitment to zebrafish tail fin wounds [16] consistent with an involvement in early, chemotactic signaling. Likewise, caspase-1 can trigger rapid eicosanoid synthesis [17], pointing to a potential link between inflammasome-mediated sensing of DAMPs, and nontranscriptional, inflammatory signaling. In the case of internal tissue damage, it appears plausible that DAMPs released by cell lysis may be retained as extracellular gradients near the injury site for a while. The same is more difficult to envision for epithelial wounds exposed to a large volume of environmental or luminal fluid, such as fish epidermis or stomach mucosa. Here, DAMPs are likely to be instantaneously rinsed out and diluted. Are there mechanisms that could provide more reliable damage detection under such circumstances? Recent concepts of epithelial wound detection acknowledge the role of prompt and continous enzymatic synthesis of paracrine regulators by sublethally stressed cells at the injury site. Two important classes of such signals are reactive oxygen species (ROS) and polyunsaturated fatty acids (PUFAs), namely arachidonic acid (AA) in animals, and linolenic acid (LA) in plants. Each class is triggered by elevation of cytoplasmic calcium concentration (Ca^{2+}).

Cell stress signaling by ROS

ROS production is a conserved wound response of plants and animals [18]. ROS are a group of small, oxygen containing molecules generated as constitutive by-products of aerobic metabolism, or after cell stimulation. Primary sources of cellular ROS are mitochondria and NADPH oxidases. ROS ions or radicals (O^{2-}, $HO^{\bullet}$) are highly reactive and oxidize any nearby biomolecule they encounter. Hence, they cannot diffuse far from their source, and are little suited to act as paracrine signals. In contrast, H_2O_2 is less reactive and can travel over microns, or tens of microns in the cytoplasm or the interstitial space, respectively. In principle, this allows H_2O_2 to convey long-range signals. H_2O_2 can reversibly oxidize thiol containing amino acids and thereby alter protein function [19]. Regulatory 'thiol switches' occur in various signaling proteins, including tyrosine kinases, phosphatases, and small GTPases, and are involved in rapid wound signaling. In injured zebrafish larvae, the Ca^{2+}-dependent NADPH oxidase Duox generates an H_2O_2 gradient that extends ~150 μm from the wound site into the unwounded tissue, and mediates rapid leukocyte recruitment [13]. A similar mechanism was described in *Drosophila* [20•]. Also plants use a Ca^{2+}