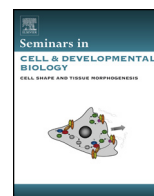




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Review

Wound redox gradients revisited

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ABSTRACT

Evidence emerges that redox gradients regulate morphogenesis, inflammation, regeneration, and healing of tissues. At the example of redox signaling during the zebrafish wound response, I briefly discuss current ideas on how such patterns might be sensed and spatially regulated to guide physiological processes over distances in animals.

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1. Introduction

In the early 20th century, Charles Manning Child's "gradient theory" [1] proposed that metabolic redox patterns regulate morphogenesis. After the discovery of genes as drivers of tissue patterning, Manning's ideas became a side note of science history. To date, the question whether and how gradients of oxygen and its reactive metabolites regulate organismal biology is gaining renewed attention. Wound-induced redox patterns, which are conserved from plants to animals, provide an exemplary, post-developmental case for redox signaling on tissue scales [2]. Owing

to its accessibility for intravital imaging approaches, transparent zebrafish larvae are an attractive system to study function and spatiotemporal regulation of these patterns during wound healing.

2. Wound redox gradients in zebrafish larvae

We became interested in biological redox patterns through our work on mitochondrial respiration in frog egg extracts [3,4]. In this system, mitochondria generate cytoplasmic redox gradients over tens to hundreds of microns. Such length scales are meaningful for spatial regulation of tissues, or unusually large cells, such as frog oocytes. The extract experiments inspired us to test whether redox gradients also occur in intact animals. By imaging the H_2O_2 biosensor HyPer in live zebrafish larvae, we found that tail fin

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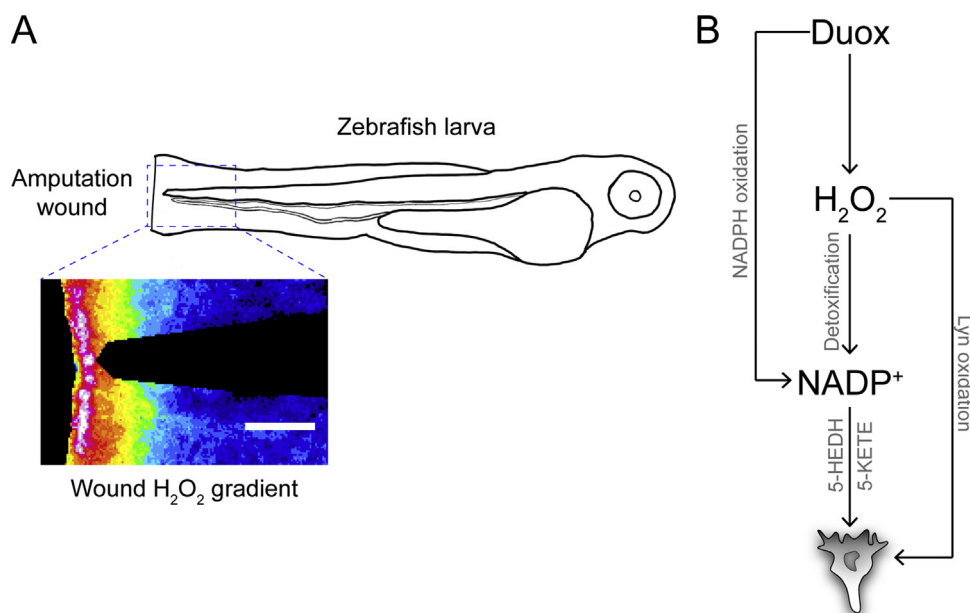


Fig. 1. (A) Tail fin wounding of zebrafish larvae generates an H_2O_2 gradient that reaches from the wound site into the tissue. Shown, representative image of the H_2O_2 gradient at a zebrafish amputation wound obtained by intravital HyPer ratio-imaging. Red, high $[H_2O_2]$. Blue, low $[H_2O_2]$. Scale bar, 100 μm . (B) Hypothetical scheme of wound redox signaling through NADPH oxidases. NADPH oxidases may signal through increasing both intracellular $NADP^+$ and extracellular H_2O_2 . H_2O_2 detoxification through antioxidant enzymes also increases intracellular $NADP^+$. $NADP^+$ stimulates lipid chemoattractant production (5-KETE) through an $NADP^+$ -dependent dehydrogenase (5-HEDH). Lyn oxidation (possibly within intracellular nanodomains) is also required for chemotactic wound signaling to leukocytes. Note the feed-forward loop structure of the system.

tip amputation elicits a ~ 150 – $300 \mu m$ wide H_2O_2 gradient that extends from the wound margin into the tissue (Fig. 1A). The gradient is generated by the Ca^{2+} -activated epithelial NADPH-oxidase $Duox$ [5], which transfers electrons from cytoplasmic NADPH onto molecular oxygen outside of the cell. The generated superoxide is dismutated into H_2O_2 , which diffuses within the extracellular space and enters cells [6]. Inhibition of $Duox$ suppresses the H_2O_2 gradient and wound recruitment of distant leukocytes [7], suggesting that $Duox$ mediates long-range signaling.

3. Signaling through wound redox gradients

Understanding long-range communication through redox gradients demands addressing two separate questions: How are these patterns sensed, and how are they generated within tissues?

So far, two non-exclusive ideas have been put forward to explain how leukocytes sense wound redox patterns (Fig. 1B): Directly, through H_2O_2 -mediated protein oxidation in leukocytes [8], or indirectly, through enzymatic oxidation of lipids that diffuse and trigger leukocyte chemotaxis through G-protein-coupled receptor signaling [9]. Direct H_2O_2 sensing has been reported to occur through Lyn —a membrane-bound non-receptor tyrosine kinase in leukocytes, which is activated by transient cysteine oxidation [8] (Fig. 1B). Active, oxidized Lyn is required for leukocyte recruitment to zebrafish and drosophila wounds [8,10]. Transient oxidation of regulatory “thiol switches”, such as the cysteine 466 in zebrafish Lyn [8], constitutes one of the better studied redox signaling mechanisms to date. Proteins susceptible to this type of regulation often contain deprotonated, and thus more reactive thiols at functionally important sites [11,12]. Signaling specificity is thought to emerge from the higher oxidant reactivity of “regulatory thiols” as compared to other thiols in the proteome environment, and from the selective accessibility of regulatory thiols to a specific reactive oxygen species (ROS), for example H_2O_2 . Some of the most reactive cysteines are found in professional antioxidant peroxidases [13,14], or specialized H_2O_2 -sensitive transcription factors, such as the bac-

terial protein OxyR [15]. Their high reactivity has been exploited for H_2O_2 biosensor design through fusion with fluorescent domains. HyPer [16], which is based on OxyR, and Orp1-roGFP2 and roGFP2-Tsa2, which are based on yeast peroxidases [17,18], detect low, physiological amounts of H_2O_2 only because they effectively compete with cellular antioxidants. Judging from proteins whose H_2O_2 reactivity is known, redox sensitive, non-antioxidant proteins are oxidized far slower by H_2O_2 than professional antioxidants or OxyR. For instance, the rate constant of the redox-regulated tyrosine phosphatase 1B (PTP1B) for its reaction with H_2O_2 is ~ 7 times higher than that of free cysteine, but still 6 orders of magnitude lower than that of peroxiredoxin [19]. Accordingly, thiol switches of non-antioxidant proteins may only become oxidized when antioxidant activity is locally or temporally suppressed. Peroxiredoxins may be transiently depleted by high pulses of oxidants or locally inactivated by phosphorylation, for example, near membranes. This is thought to create discrete time windows or spatial nanodomains permissive for redox signaling to non-antioxidant proteins [20,21]. Whether, when and where antioxidants are inactivated during physiological wound redox signaling remains experimentally little addressed. To this end, we recently developed an imaged-based method that measures antioxidant reaction kinetics from time-lapse HyPer measurements in wounded zebrafish larvae [22]. Our data suggest that H_2O_2 inactivates antioxidants within $\sim 30 \mu m$ of the wound margin. Interestingly, we found no evidence that the wound-induced H_2O_2 gradient breaches antioxidant barriers at larger distances. This suggests that Lyn must be exempted from general antioxidant competition for leukocytes to directly sense H_2O_2 from $> 30 \mu m$ away. Future experiments should explicitly test this possibility.

Not all redox signaling mechanisms are antagonized by antioxidants: By breaking down H_2O_2 , antioxidants themselves may transmit signals through redox chains [23,24]. Such “redox relay signaling” may contribute to wound chemotaxis of leukocytes in zebrafish (Fig. 1B). $Duox$ consumes O_2 and NADPH to generate H_2O_2 and $NADP^+$. $NADP^+$ also accrues during H_2O_2 detoxifica-

tion by thioredoxin and glutathione reductases of the antioxidant chain. Much attention has been focused on the role of H_2O_2 as diffusible redox signal. However, Duox-induced increases of cytoplasmic NADP^+ may be of regulatory importance, too. Biosensor imaging in zebrafish larvae suggests that the wound redox gradient is paralleled by a gradient of cytoplasmic NADP^+ , decreasing from the wound margin into the tissue [25]. Enzymatic reactions that require NADP^+ as cofactor may be sensitive to such a gradient. For example, the microsomal NADP^+ dehydrogenase 5-HEDH, uses NADP^+ to oxidize the hydroxyl group of the lipid mediator 5-HETE into a keto group [26–29]. This generates 5-KETE—an oxidized lipid product that is strongly chemotactic for neutrophils and eosinophils [30]. 5-KETE attracts neutrophils to zebrafish wounds [9]. Through mediating intracellular peroxiredoxin oxidation, diffusing H_2O_2 could propagate increased $\text{NADP}^+/\text{NADPH}$ ratios to neighboring cells to activate 5-HEDH and 5-KETE synthesis around epithelial wounds. Per this model, both H_2O_2 and 5-KETE diffusion would contribute to the overall range of chemotactic wound signaling. The model implies a novel role for NADPH oxidases as regulators of NADP^+ dependent metabolism (e.g., of inflammatory lipids), in addition to their well-known role as ROS generators. It will be intriguing to test the physiological relevance of this concept in the future.

4. Spatial regulation of wound redox gradients

Per the above considerations, H_2O_2 tissue diffusion plays a central, paracrine role, either through mediating oxidation of regulatory protein thiols or lipid oxidation via $\text{NADP}^+/\text{NADPH}$ ratio changes at distances. But how are H_2O_2 diffusion patterns regulated? Biophysical aspects of redox signaling have remained little studied, although they could help to quantitatively understand H_2O_2 's tissue signaling roles [31]. Like water, H_2O_2 diffuses fast. But its signaling range within the tissue is limited by sink reactions that constantly remove H_2O_2 from the extracellular space. Namely, fast extracellular H_2O_2 diffusion, is counterbalanced, at least in part, by aquaporin-mediated uptake into cells and peroxiredoxin-thioredoxin-mediated breakdown within them [22]. Whereas gradients generated by H_2O_2 diffusion alone are expected to be transient and spatially unstable, reaction-diffusion establishes H_2O_2 steady state gradients of discrete length scale that are robustly maintained in the tissues over tens of minutes, that is, presumably long enough to convey information on tissue injury over distances. This highlights another regulatory role of antioxidants as active mediators of spatial redox patterning.

5. Conclusion

A century ago, Charles Manning Child only had toxic live dyes, such as methylene blue, to study redox gradients, and little means to determine their function [32]. Today, we can harness a growing panel of genetically encoded biosensors and the power of genetics and pharmacology. Combined with quantitative thinking, these approaches may elevate tissue redox patterns from the niche of fascinating phenomenology into the realm of mechanistic physiology.

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