Seminars in Cell & Developmental Biology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Wound redox gradients revisited

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ARTICLE INFO

Article history:
Received 15 June 2017
Received in revised form 19 July 2017
Accepted 21 July 2017
Available online xxx

Keywords:
Redox gradient
Metabolic gradient
Wound healing
Reactive oxygen species
Signaling
Neutrophil
Chemotaxis
NADPH oxidase
Zebrafish
Biosensor
Imaging

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.

Contents

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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http://dx.doi.org/10.1016/j.semcdb.2017.07.038
1084-9521/

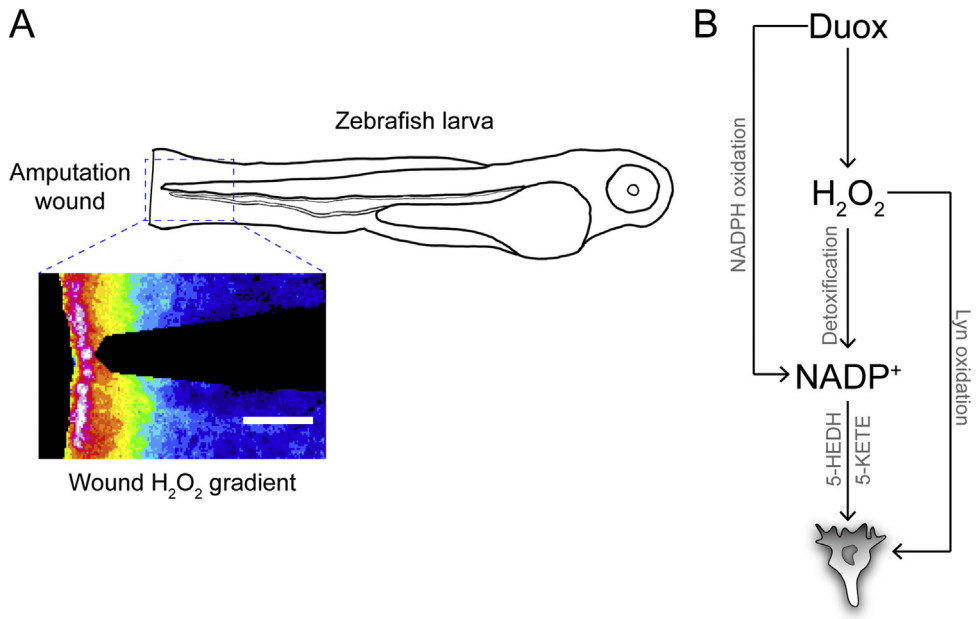

Fig. 1. **(A)** Tail fin wounding of zebrafish larvae generates an H_2O_2 gradient that reaches from the wound site into the tissue. Shown, representative image of the H_2O_2 gradient at a zebrafish amputation wound obtained by intravital HyPer ratio-imaging. Red, high [H_2O_2]. Blue, low [H_2O_2]. Scale 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 μm 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 bacterial 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 ~30 μm 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 μm 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-

Please cite this article in press as: P. Niethammer, Wound redox gradients revisited, Semin Cell Dev Biol (2017), http://dx.doi.org/10.1016/j.semcdb.2017.07.038