

● PERSPECTIVE

Nerves and hydrogen peroxide: how old enemies become new friends

ROS and nerves play together during the regeneration process: For many years, the role of reactive oxygen species (ROS) in neurobiology has mainly focused on its pathological implications in neurodegenerative diseases. Contrasting with this view, ROS were recently put forward as key positive signals for axon growth and repair, highlighting beneficial functions of ROS signalling in the vertebrate adult brain (Borquez et al., 2016). Nerve injury is often associated with damage of the neighbouring tissues. It was demonstrated, first in larvae and then in adult vertebrates, that the increase of ROS production induced by tissue lesion stimulates axon growth (Rieger and Sagasti, 2011; Gauron et al., 2013; Meda et al., 2016). Tissue re-innervation, starting with axon regeneration, is a prerequisite to launch the regenerative program in damaged tissues and organs (Kumar and Brockes, 2012). As ROS are also required for regeneration to proceed (Gauron et al., 2013), simultaneous control of tissue and axon regeneration by a same redox signal would be a parsimonious way to coordinate the reformation of a fully functional

tissue (Meda et al., 2016) (Figure 1).

H₂O₂ stimulates axon growth during development and adult regeneration: Appendage and organ regeneration are often described as a replay of the developmental process. The role of hydrogen peroxide (H₂O₂) during morphogenesis was recently addressed in zebrafish thanks to the engineering of a transgenic fish line harbouring ubiquitous expression of the ratiometric HyPer sensor to monitor H₂O₂ levels *in vivo*. H₂O₂ levels are highly dynamic, both spatially and temporally. They reach their maximum during early developmental stages, somitogenesis and organogenesis, and decrease at the end of morphogenesis down to minimal levels that persist in the adult. Within the embryo, the brain displays highest levels of H₂O₂. Soaking the embryos in a solution of NADPH oxidase pan-inhibitor (main enzymes responsible for H₂O₂ production) dramatically reduces H₂O₂ levels and impairs retinal ganglion cells axonal projections toward the tectum. Interestingly, this defect is rescued by either exogenous application of H₂O₂ or activation of the Hedgehog pathway (Gauron et al., 2016). This situation is reminiscent of axonal growth during adult regeneration, which in the same way is controlled by the interplay between H₂O₂ and Hedgehog (Meda et al., 2016) (Figure 2).

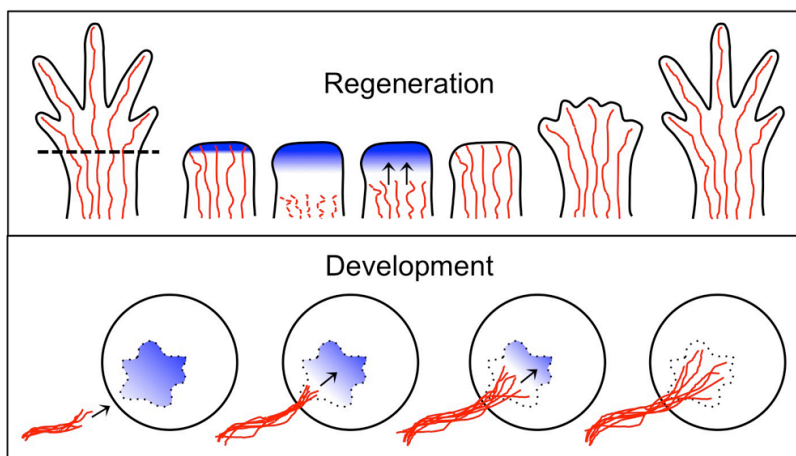


Figure 1 Nerves and hydrogen peroxide interact during regeneration and development.

The interplay between nerves and hydrogen peroxide during the regeneration process is here represented through the scheme of a regenerating paw (upper part). Immediately after amputation (dashed line), H₂O₂ accumulation (blue) is detected in the stump epidermis. Few hours after amputation, the Wallerian degeneration begins and the nerves (red lines) recede along the stump; meanwhile hydrogen peroxide starts to accumulate at the tip. The newly established H₂O₂ gradient then attracts the nerves, which regrow toward the tip of the stump, until they re-innervate the entire appendage. Nerve arrival switches off the production of H₂O₂, which is no longer detected, and allows the regeneration of the missing part of the paw to proceed. During development (bottom part) the developing tissues/organs of the embryo (white circle) exhibit some areas with higher concentrations of H₂O₂ (blue area), which acts as an attractant for nerves (red lines). As nerves populate their target area, the concentration of H₂O₂ gradually decreases down to levels observed in the surrounding tissues, concomitantly with the completion of the innervation process.

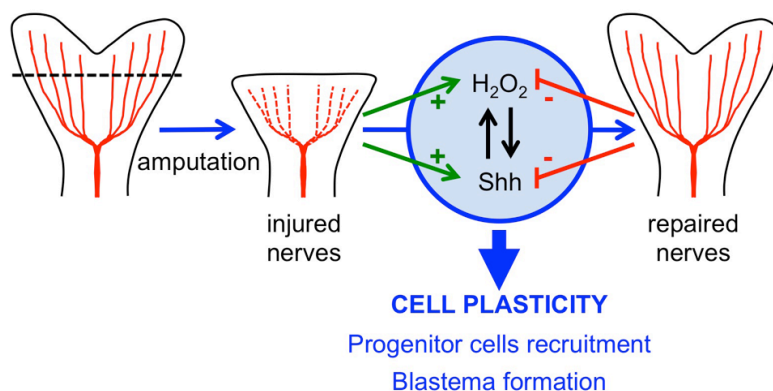


Figure 2 Nerves, H₂O₂ and Shh play together during adult zebrafish fin regeneration.

Following amputation, the injured nerves induce the production of H₂O₂ through the activation of the Hedgehog (Shh) pathway, thus providing an environment that promotes cell plasticity and pro-regenerative processes, such as progenitor recruitment and blastema formation. The re-innervation of the regenerating appendage successively stops the production of H₂O₂, bringing back the redox environment to its original state.



The identity of the targets of ROS signalling mediating axon regeneration remains an open question that, to be solved, will need to decipher the redox code of protein modification (Jones and Sies, 2015). Cytoskeleton proteins, of which dynamic assembly is regulated by ROS signalling (Wilson and Gonzalez-Billault, 2015), are attractive candidates.

Nerves control H₂O₂ levels: Since many decades, nerves were shown to be key players in metazoan regeneration and tissue repair. Back in the 1950's, M. Singer proposed the existence of a neurotrophic factor, the "factor X", produced by the nerves and diffusing in the damaged tissue, required to initiate and guide the progression of the regeneration process (Kumar and Brockes, 2012 and references therein). Recently, a denervation strategy in adult zebrafish has revealed that nerves control ROS levels both in physiological conditions and after injury (Meda et al., 2016). In healthy tissues, nerves maintain low ROS levels thanks to Schwann cells, below the levels required for axon and organ regeneration (Meda et al., 2016). After amputation, injured nerves activate Shh signalling in Schwann cells that, in turn, is responsible for H₂O₂ production in the wounded epidermis (Meda et al., 2016). It is tempting to propose that Shh might correspond to the "factor X" proposed by Singer in 1954, acting on H₂O₂ production in the nerve regenerative environment.

Altogether, these findings support the existence of a feedback loop between nerves and H₂O₂, in which nerves control H₂O₂ levels in the tissue, which in turn participate in axon regrowth after injury. These data also lead us to propose that the redox environment of peripheral nerve endings might be a good target for the manipulation of adult cell plasticity (Figure 2).

Targeting nerves/redox levels loop in neurodegenerative diseases: Chronic wound and tumour irradiation are often associated with neuropathies and in both cases, nerve degeneration is due to an inaccurate cross-talk between axon and glia (Zenker et al., 2013). Manipulation of Hedgehog signaling has been shown to reverse diabetic neuropathy (Calcutt et al., 2003), but only few reports have addressed the involvement of H₂O₂ in this pathology (Pop-Busui et al., 2013; Papanas and Ziegler, 2014). Based on our recent findings showing that the cross-talk between neurons and glia operating during vertebrate regeneration involves the combination of redox and Hedgehog signalling, we propose that interactions between these two pathways should be considered in other situations. In regenerative processes, although inflammation-induced ROS production could participate in axon recovery, the recent identification of a feedback loop between H₂O₂ and axon growth might plead for more cautious approaches, due to the extreme sensitivity of nerves to ROS levels.

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