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Review article

Do you have the nerves to regenerate? The importance of neural signalling in the regeneration process

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ABSTRACT

The importance of nerve-derived signalling for correct regeneration has been the topic of research for more than a hundred years, but we are just beginning to identify the underlying molecular pathways of this process. Within the current review, we attempt to provide an extensive overview of the neural influences during early and late phases of both vertebrate and invertebrate regeneration. In general, denervation impairs limb regeneration, but the presence of nerves is not essential for the regeneration of aneurogenic extremities. This observation led to the "neurotrophic factor(s) hypothesis", which states that certain trophic factors produced by the nerves are necessary for proper regeneration. Possible neuron-derived factors which regulate regeneration as well as the denervation-affected processes are discussed.

1. Introduction

Regeneration is a diverse concept, defined differently depending on the context, covering processes from tissue repair to asexual reproduction. Basically, each type of regeneration can be described as the ability of an organism to repair and regrow lost or damaged tissues, structures and even entire extremities without the formation of scar tissue and with functional integration of the regenerate in the pre-existing tissues (Oviedo and Beane, 2009). Although the regeneration capacity of humans is limited to the repair of cuts in our skin, healing of broken bones, regeneration of lost digit tips and parts of our liver, regeneration is not an uncommon feature. In fact, it is a trait that is widely distributed in the animal kingdom, and virtually each animal class has at least one representative with good regeneration capacities (Pagan, 2014). A lot of organisms can regenerate, at least partly, during early life stages, but lose this ability due to metamorphosis or ageing (Seifert and Voss, 2013). Some organisms maintain excellent regenerative abilities throughout their lives. These animals, both vertebrate (f.e. *Xenopus* species, axolotl, salamanders, and zebrafish) and invertebrate organisms (f.e. *Hydra*, planarians), are commonly used as model organisms in regeneration research (Sanchez Alvarado, 2000; Brockes and Kumar, 2008; Fior, 2014; Gurtner et al., 2008; Li et al., 2015). They are crucial to acquire each piece of information concerning the regeneration process, from involved genetic responses to cellular signalling. It Is only by doing this that regenerative medicine can be successfully achieved and new insights in various pathological conditions can be discovered, since regenerative tissues and organisms have the potential to overcome degenerative disorders (Alzheimers disease, Parkinsons disease) or cancer (Stevens et al., 2015). Important features of the regeneration process identified in various of these model organisms include the time point(s) of the proliferation peaks, the origin and migration of involved progenitor cells, the importance of apoptosis for regeneration to proceed, and the genes and signalling pathways involved (Sanchez Alvarado, 2000; Carlson, 2007; Vriz et al., 2014). Among these factors, innervation comes forward as a crucial parameter in successful regeneration. However, its exact role and the underlying mechanisms and factors remain largely unknown. The reason for this lack of knowledge is the fact that most of the research on this topic was published in the 1950s up to the 70s, when the necessary molecular techniques were simply not available. Although important contributions on the role of the nervous system for successful regeneration were made in

Abbreviations: AEC, apical epithelial cap; AGP, anterior gradient protein; BDNF, brain-derived neurotrophic factor; Cnox-2, paraHox *gsx* homologue gene; DUOX, dual oxidase; EGR, early growth response; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GDNF, glial cell-derived neurotrophic factor; GGF, glial growth factor; GJ, gap junction; HGF, hepatocyte growth factor; Hh, hedgehog; IGF, insulin-like growth factor; JNK, c-jun N-terminal kinase; Krt8, keratin 8; Lef1, lymphoid enhancer-binding factor 1; MAPK, mitogen-activated protein kinase; Mkp3, MAPK phosphatase 3; NADPH, nicotinamide adenine dinucleotide phosphate; NF, neurotrophic factor; Nip1, numb-interacting protein; NOX, NADPH oxidase; Pxn-2, peroxidasin 2; ROS, reactive oxygen species; SCF, scatter factor; Sdf1, stromal cell-derived factor 1; WE, wound epidermis; Wnt, wingless-type MMTV integration site

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the mid 1900s, a lot of information is still missing. During the last years, research on this topic continued or is ready to be re-investigated in more molecular contexts. In this review, we represent the state of the art on the interconnection of innervation and (in)vertebrate regeneration. We give an overview of potential signalling factors and pathways, including the possible involvement of the cellular redox state.

2. The importance of innervation in vertebrate regeneration

2.1. A brief description of vertebrate extremity regeneration

The general process of appendage regeneration (such as regeneration of limbs, tails and fins) proceeds in distinct phases (Fig. 1) (Simoes et al., 2014; Stocum, 2011; Kumar and Brockes, 2012). After amputation, epithelial cells first reorganise and migrate to the wound site in order to form the wound epidermis (WE) and close the wound. Via cell migration, the WE acquires additional cell layers and eventually establishes a specialized epidermis called the apical epithelial cap (AEC) (Simoes et al., 2014; Stocum, 2011; Kumar and Brockes, 2012). Next, in the mesenchymal tissue beneath the AEC the extracellular matrix of the tissues is degraded by proteases, liberating stem cells as well as mononucleate myofiber fragments, chondrocytes, fibroblasts and Schwann cells, which all start to dedifferentiate, migrate to the amputation plane and re-enter the cell cycle to give rise to the blastema (an undifferentiated cell mass, which will start to differentiate and in which the missing structures will be formed) (Simoes et al., 2014; Stocum, 2011).

In a last phase, interactions between the AEC and the blastema ensure growth and patterning of the regenerate until the formation of the missing structure is completed (Fig. 1) (Simoes et al., 2014; Stocum, 2011). The establishment and outgrowth of the regenerate are under the control of many factors, including the presence of nerves at the wound site. Due to the damage caused by the injury, nerves degrade, after which sensory neurons rapidly regenerate in the AEC, while motor neurons regenerate between the subjacent blastema cells (Stocum, 2011; Kumar and Brockes, 2012; Salpeter, 1965; Lentz, 1967). Both of these processes seem to be crucial for proper regeneration to proceed, since denervation results in various regeneration defaults depending on the extent and time of denervation (Brockes and Kumar, 2008; Carlson, 2007; Simoes et al., 2014; Stocum, 2011; Kumar and Brockes, 2012; Kumar et al., 2007). In the following section, the different aspects of the nerve dependence of the regeneration process are discussed.

2.2. Regenerative capacities are affected by denervation

Although many model organisms have been used to investigate the regeneration process, the earliest and most intensively studied example which describes the necessity of existing nerves during regeneration is that of the salamander limb. Researchers have used this model system in past and ongoing research to illuminate the necessity of neuronal presence – the abundance of innervation and the time of denervation rather than the type or activity of the nerves – for successful regeneration (Fig. 2) (Carlson, 2007; Stocum, 2011; Thornton, 1970; Mullen et al., 1996; Seifert et al., 2012; Singer, 1952). In the following section, we discuss their most important contributions on this topic in a chronological manner.

Nerve dependence during salamander regeneration was described for the first time in the 1823 – even before the establishment of the cell theory – by Tweedy John Todd (Todd, 1823). He noticed that salamanders experienced regeneration defaults after transection of the sciatic nerve in their limbs. Denervation before amputation resulted in healing with the formation of scar tissue, while regeneration was completely inhibited or retarded if denervation was performed after wound healing. If the nerve was transected after blastema formation, the regenerate remained static or regressed. Although these “simple” experiments provided early proof for the importance of innervation during vertebrate regeneration, it was not until the early 1900s that research on this topic continued.

In the early 1940s, Butler and Schotté (1941) performed denervation experiments on larval urodele limbs (*Ambystoma punctatum*, *Ambystoma opacum*, and *Triturus viridescens*) and noticed

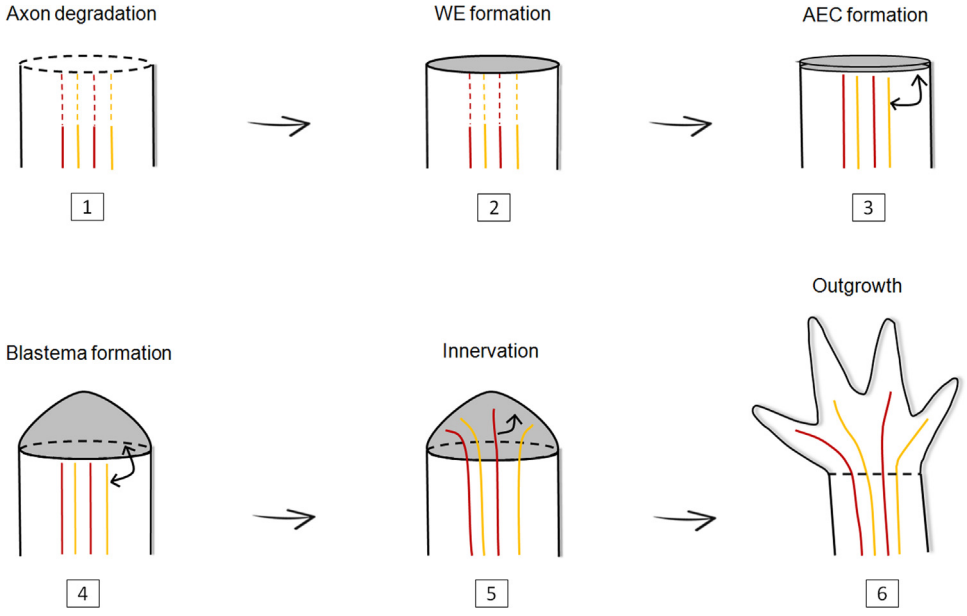

Fig. 1. The general different phases of regeneration and the involvement of neural interaction. (1) Following amputation or injury, axonal degradation (as presented by the dotted lines) appears. Different types of nerves are presented by the different colours of the lines. (2) Directly thereafter, the wound epidermis (WE) is formed and the wound is closed. (3) The WE expands and the formation of the apical epithelial cap is established. Interaction between the regenerating axons and the AEC is necessary for proper blastema formation. (4) The blastema is formed through proliferation of progenitor cells and these blastema cells promote the innervation of the regenerate (5). (6) Innervation and signalling between the neurons and the blastema is necessary for successful outgrowth and patterning of the regenerate.

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