Review

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Nerve dependence in tissue, organ, and appendage regeneration

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Many regeneration contexts require the presence of regenerating nerves as a transient component of the progenitor cell niche. Here we review nerve involvement in regeneration of various structures in vertebrates and invertebrates. Nerves are also implicated as persistent determinants in the niche of certain stem cells in mammals, as well as in *Drosophila*. We consider our present understanding of the cellular and molecular mechanisms underlying nerve dependence, including evidence of critical interactions with glia and non-neural cell types. The example of the salamander aneurogenic limb illustrates that developmental interactions between the limb bud and its innervation can be determinative for adult regeneration. These phenomena provide a different perspective on nerve cells to that based on chemical and electrical excitability.

Introduction

Nerves play an important role in various contexts of metazoan regeneration and tissue repair. This activity is considerably less familiar to neuroscientists than the signalling roles associated with chemical and electrical transmission. Our review introduces this topic with an emphasis on current findings. The neuron is a securely post-mitotic cell type, and nerve cells do not in general contribute directly to the regenerating tissue, but neuronal processes are often present among the cells of the niche that supports regeneration. This contribution can be removed by surgical denervation or by genetic ablation, and the effect of these manipulations on the outcome of regeneration is often marked. Although the activity of nerves is most obvious in terms of the proliferation of regenerative cells, there are other effects as well. This contribution to the mechanisms underlying regeneration and repair appears to be an unconventional property in that it does not usually involve electrical or neurotransmitter signalling.

Nerve dependence was discovered in the context of salamander limb regeneration [1]. Several other examples of salamander, *Xenopus* and fish regeneration continue to be investigated in relation to this property, or seem to be ready for renewed study. Prevention of innervation during development of the salamander limb leads to a fascinating exception to nerve dependence, referred to as the aneurogenic limb (ANL), and we review a coherent explanation for this. We also consider several demonstrations of nerve dependence in mammals, as well as the possibility that other currently unsuspected examples of regeneration may have this property. Finally, the role of nerves in various cases of invertebrate regeneration is of interest for evolution of the nervous system, as well as evolution of regeneration. Regeneration of the primary body axis in some invertebrates appears to be closely related to asexual reproduction by fission, and regulation of these processes may be an important attribute of neural networks whose significance has been underestimated.

Nerves and salamander limb regeneration

Limb regeneration in salamanders proceeds by formation of the blastema, a mesenchymal growth zone of local progenitor cells that is adjacent to a specialised wound epithelium (WE) (Figure 1a). Limb denervation at the level of the brachial plexus appears to inhibit neither WE formation by cell migration nor generation of the initial cohort of blastemal progenitors. It does curtail proliferation of blastema cells, and this effect is mediated by the interaction of nerves with the WE [2]. Regenerating axons and the WE are key elements of the niche for limb regeneration, and are appropriately transient cellular phenotypes for regulating episodes of adult regeneration. Axons cease to regenerate after meeting their targets and the WE differentiates into normal epidermis. The activity of regenerating axons on the blastema is not dependent on action potentials or release of acetylcholine, and can be mediated by either an exclusively sensory or an exclusively motor population that is present in adequate quantities [3–5]. The accessory limb model (ALM), in which a deflected nerve induces formation of a limb, is considered in Box 1.

In the case of newt limb regeneration, the secreted newt anterior gradient (nAG) protein (sometimes referred to as AGP [2], or AGR2 for the mammalian orthologue [6]) plays a role in nerve dependence [7]. AG proteins were discovered in relation to the specification and activity of the cement gland, a mucus-secreting anterior structure in the *Xenopus* tadpole [8]. Peripheral axons regenerate after limb transection and markedly upregulate AG expression in their ensheathing Schwann cells at the end of the stump. This is followed by AG expression in dermal glands underlying the WE (Figure 1a). If the limb is denervated before amputation, this prevents AG expression in both locations. Expression of the nAG protein after electroporation of an expression plasmid into a denervated limb blastema leads to induction of positive gland cells under the WE [7]. It is possible that the normal appearance of the glands after

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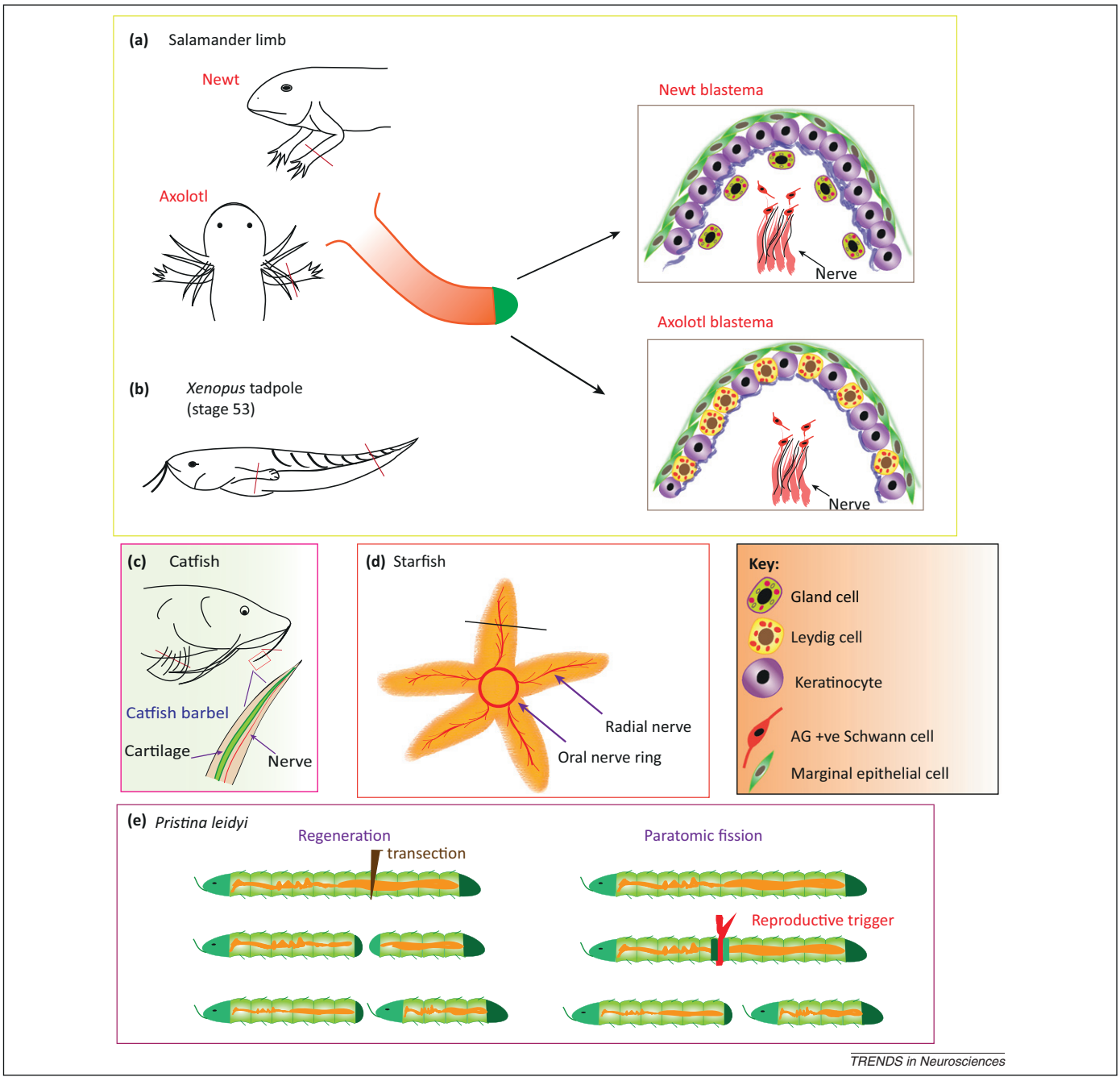

Figure 1. Examples of nerve-dependent regeneration in various phyla. **(a)** Regeneration of the forelimb in the newt and larval axolotl. After amputation of the limb, regeneration proceeds by formation of a blastema (green). The panel on the right shows diagrammatic illustrations of the blastema. In the newt, anterior gradient (AG) protein is expressed in Schwann cells at the end of the nerve and in gland cells underlying the wound epithelium [7]. In axolotls, Leydig cells of the wound epithelium, in addition to Schwann cells, express AG. **(b)** *Xenopus* tadpoles are capable of limb regeneration until metamorphosis at stage 53. Tail amputation results in regeneration of the spinal cord, as well as a full-length tail. **(c)** The catfish can regenerate its sensory barbels after injury. The barbel is supported by a cartilaginous core and nerves run along the axis of the tissue to the sensory receptors. **(d)** In starfish the oral nerve ring is critical for arm regeneration. Each arm of the starfish is innervated by radial nerves, which radiate from the oral nerve ring. **(e)** Regeneration of the worm *Pristina leidyi* occurs after transection, as well as after paratomic fission [58]. Favourable environmental conditions trigger fission at the middle of the body. A fission zone containing a presumptive head and tail forms before fission, and scission of the body occurs through the mid-plane (red). After transection of the worm, the anterior segment regenerates a tail, whereas the posterior fragment regenerates a new head.

amputation depends on the inducing activity of nAG that is released from terminal Schwann cells, an activity that has been demonstrated for *Xenopus* AG protein in relation to the cement gland [9]. nAG expression after electroporation is able to rescue 50% of denervated limbs to complete the proximodistal axis and form digits [7].

These results raise several issues of functional and comparative anatomy [10]. The gland cells under the WE are not ducted to the exterior, but apparently discharge into the blastema via a holocrine mechanism. Limb regeneration has been studied in both the adult newt and the paedomorphic larval axolotl. The skin of the axolotl has dermal glands but these are absent under the WE, although the glands just proximal to the amputation plane do upregulate AG expression. Secretory Leydig cells within the axolotl WE stain positive for axolotl AG (Figure 1a) and this expression was diminished by prior denervation [10]. The possibility that other activities such as fibroblast

Box 1. Nerve-induced accessory limb formation

It is possible to induce a new limb in a larval or adult salamander without amputation of an old one (Figure I). The ectopic limb is generally deficient in structural elements such as the humerus and elbow [61]. Comparative gene analysis has been performed on regenerating and accessory limbs in the axolotl, and many of the regeneration-associated genes are common to both [62]. Tissue labelling experiments have shown that dermal fibroblasts contribute to the formation of an accessory limb [61].

Denervation of the host limb curtails growth and results in regression of the blastema [61], which emphasises that regeneration of the deflected nerve is obligatory for proliferation and growth of the accessory limb blastema [63]. It has been shown that the axons invade the WE, and signalling from the nerve induces early expression of the buttonhead-like zinc-finger transcription factor *Sp9* and regulates cell cycle withdrawal in basal keratinocytes of the apical epidermal cap [64]. In a normal limb blastema, the WE is extensively innervated by sensory axons, whereas motor axons regenerate between blastemal cells and do not invade the apical cap or WE [65–67]. Nonetheless, either motor or sensory nerves in adequate numbers are sufficient to satisfy the nerve requirement for regeneration [68,69]. Earlier studies showed that invasion of nerves in the WE, although characteristic of normal regeneration, is not necessary for support of limb regeneration. There are experimental circumstances in which the epithelium is densely innervated yet regeneration does not occur [70]. These studies were performed in the adult newt and there could be a difference compared to the axolotl in this respect. In the ANL, formation of a WE that is capable of supporting regeneration occurs in the absence of nerves (see Figure 2 in main text). In an accessory limb blastema, where a large branch of peripheral nerve is relocated to a wound boundary, there may be an enhanced possibility for close interaction of regenerating axons with the WE [71]. It has been proposed that FGF may be a nerve-derived signal that is involved in conjunction with matrix metalloprotease activity in the formation of accessory limb blastemal cells [12].

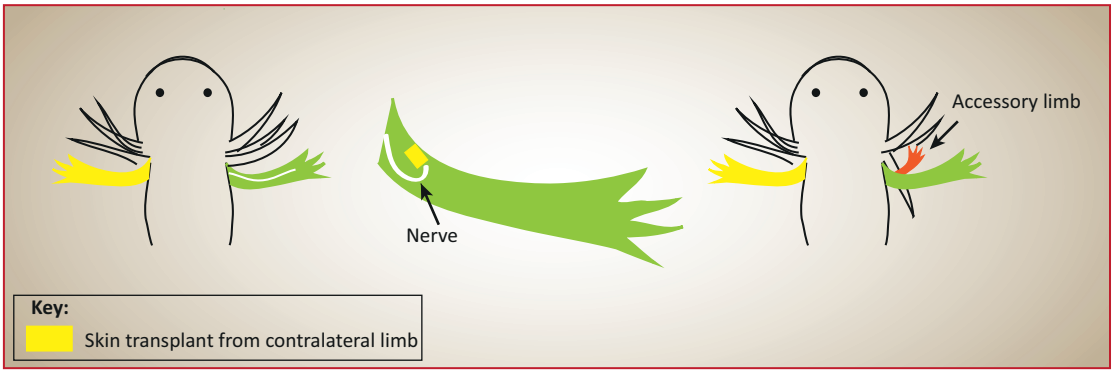

Figure I. Formation of ectopic limbs in axolotls. If a large nerve branch is transected and the cut end is deflected under a skin wound in the regenerative territory, this can induce formation of a limb or other appendage, depending on the location of the wound [72]. Deflection of the nerve under normal skin does not induce a limb, but if the nerve is positioned at the margin of the wound, the regenerating axons interact with the WE formed at that location. The preferred method for production of accessory limbs in axolotls involves transplantation of a skin patch (yellow) from the contralateral limb to create a local axial disparity [61,73].

growth factor (FGF) [11–13] and transferrin [14] are relevant for nerve dependence remains open. These experiments provide an explicit model of nerve dependence in the newt whereby regenerating axons interact with the WE through the diffusible signal nAG, which has the ability to induce the formation of dermal glands that in turn release nAG and possibly other signals.

How does nAG exert its effects on the blastema? It is possible that induction of the dermal glands in the newt leads to release of gland-derived signals in addition to nAG that act on blastemal cells. Nonetheless, when newt limb mesenchymal blastemal cells were isolated by gentle dissociation [15] and plated in culture, exposure to recombinant nAG stimulated their entry to S phase [7]. nAG was originally identified in salamanders as a binding partner for the GPI-anchored three finger protein (TFP) called Prod1, which is expressed on the surface of blastemal cells [16]. Prod1 is a salamander-specific member of the TFP family [17], so extension of the Prod1–nAG interaction to other vertebrates would require another receptor, possibly a taxon-specific TFP, in place of Prod1. The activity of nAG on the S phase labelling of blastemal cells is blocked by antibodies against Prod1, but details of the downstream signalling remain to be elucidated, although it is known that Prod1 can activate the epidermal growth factor (EGF) receptor in cultured cells [18]. AGR2 is upregulated in many examples of mammalian adenocarcinoma and acts on human oesophageal tumour cells to stimulate proliferation by inducing the expression of amphiregulin, a growth-promoting EGF receptor ligand [6]. This is mediated by activation of the Hippo signalling pathway co-activator YAP1. It thus promotes growth by regulating the Hippo and EGF pathways, and it will be interesting to determine if these activities operate during limb regeneration.

Anuran and fish regeneration

Limb regeneration in *Xenopus* during the early stages of larval development is independent of nerves, and only shows a denervation effect at the end of larval life (Figure 1b) [19]. Limbs do not regenerate after metamorphosis, but form a hypomorphic outgrowth or 'spike' of cartilage after amputation [19,20]. The proliferation of limb blastema cells during the early stages of outgrowth in the *Xenopus* froglet is relatively insensitive to nerve ablation after uprooting of the nerve trunk. Later stages show decreased S-phase labelling of blastema cells and this is correlated with diminished expression of FGF8 and 10 in the WE and blastema [20]. Classical studies revealed that tail regeneration in urodeles depends on the presence of adjacent spinal cord [21]. Its importance for tail regeneration in the *Xenopus*

tadpole has been demonstrated (Figure 1b). Spinal cord ablation does not affect the early steps of regeneration, including wound healing and generation of progenitor cells, but proliferation of the latter was somewhat reduced and the regenerated tail was twisted. One or more FGFs expressed in the spinal cord may play a role in the normal proliferation of progenitor cells, as evidenced by implantation of growth-factor-coated beads [22]. Tail amputation apparently activates the proliferation of neural precursor cells expressing *Sox2*, and overexpression of a dominant negative *Sox2* construct inhibits regeneration not only of the spinal cord but also of the tail [23]. Thus, regeneration of the spinal cord may be a step that is critical for subsequent regeneration. Nerve dependence in fish is familiar from classical studies of regeneration of the fin [24] and the sensory barbel [25], and both issues are under investigation in the zebrafish. A study has analysed both the development and regeneration of the zebrafish barbel, as well as its innervation by branches of the facial nerve [26]. This work has established that the barbel can regenerate after repeated injury and in senescent fish.

The aneurogenic limb

Regeneration of an adult structure is a sequel to its generation during embryonic development and subsequent growth, and studies of regeneration have often addressed the issue of similarities and differences between development and regeneration [27]. Outgrowth of the embryonic limb is not dependent on nerve axons, and nerve dependence has provided a distinction between development and regeneration [28]. By contrast, the phenomenon of the salamander ANL provides the most striking example known in which developmental interactions actually determine the mechanism of adult regeneration (Figure 2). A large section of the neural tube can be removed from a salamander embryo so that the limb develops without innervation. If such an ANL is transplanted to the flank of a normal larva, it can be functionally innervated by the host. This was an important demonstration by Ross Harrison that nerves and their targets did not have to be in embryonic proximity to establish connections [29]. The ANL can regenerate in the absence of a nerve supply [30–32] and becomes nerve-dependent for regeneration after transplantation and innervation [33].

Recent work has provided an explanation of these phenomena in the spotted salamander *Ambystoma maculatum* involving regulation of AG expression in the epidermal niche [34]. Before the arrival of the nerve at stage 38, AG expression is readily detectable in cells of the larval epidermis, some of which are glandular in nature as evidenced by electron micrographs (Figure 2b,ii). After stage 38, the arrival of nerves leads to diminished staining with antibodies to AG and a decrease in the incidence of gland cells (Figure 2a). By contrast, the ANL shows strong epidermal AG expression and a high incidence of gland cells such that by stage 46 the difference compared to the normal limb is marked (Figure 2b). When the ANL is transplanted, subsequent innervation leads to marked downregulation of AG expression in the epidermis (Figure 2d). After amputation of an ANL, the early blastema is already strongly positive for AG expression in the WE, in contrast to the early WE of a normal limb (Figure 2c) [34]. The latter requires the priming activity of regenerating axons to establish AG expression as described above [7].

These observations identify AG downregulation in the larval epidermis as a critical event in establishing the nerve dependence of limb regeneration. This downregulation after innervation was not observed in development of the limb in *Xenopus* tadpoles, and it may be a developmental modification that evolved in relation to limb regeneration [34]. Although development is frequently compared to regeneration, as mentioned above, there are presently no other examples of developmental novelties that are associated with the mechanism of adult regeneration. The results for the ANL raise the question of how the nerve irreversibly downregulates AG expression in the larval epidermis such that this state persists through adult life. If the limb is denervated, AG expression is not upregulated [34], whereas other effects of peripheral nerves on their targets, for example the downregulation of acetylcholine receptor synthesis in skeletal muscle fibres, are reversed after denervation [35]. This imprinting of the epidermis is critical for nerve dependence and it may be imposed on the stem cell compartment, because the epidermis is subject to continuous cellular turnover [34].

Nerves, mammalian regeneration, and mammalian niches

Regeneration of holes in the pinna of the ear is found in a few mammalian species such as rabbits, but there is great interest in this ability found in certain mouse strains such as Murphy Roths Large (MRL) (Figure 3a) [36]. Regeneration in MRL proceeds via WE formation with characteristic ingrowths into an underlying blastema-like structure (Figure 3a,ii), that regenerates cartilage and epithelial structures with no evidence of scarring. The control strain C57 BL/6 regenerates more slowly and does not exhibit the remarkable fusion of opposing epithelial margins required to close the wound as seen in MRL. Innervation of the pinna by the auricular nerve (a branch of the facial nerve) enters at the base of the ear, so that the proximal margin of the hole is further from the ear tip than the distal margin (Figure 3a,i) [37]. There is a difference in the rate of re-innervation after injury between MRL and C57 BL/6, such that the axon density in the early blastema is twofold higher at the proximal margin in the former strain [37]. The distal margin is less innervated in both strains. If the pinna is denervated prior to hole punch injury, regeneration at the proximal margin produces a re-epithelialised edge but there are no features of a blastema-like structure and no regeneration of hair follicles or sebaceous glands. The effect on the distal margin is even more marked in that there is a lack of wound healing and formation of a necrotic zone that extends to the ear tip (Figure 3a,ii) [38]. It is noteworthy that a delay occurs between healing of proximal and distal margins in the normally innervated case, possibly reflecting the initial differences in axonal density and suggestive of a gradient across the wound area [38]. The regenerating auricular nerve is thus essential for normal wound healing and for a regenerative outcome in this context.

There are now several examples in which nerves play a critical role in the niche of mammalian stem cells. It has been proposed that the adult haematopoetic stem cell

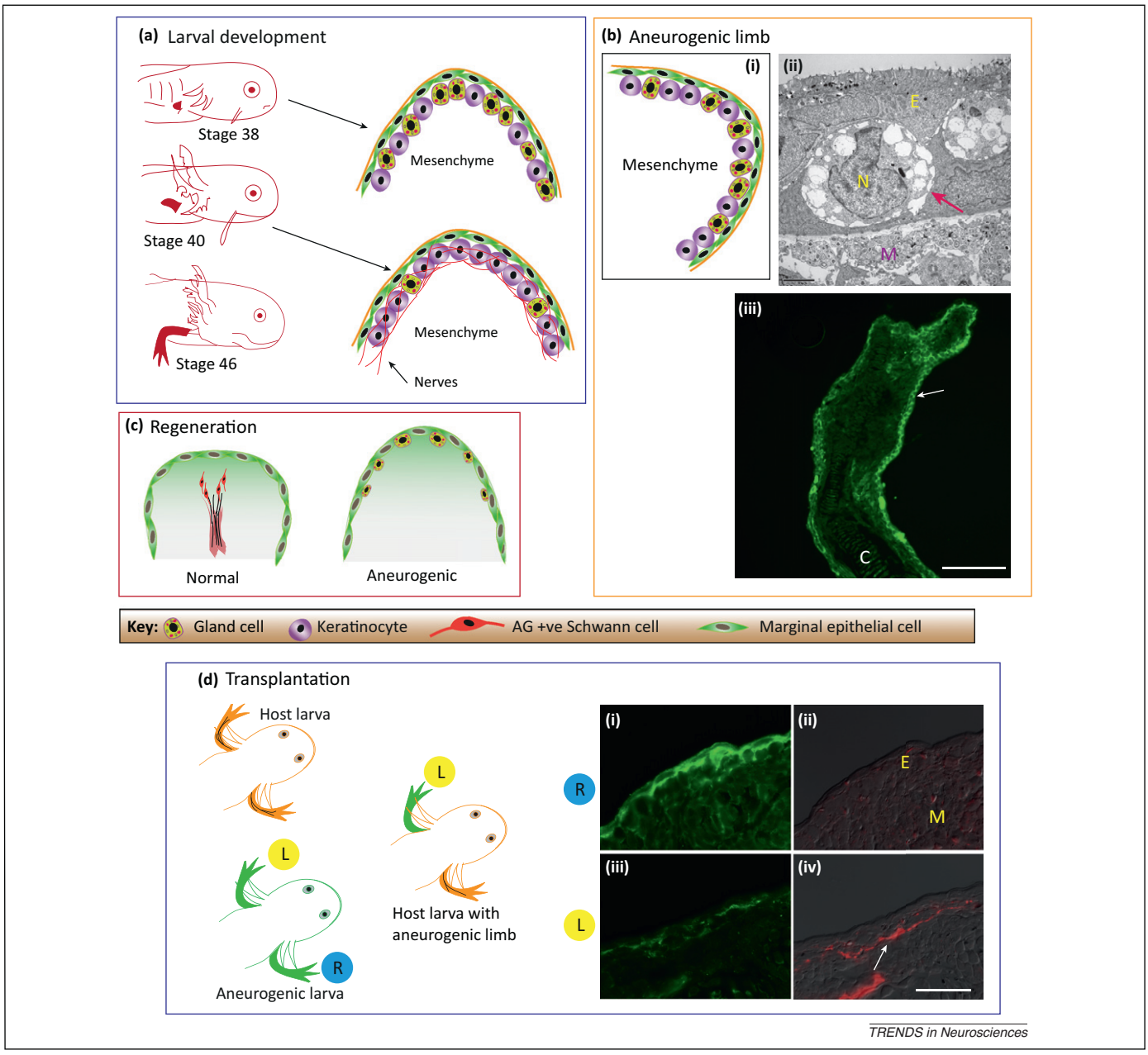

Figure 2. Limb development and regeneration in the presence and absence of nerves in the spotted salamander *Ambystoma maculatum*. **(a)** During normal development of the limb, nerves enter the limb bud after stage 38, and by stage 46 the number of unicellular glands in the epidermis that express anterior gradient (AG) has markedly decreased [34]. **(b)** During aneurogenic development, the number of glands (**ii,** red arrow) remains high, as determined by electron microscopy(**i, ii**), and overall AG expression in the epidermis is also high, as detected by staining with antibodies to AG (**iii,** green fluorescence, arrow) [34]. **(c)** The consequence of this difference for limb regeneration is that the normal blastema has an early wound epithelium (WE) that lacks AG expression whereas the aneurogenic WE is positive for gland cells. **(d)** After transplantation of a left aneurogenic limb in place of a normal limb, the control right limb from the donor aneurogenic larva maintains high AG expression in the epidermis (**i,** green fluorescence) in the absence of nerves, as detected by staining with antibodies to acetylated tubulin (**ii,** red fluorescence), whereas AG downregulation its observed in its transplanted counterpart (**iii**) after innervation (**iv**) [34]. Scale bars: bii, 5 μm; biii, 100 μm; di–iv, 50 μm. Abbreviations: C, cartilage; E, epidermis; M, mesenchyme; N, nucleus.

(HSC) niche in the bone marrow (BM) consists of distinct vascular and endosteal niches associated with activated and dormant HSCs, respectively [39,40]. The endosteal region is located in the immediate proximity of osteoblasts that line the bones (Figure 3b). A critical ligand in maintenance of the dormant phenotype is transforming growth factor (TGF)-β, which is generated by activation of a latent complex. The presence of activated TGF-β, along with integrin β8, which is an established co-regulator of activation, is detectable in Schwann cells associated with non-myelinated sympathetic axons (Figure 3b) [41]. Unilateral transection of postganglionic sympathetic lumbar nerves reduced the number of TGF-β-producing Schwann cells in the BM. It also reduced the number of HSCs in the BM to 20% of that on the contralateral side, whereas other BM niche cell types were unaffected. This decrease was reflected in standard HSC repopulation assays comparing denervated and control populations. Interestingly, HSCs from the denervated context showed higher S-phase labelling, a finding consistent with the interpretation that the Schwann cell component of the niche is responsible for maintenance of HSC dormancy, and this phenotype was reversed by infusion of TGF-β into the denervated mice [41]. It is not yet clear if the regulation of endosteal

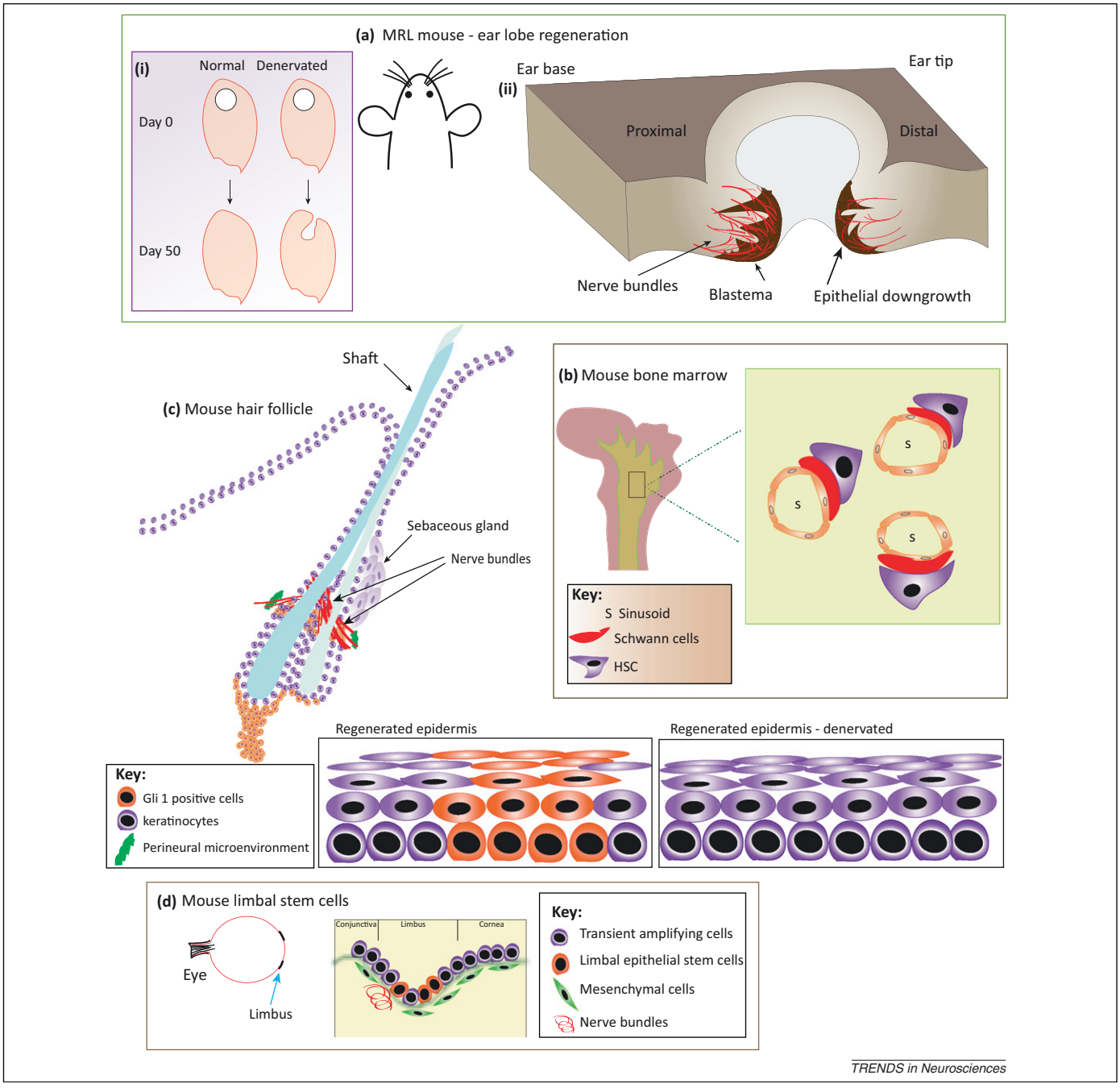

Figure 3. Innervation and mammalian regeneration. **(a)** Regeneration of the pinna in the Murphy Roths Large (MRL) mouse. **(i)** Injury induced by an ear punch is completely healed by day 50 in the presence of normal innervation [37]. Transection of the nerves to the ear lobe prior to ear punch results in abnormal wound healing and necrosis of the tissue [38]. **(ii)** Diagrammatic representation of a regenerating ear-punch hole. Quantitative image analysis has shown that the density of innervation is higher in the proximal ear base of the pinna compared to the distal tip [37]. Epithelial downgrowth into the regenerating tissue is an interesting feature of the blastema. **(b)** Association of haematopoetic stem cells (HSCs) and Schwann cells in bone marrow. The endothelial cells and non-myelinating Schwann cells lie in close proximity along the sinusoid. The majority of the HSCs are in contact with Schwann cells. Denervation of the postganglionic fibres results in a reduction in HSCs [41]. **(c)** Innervation of the telogen hair follicle. The upper bulge of the hair follicle is extensively innervated by cutaneous fibres. This domain of the perineural microenvironment is populated by *Gli1*-positive multipotent stem cells, which receive Shh from the nerves. During regeneration, these cells behave as epidermal stem cells. Denervation of the cutaneous nerves results in loss of *Gli1* epidermal stem cells in the regenerated epidermis because of a lack of nerve-induced signalling [42]. **(d)** The cornea is densely innervated and limbal corpuscular nerve endings are located immediately beneath the epithelium. Innervation is essential for maintenance of corneal limbal stem cells in mice [43].

Schwann cells by sympathetic axons is dependent on neural activity.

Regeneration of the mouse hair follicle depends on stem cells in the bulge region (Figure 3c). The transcription factor Gli1 is an established target of hedgehog (Hh) signalling, and *Gli1* reporter mice were used to identify expression in two distinct domains in the upper and lower regions of the bulge [42]. *Gli1* expression in the upper but not the lower domain depends on sensory nerves that wrap around this region (Figure 3c). Sonic Hh expression can be identified in the relevant population of dorsal root ganglion neurones projecting to hair follicles. If this input is removed by surgical denervation, then *Gli1* expression is selectively lost from the upper bulge cells, which persist

after denervation. These cells can contribute both to hair follicle regeneration and to wound healing and epidermal regeneration. The effect of denervation on the latter property is to allow unaltered migration of cells into wounds but to inhibit, by approximately fivefold, the persistence of cells by 2 months after wounding (Figure 3c). Thus, innervation of the follicle is a critical aspect of the perineural niche, and nerves signal to maintain *Gli1* expression in their local target stem cells [42]. It is possible that there are other nerve-derived signals in addition to Hh that are necessary to maintain cell function.

The role of nerves in a niche of considerable clinical importance has been investigated in relation to corneolimbal epithelial stem cells (Figure 3d) [43]. The effect of sensory denervation was investigated using lesions of the ophthalmic branch of the trigeminal nerve, which led to a loss of stem cell markers and a two- to fourfold loss of stem cells [43].

Nerve dependence in invertebrates

The invertebrate phyla provide examples that echo the different themes described in vertebrates. For example, in echinoderms the regeneration of a starfish arm proceeds through stages that are absolutely dependent on the presence of a regenerating radial nerve at the amputation plane (Figure 1d). Transection of either the radial nerve or the more central nerve ring has effects that are comparable to those considered for the salamander limb [44]. The theme of nerve-dependent niches is illustrated by a study on blood cell homing and survival in the *Drosophila* larva [45]. The niches are located at anatomically secluded epidermal muscular pockets of the larval body wall, and provide a site for proliferation of differentiated larval haemocytes. These patches coincide with defined clusters of peripheral neurons, and depletion of neurons, either by genetic ablation or in atonal mutants, leads to a decrease in the number of haemocytes. It is possible to induce supernumerary peripheral neurons and to demonstrate attraction of haemocytes to ectopic neurons in an area normally devoid of both cell types [45]. It is unclear at present if this involves glia or other closely associated cells. The parallels between blood cell niches in *Drosophila* and mammals are notable [46].

Planaria are an important system for the study of regeneration. This follows the establishment of genomic and gene knockdown approaches in a context with an extensive background of classical investigation [47]. The extent to which planarian regeneration is under the influence of the nervous system is still unclear. Several studies have attempted to correlate regeneration of the central nervous system (CNS) with the morphogenesis of the regenerated tissue. In one case, knockdown of the axon guidance receptor *robo* led to failure of normal reconnections between the cephalic ganglia and ventral nerve cords. This induced differentiation of ectopic pharynges and dorsal outgrowths [48,49] reminiscent of the induction of ectopic limbs in the salamander (Box 1). A related study has reported on the effects of surgical disruption of the ventral nerve cord in relation to the altered fate and axial polarity of regenerated tissue [50]. In another study, establishment of posterior specification in transversely amputated planaria was dependent on *Patched*-mediated Hh signalling. The authors suggested that the source of Hh was the ventral nerve cord, where it is expressed by neural cells along the anteroposterior axis [51]. Planarians might be an example in which nerves are instructive for morphogenesis during regeneration, an interpretation that is somewhat counter to evidence of their permissive role in the vertebrate examples. These issues should be clarified by further work in both contexts.

In the cnidarian *Hydra*, nerves are generated from interstitial stem cells and this lineage can be eliminated by colchicine treatment or by other methods [52,53]. The resulting nerve-free 'epithelial' polyps are unable to capture or ingest food but can be maintained by feeding. They can regenerate after transection, although this generally takes longer than in the normal case. There is some evidence that the epithelial cells might be reprogrammed in the absence of nerve cells [54,55], and parallels with the ANL leave open the possibility that regeneration may normally be nerve-dependent. Decapitation of *Hydra* induces a rapid increase in nerve cell generation [56] and a similar, somewhat delayed, increase occurs after mid-gastric transection [57]. In a study of head regeneration, the gene *cnox2* was downregulated by RNAi. This is a *hox* gene expressed in neuronal progenitor cells, and its downregulation disrupted neurogenesis and led to a marked delay in head regeneration. It appears that in wild-type *Hydra*, cnox-2 function is necessary for regeneration and a slower program is activated when it is missing [57]. From an evolutionary perspective, *Hydra* remains a critical system for understanding the role of nerves in regeneration.

Regeneration of the primary body axis in invertebrates is closely related to the various modes of agametic reproduction and in particular to fission, in which an individual physically divides its body to reproduce. Fission in annelids is always found within regenerating clades and never within non-regenerating clades, leading to the view that it is derived from regeneration [58]. The first systematic comparison of the two processes was reported for the annelid *Pristina leidyi*, and several similarities were identified (Figure 1e). One in particular was not shared with embryonic development: numerous peripheral nerves grow over the regenerating blastema, and this is also observed during fission [58]. There is considerable evidence of nerve dependence of annelid regeneration [59] and this may be shared with fission, although direct evidence of this point is not yet available.

Concluding remarks

It seems likely from this survey that nerve dependence reflects a variety of mechanisms. It may have evolved in different contexts to ensure that regeneration of a structure always proceeds with concomitant regeneration of the nerve supply, and therefore the regenerate becomes functionally innervated. The interactions described above between developmental innervation and the epithelium of the larval limb bud in the salamander are consistent with such a view, in that nerve dependence is imposed on an alternative mechanism seen in the ANL. This might suggest that it is the signalling properties underlying

functional innervation that are central to understanding the selective aspects of nerve dependence and its origin in phylogeny. An alternative possibility is that the neuron evolved first to fulfil a growth regulatory role in relation to regeneration and asexual reproduction. Its post-mitotic status, and hence the fixed number of nerve cells in an adult organism, may have been critical to define the limits of growth in some species, whereas its ability to extend and regenerate axons allowed it to control growth at a distance. This growth-regulating aspect of neurons may deserve at least some consideration in discussions of the emergence of this cell type [60].

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