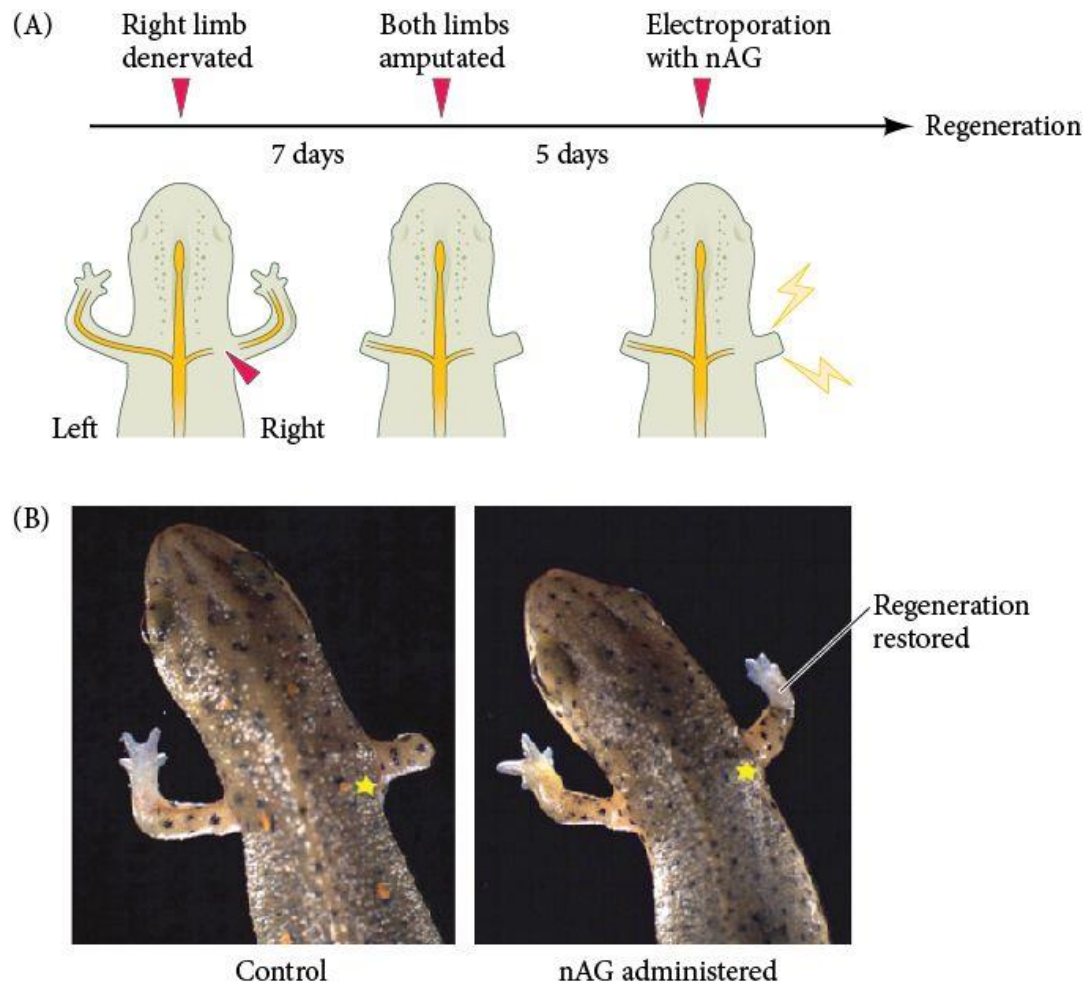


Newt Anterior Gradient Protein

As we discussed in Chapter 15, neural activity with target cells is required for synapse maturation and neuronal survival. Thus, it was initially postulated that neural activity might be the necessary stimulus for limb regeneration. However, experiments have shown that neural conductance (action potentials and release of acetylcholine) is not required to promote salamander limb regeneration (Sidman and Singer 1951; Drachman and Singer 1971; Stocum 2011). If neural activity is not required, then what are the nerve axons providing to the limb blastema? These neurons are believed to release factors necessary for the proliferation of the blastema cells (Singer and Caston 1972; Mescher and Tassava 1975). There have been many candidates for such a nerve-derived blastema mitogen, but probably the best candidate is newt anterior gradient protein (nAG). This protein can cause blastema cells to proliferate in culture, and it permits normal regeneration in salamander limbs that have been denervated (Figure 1; Kumar et al. 2007a). If activated nAG genes are electroporated into the dedifferentiating tissues of amputated limbs that have been denervated, the limbs are able to regenerate. If nAG is not administered, the limbs remain stumps. Moreover, nAG is only minimally expressed in normal limbs, but it is induced in the Schwann cells that surround the regenerating axons within 5 days of amputation.

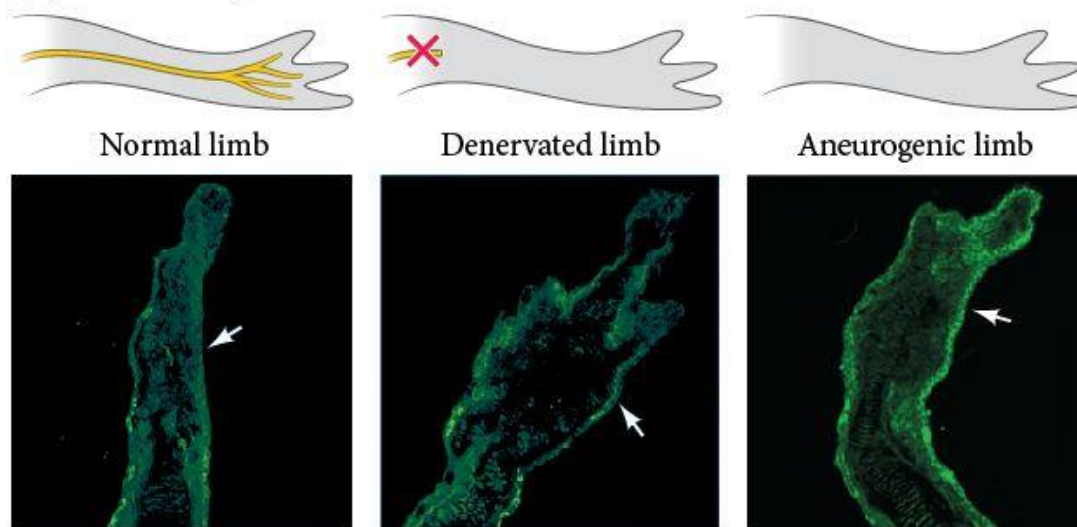


After A. Kumar et al. 2007. *Science* 318: 772-777, courtesy of K. Poss.

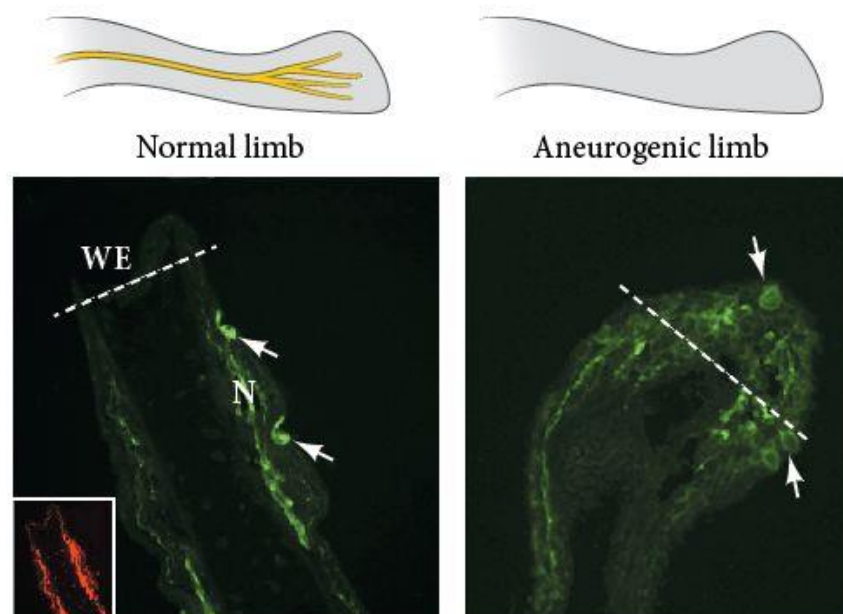
Figure 1 Regeneration of newt limbs depends on nAG (normally supplied by the limb nerves). (A) Schematic of the procedure. The limb is denervated and 7 days later is amputated. After another 5 days, nAG is electroporated into the limb blastema. (B) Results show that in the denervated control (not given nAG), the amputated limb (yellow star) remains a stump. The limb that is given nAG regenerates tissues with appropriate proximal-distal polarity.

Further support for the stimulatory role of nAG comes from the study of aneurogenic limbs. In this experiment, two embryonic salamanders were joined together by parabiosis, ensuring similar conditions and survival of both. In one of the salamanders, the neural tube was removed. Both salamanders survived this procedure, and all limbs grew successfully, but the limbs of the salamander lacking a neural tube were aneurogenic, completely devoid of any neural innervation. Based on the previous findings that denervated limbs are unable to regenerate, one would hypothesize that these aneurogenic limbs would likewise be unable to regenerate following amputation. Strikingly, these aneurogenic limbs *did* regenerate. When researchers compared the expression of nAG of normal limbs with that of both denervated limbs and these aneurogenic limbs, they discovered that the aneurogenic limbs had a uniquely high level of nAG expression in the epidermis (Figure 2A; Kumar et al. 2011). Moreover, upon limb amputation, nAG increased first in the nerve sheath of normal limbs, but was present throughout the blastema in the aneurogenic limbs (Figure 2B). These results suggest that nAG alone is the primary mitogen responsible for nerve-dependent regeneration. The receptor for nAG, Prod1, has since been discovered and found to be expressed in a proximal-to-distal gradient in the salamander limb (Morais et al. 2002; Kumar et al. 2007a and b). This ligand-receptor relationship seems to be conserved across regeneration-competent salamander species (Geng et al. 2015).

(A) Limb development



(B) Limb regeneration



From A. Kumar et al. 2011. *Proc Natl Acad Sci USA* 108: 13588-13593.

Figure 2 Differential expression of nAG in the aneurogenic salamander limb. (A) Expression of nAG protein (green) is localized to a small subset of epidermal cells in the normal and denervated limbs during development but is highly upregulated throughout the epidermis of aneurogenic limbs. Arrows indicate the epidermis. (B) Schematic representation of limbs prior to amputation. Lower images show the regenerated limbs, with a white dotted line indicating the plane of amputation; the wound epidermis (WE) is positioned distal to this line. In normal limb regeneration, nAG is upregulated only in the sheath cells of regenerating nerves (N); the inset is a low-magnification view of neurofilaments (red) in the same image. In the regenerating aneurogenic limb, nAG is upregulated throughout the limb blastema.

Literature Cited

Drachman, D. B. and Singer, M. 1971. Regeneration in botulinum-poisoned forelimbs of the newt, *Triturus*. *Exp. Neurol.* 32: 1–11.

[PubMed Link](#)

Geng, J. and 8 others. 2015. Identification of the orphan gene *Prod1* in basal and other salamander families. *Evodevo* 6: 9.

[PubMed Link](#)

Kumar, A., J. P. Delgado, P. B. Gates, G. Neville, A. Forge and J. P. Brockes. 2011. The aneurogenic limb identifies developmental cell interactions underlying vertebrate limb regeneration. *Proc. Natl. Acad. Sci. USA* 108: 13588–13593.

[PubMed Link](#)

Kumar, A., J. W. Godwin, P. B. Gates, A. A. Garza-Garcia, J. P. Brockes. 2007a. Molecular basis for the never dependence of limb regeneration in an adult vertebrate. *Science* 318(5851: 772-777.

[PubMed Link](#)

Kumar, A., P. B. Gates and J. P. Brockes. 2007b. Positional identity of adult stem cells in salamander limb regeneration. *C R Biol* 330(6-7): 485-90

[PubMed Link](#)

Mescher, A. L. and R. A. Tassava. 1975. Denervation effects on DNA replication and mitosis during the initiation of limb regeneration in adult newts. *Dev. Biol.* 44: 187–197.

[PubMed Link](#)

Morais Da Silva, S., P. B. Gates and J. P. Brockes. 2002. The newt ortholog of CD59 is implicated in proximodistal identity during amphibian limb regeneration. *Dev. Cell* 3: 547–555.

[PubMed Link](#)

Sidman, R. L. and M. Singer. 1951. Stimulation of forelimb regeneration in the newt, *Triturus viridescens*, by a sensory nerve supply isolated from the central nervous system. *Am. J. Physiol.* 165: 257–260.

[PubMed Link](#)

Singer, M. and J. D. Caston. 1972. Neurotrophic dependence of macromolecular synthesis in the early limb regenerate of the newt, *Triturus*. *J. Embryol. Exp. Morphol.* 28: 1–11.

[PubMed Link](#)

Stocum, D. L. 2011. The role of peripheral nerves in urodele limb regeneration. *Eur. J. Neurosci.* 34: 908–916.

[PubMed Link](#)

All the material on this website is protected by copyright. It may not be reproduced in any form without permission from the copyright holder.

© 2023 Oxford University Press |