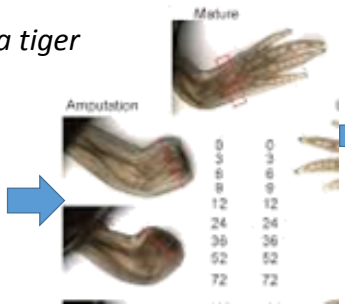
Interaksi jaringan pada proses regenerasi

- Regenerasi epimorfik
- Regenerasi jaringan

Perlu ada interaksi jaringan untuk terjadi regenerasi

Regenerasi anggota tubuh

- Interaksi jaringan diperlukan untuk:
  - Memulai epitelialisasi pada daerah yang diamputasi → tanpa epitelialisi → regenerasi tidak terjadi
- Thornton (1957) – *Ambystoma tiger*



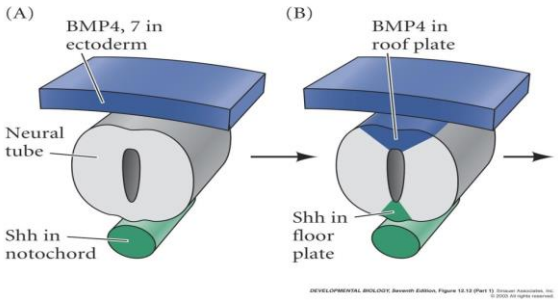
Epitel yang menutupi daerah luka dihilangkan dalam waktu 24 jam

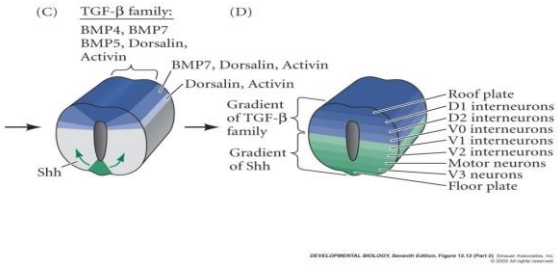
Tidak terjadi regenerasi

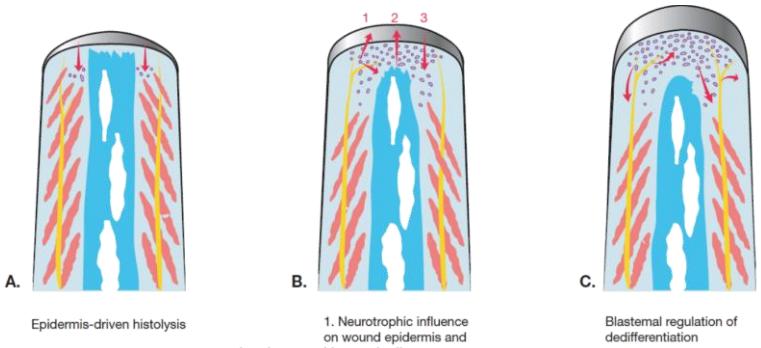
- Goss (1956) and Polležajew and Faworina (1935)
  - → mencegah epitelialisasi → insert lengan yang diamputasi ke dalam rongga tubuh atau daerah otot terdekat → tidak terjadi regenerasi
  - Jika lengan teramputasi dimasukan sesudah terjadi epitelialisasi → regenerasi terjadi
- Daerah teramputasi ditutup dengan kulit yang tebal (dermis dan epidermis) → tidak terjadi regenerasi (Godlewski, 1928; Tornier, 1906)

Tissue Differentiation

- What appears to be responsible for dorsal patterning of neural tube?

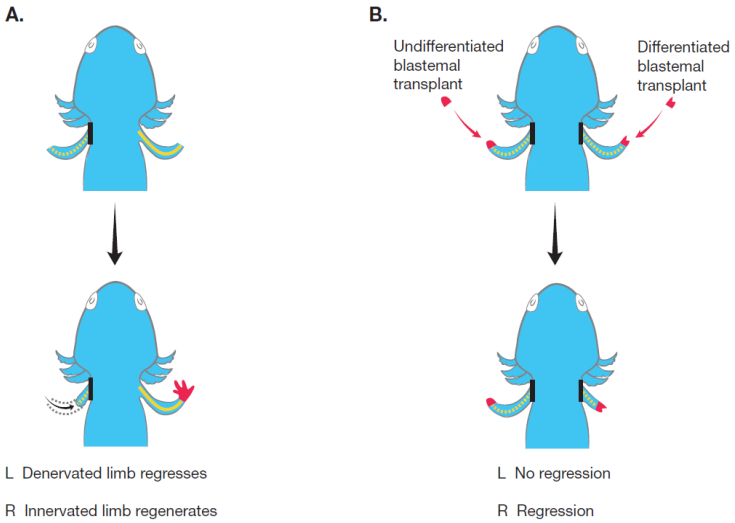


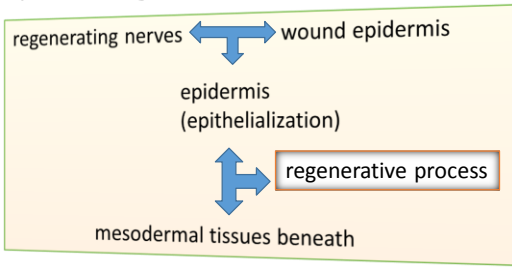




- Interaksi jaringan dalam regenerasi amphibi:
  - histolysis →
    - diinduksi oleh epidermis
    - Enzim yang dihasilkan oleh epidermis yang menstimulasi histolysis jaringan mesoderm di daerah yang dilukasi
  - Pembentukan blastema awal → dipengaruhi oleh saraf:
    - Memelihara epidermis luka dan blastema
    - Mempertahankan epidermis luka yang mempengaruhi apical epidermal cap
    - Memelihara blastema
  - Keberadaan blastema mengatur dediferensiasi dalam daerah yang dilukai

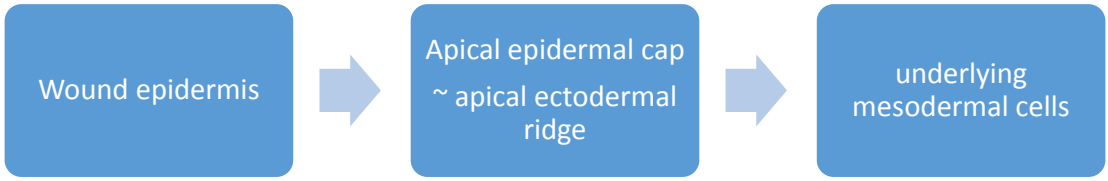
Saraf diperlukan untuk terjadinya regenerasi



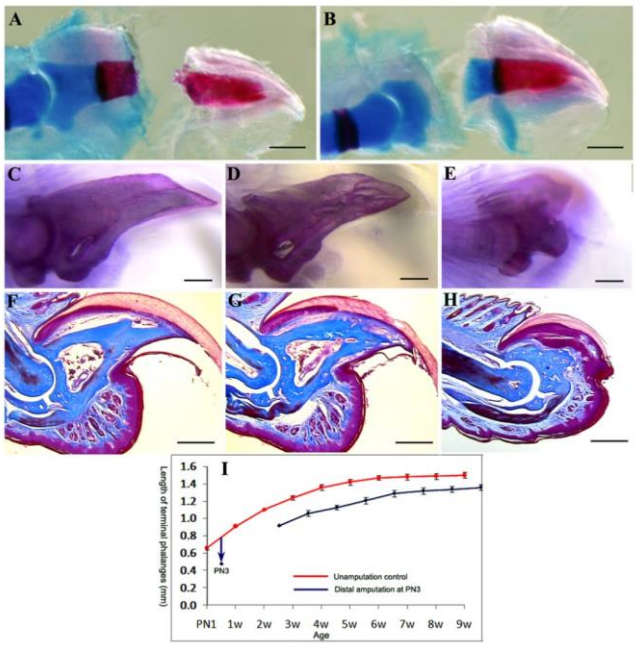


- *(A)* After denervation, an amputated limb regresses. *(B)* If an undifferentiated regeneration blastema is transplanted to the end of an amputated, denervated limb, no regression occurs. In contrast, regression occurs if the blastemal graft is differentiated. (Based on fi ndings by Schotté et al. [1941].)

FGF8



Human fingertips and mammalian limb



Regeneration of the terminal phalanx of digit tips in neonatal mice. Amputations were carried out at a distal level through bone, (**A**) and a proximal level through cartilage (**B**) at postnatal day 3 (PN3). Control unamputated digits were used for comparison (**C, F**). After 6 weeks, digits were analyzed using whole mount bone stain with Alizarin Red S (**C–E**) and histological analysis with Mallory's triple stain (**F–H**). Proximal amputations show no signs of regeneration (**E, H**). Distal amputations regenerate anatomically normal digit tips (**D, G**), however the length of the terminal phalanx of these digit tips never reach that of unamputated control digits (**I**). Scale bars: A,B – 200μm; C–E – 300μm; F–H – 400μm.

Han etal., 2008, Dev Biol. 315(1): 125–135

- Fingertip regeneration → correlated with msx-1

Bmp-4

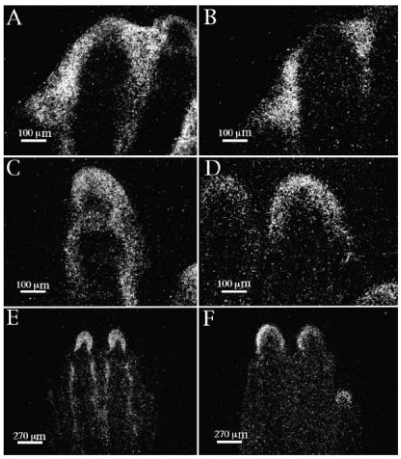


Fig. 2. Dark-field illumination of sections of developing digits hybridized with probes specific for *Msx1* (A,C,E) and *Msx2* (B,D,F) transcripts. (A) Stage 9 limb bud showing *Msx1* transcripts at the digit tip and in the interdigital regions. (C) Stage 10/11 limb showing the presence of *Msx1* transcripts associated with the digit tip, and in tissues surrounding the distal phalangeal elements, including the distalmost phalangeal articulation. (E) Stage 12 mouse limb showing *Msx1* transcripts localized distally in the forming nail bed, and laterally in the perichondrial region of phalangeal elements. (B) Stage 9 limb showing *Msx2* transcripts in the interdigital region, and associated with a thin band of cells at the distal tip of the digit. (D) Stage 10/11 limb showing *Msx2* transcripts at the distal digit tip. (F) Stage 12 limb showing *Msx2* transcripts in the forming nail bed.

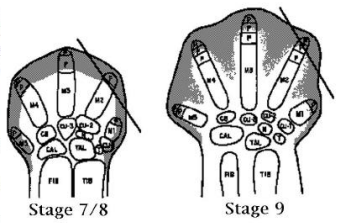


Fig. 1. Fate maps and cartilage patterns of developing mouse limbs. Stage 7/8 and 9 limbs are depicted in fate maps redrawn from Muneoka et al. (1989). The fate maps are overlaid with the approximate expression domains of *Msx1* at these stages to give a clearer image of the correlation between expression boundaries of *Msx1* and amputation levels used in the present study. The lines over digits indicate the proximal extent of *Msx1* expression in digit 2 for each stage shown. Thus, distal level amputations were performed at levels slightly closer to the digit tip and proximal level amputations were performed at more proximal levels where the majority of the presumptive digit was removed (see Materials and Methods). The stage 11 limb shows the cartilage pattern following whole mount Victoria blue staining. This limb shows the presence of cartilage condensations of the presumptive digit pattern.

Reginelli, et al., 1995

Human fingertips and mammalian limb

- Human – young children :
  - Amputation – healing:
    - Treating with a skin flap ➔ inhibit limb regeneration ~ in amphibians
    - Allow a wound epithelium to grow → finger tip regeneration

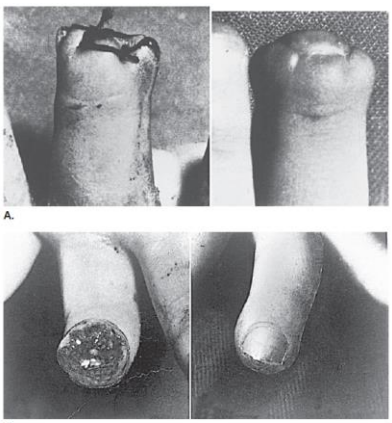
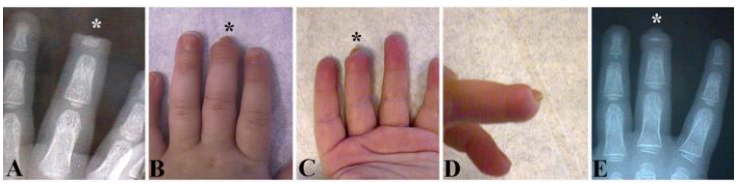


FIGURE 5-3 Human fingertip regeneration. *(A)* Treatment of amputated fingertip in 3-year-old girl by classic skin flap method (left). Four months after the operation (right), the finger has healed with a blunt stump. *(B)* Conservative treatment of an amputated fingertip in a 22-month-old boy. Good regeneration has occurred by 11 weeks after amputation. (Reprinted from Illingworth CM: (1974) Trapped fingers and amputated finger tips in children. *J Pediatr Surg* 9:853–858, by permission.)



A case of regenerative failure after proximal amputation injury of the fingertip (*) of a 2-years old child that was conservatively treated.**A**: Radiograph at the time of injury indicated about 70% of the terminal phalangeal bone was lost. **B–E**. Fingertip 10 months after injury. **B**: Dorsal view, **C**: Ventral view and **D**: Lateral view. The wound healed to form a small bump with normal contour and sensibility (**B–D**), but there was no elongation of the terminal phalangeal bone (**E**).

Antler regeneration

- Early antler regeneration →
  - without blastema regeneration
  - Formation of anterior and posterior growth centers stemming from periosteum of the pedicle
- Growing antler:
  - Richly innervated with sensory nerve fibers
  - Does not occur after denervation

Annual growth of antlers of a sika deer: *(A)* May 7; *(B)* May 13; *(C)* June 4; *(D)* June 15; *(E)* August 23; and *(F)* January 6.

Antler regeneration

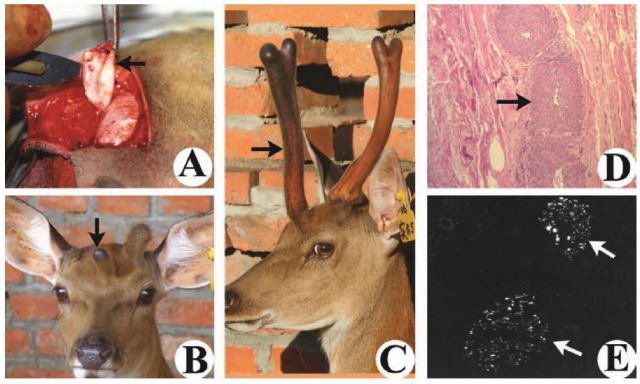


Figure 13. Origin of the external antler components. (**A**) Antlerogenic periosteum (arrow). (**B**) First year ectopic spike antler (arrow) initiated from the transplanted periosteum on the forehead region. (**C**) Regenerated ectopic antler (arrow) in the second year. (**D**) Blood vessels (arrow) in the vascular layer of the regenerated ectopic antler. Note these blood vessels have all the features of antler blood vessels. (**E**) Nerve fibers (arrows) in the vascular layer of the regenerated ectopic antler.

- growing antlers and pedicles consist of:
  - Internal (cartilage and bone) and
  - external components (skin, blood vessels, and nerves).
- the regeneration of both internal and external components relies on the presence of pedicle periosteum (PP).
- PP cells
  - express key embryonic stem cell markers (Oct4, Nanog, and SOX2)
  - multipotent, ➔ antler stem cells.

Li, 2012. Birth Defects Research (Part C) 96:51–62

MAMMALIAN EAR HOLE REGENERATION

- ear hole regeneration has been found in only a few mammalian species, most notably rabbits, cats, and a certain variant of mice (MRL mice).
- mammals that do not regenerate ear holes include normal mice, rats, guinea pigs, chinchillas, hamsters, gerbils, opossums, armadillos, Patagonian cavies, dogs, sheep and deer. (Goss (1983),

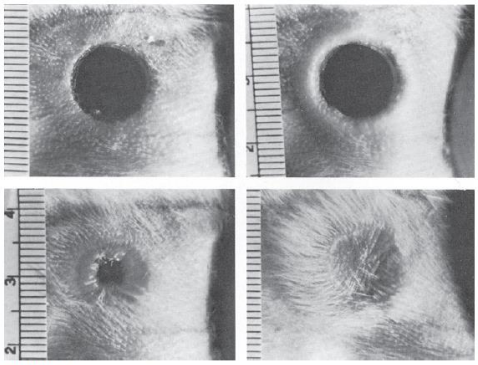
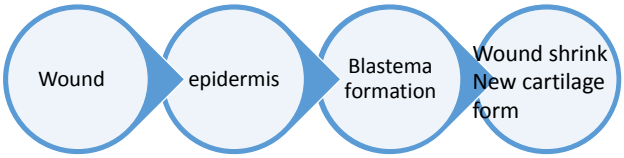
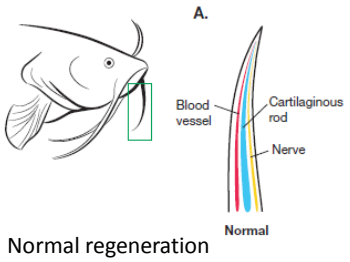


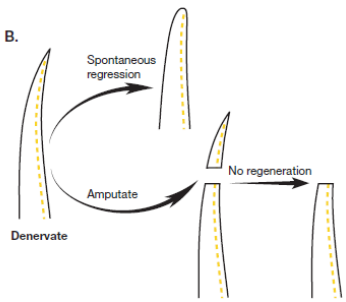
FIGURE 5-5 Regeneration from the margins of a 1-cm² hole cut through the full thickness of a rabbit ear. (top left, reading like a book) One day, 1 week, 4 weeks, and 8 weeks after surgery. (Reprinted from Goss, R.J. 1983. *Deer Antlers: Regeneration, Function, Evolution.* New York: Academic Press, by permission.)



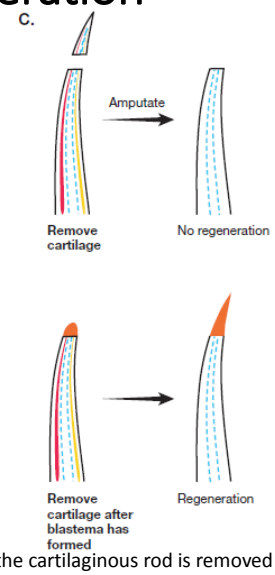
Catfish barbel regeneration



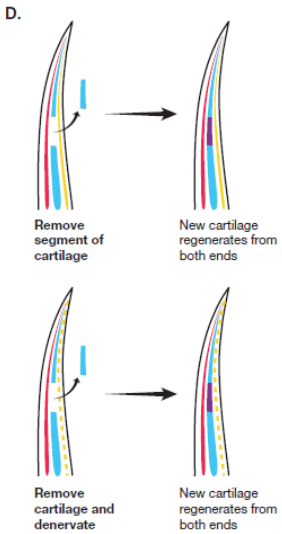
Normal regeneration



After denervation, the barbel regresses; after amputation, no regeneration occurs

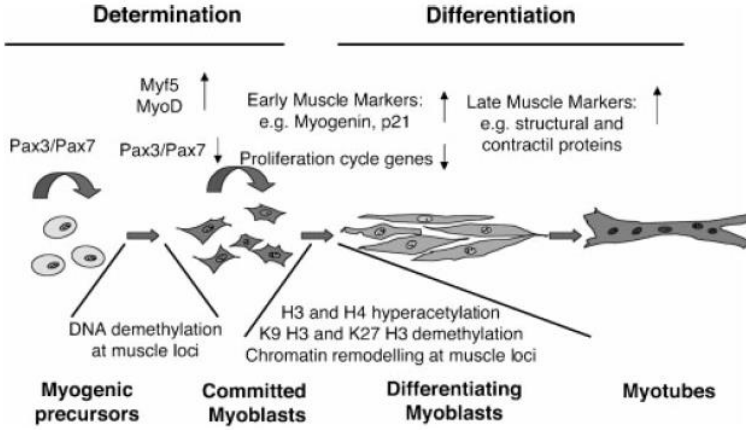


If the cartilaginous rod is removed at the time of amputation, regeneration fails to occur; if the cartilage is removed after a blastema has formed, regeneration takes place



In both normal and denervated barbels, cartilage regenerates in the gap after removal of a segment of the cartilaginous rod.

Mammalian muscle regeneration



A schematic representation of the sequential stages that underlie skeletal myogenesis. Determination of the myogenic lineage is conferred by the expression of the early myogenic bHLH proteins, MyoD and Myf5, likely due to DNA de-methylation at their regulatory regions. These muscle regulatory factors (MRFs) initiate myogenesis by activating transcription of muscle genes at previously silent loci, via the combined histone acetylation and demethylation of particular lysines, and the local chromatin remodeling. These events are promoted by the recruitment of chromatin-modifying enzymatic complexes by sequence-specific myogenic activators (e.g. myogenic bHLH and MEF2 proteins.)

Mammalian muscle regeneration

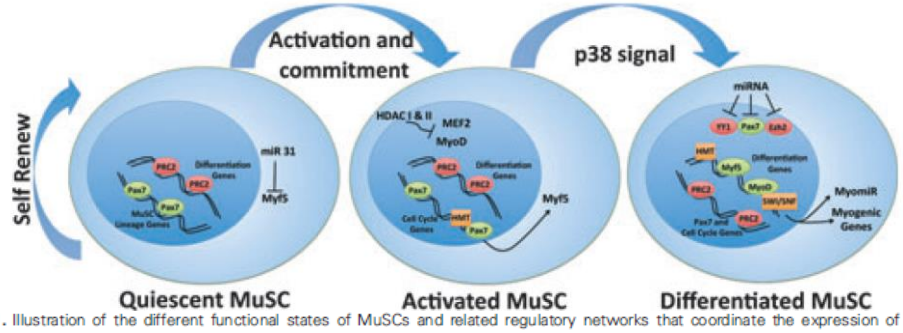


Fig. 1. Illustration of the different functional states of MuSCs and related regulatory networks that coordinate the expression of different subsets of genes at each stage. In quiescent cells, the muscle stem cell lineage is maintained through the cooperation of Pax7 and PRC2 that promote the expression of MuSC lineage genes and repressed muscle differentiation genes, respectively, and through miR31 that blocks Myf5 translation. Upon activation on muscle injury, exposure to regeneration cues promotes asymmetric division, giving rise to differentiation-committed MuSCs in which Pax7 activates the expression of cell-cycle genes and, with the collaboration of the Wdr5–Ash2L–MLL2 histone methyltransferase complex (HMT) promotes Myf5 transcription. At the same time, HDAC class I and II contribute to repress the activity of MEF2 and MyoD and hold the cells at the stage of proliferating myoblasts. Subsequent exposure to differentiation signals triggers pro-myogenic cascades (e.g. p38) and causes the recruitment of SWI/SNF complex on muscle loci, the deposition of H3K4 marks on Myog and CKM by the HMT complex and the downregulation of the PRC2 enzymatic subunit EzH2. MyoD and SWI/SNF complex also promote the expression of Myomirs. In addition, different miR contribute to the repression of Pax7 YY1 and Ezh2. The overall decrease in Ezh2 levels lead to derepression of muscle genes and formation of limiting amounts of EzH2-based PRC2 complexes that extinguish the expression of Pax7 and cell-cycle genes.

FEBS Journal 280 (2013) 4014–4025

Mammalian muscle regeneration

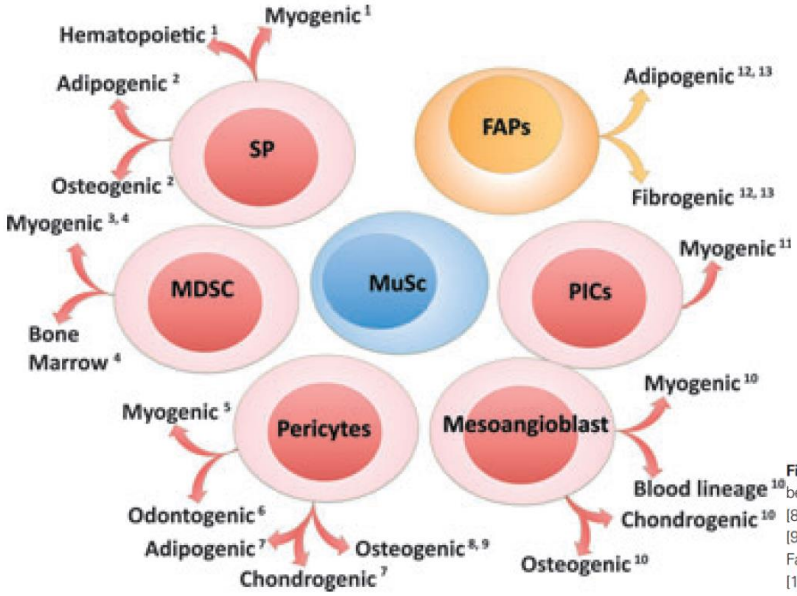


Fig. 2. Schematic representation of the possible lineages that can be adopted by the different muscle-resident cells. 1, Gussoni *et al.* [8]; 2, Uezumi *et al.* [7]; 3, Qu-Petersen *et al.* [96]; 4, Cao *et al.* [97]; 5, Dellavalle *et al.* [90]; 6, Alliot-Licht *et al.* [103]; 7, Farrington-Rock *et al.* [102]; 8, Schor *et al.* [100]; 9, Doherty *et al.* [101]; 10, Minasi *et al.* 2002 [104]; 11, Mitchell *et al.* [12]; 12, Joe *et al.* [6]; 13, Uezumi *et al.* [7].

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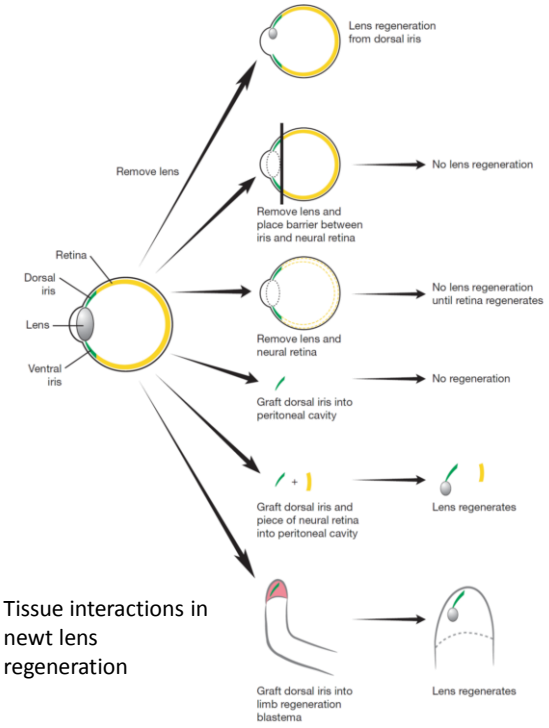
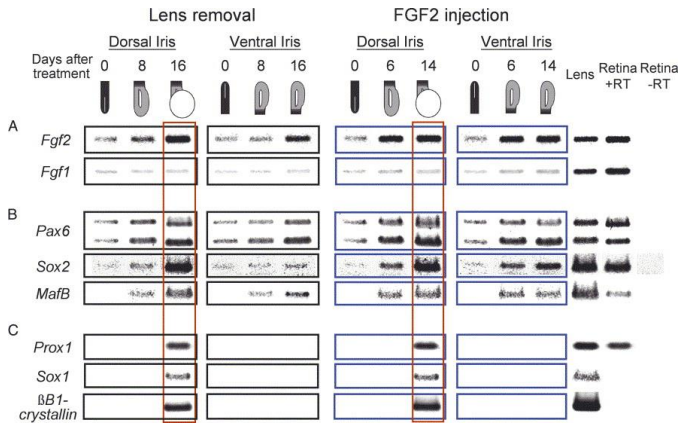
- At the cellular level
  - interaction is between myogenic cells and the basal lamina that surrounds them.
  - Satellite cells → produce growth factors for their proliferation and their ultimate fusion into myotubes ➔ GF bounds to the basal lamina and the endomysial connective tissue until their release during the regenerative process.
- At the tissue level
  - muscle requires a nearby vascular supply for regeneration
  - The angiogenic response to muscle damage supplies the damaged muscle fibers with both the macrophages necessary for removal of the necrotic debris and the oxygen and nutrients needed for survival.
  - Damaged muscle possesses angiogenic properties that stimulate vascular ingrowth into ischemic areas (Phillips et al., 1991).
- Innervation:
  - Early muscle regeneration →absence of innervation
  - Full differentiation of regenerating muscle fi bers requires the presence of motor innervation



Tissue interactions in newt lens regeneration

Lens regeneration in newts

- Stimulating factor→ FGF
- Inhibitory factor



Hayashi, et al. 2004