Chapter 4
Conclusion and Perspectives: Implications for Human Regeneration

The introductory discussion on the biological conditions that might have allowed the evolution of tail regeneration in lizards (Sect. 1.2) indicated that the mechanisms for organ regeneration may be induced by medical treatments that utilize knowledge of the biological process responsible for organ regeneration in amniotes. These medical interventions should aim to limit inflammation, to increase the number of stem cells, and to stimulate dermal–epidermal interactions by removing the early formation of the barrier represented by a stable basement membrane between epidermis and dermis. Lizards represent a good model to study all these conditions, especially when comparing inflammation and scarring in the limb (or in the cauterized tail) with healing in the normal tail. Therefore, the lizard model is useful for studies that aim to detect the factors involved in the delicate equilibrium between regeneration (the environment present in the normal tail stump) and scarring (the environment present in the limb stump or in the manipulated tail stump).

Although detailed cytological information on the process of tail and limb regeneration in lizards is now available, there is little molecular information on specific genes and proteins activated during regeneration. Furthermore, the role of these genes in the process of regeneration in lizards remains completely unknown (Liu et al. 2006; Jiang et al. 2007).

In conclusion, the use of the lizard model in research on the mechanisms of tissue and organ regeneration is particularly interesting. Among others, three main topics are presently under analysis: (1) the presence of potent antimicrobial molecules produced after wounding; (2) the mechanisms limiting limb and digit regeneration; (3) the mechanisms implicated in the limited regeneration of the lumbar and thoracic spinal cord.

1. The innate immunity of wounded tissues of lizards to avoid infection and then septicemia may lead to the discovery of potent antimicrobial molecules like the defensins or cathelicidins, effective antimicrobial peptides present in most vertebrates (Zasloff 2002). Studies in this direction are presently under way to characterize possible antimicrobial molecules present in azurophil granules of granulocytes and dense granules or the cytoplasm of wound keratinocytes (Fig. 2.1b, 2.14b, c).

2. Future studies on lizard tissue regeneration should focus on the molecules exchanged between the apical cup or the wound epidermis and the underlying mesenchyme, an interaction that maintains the apical center for the growth of the tail. One of these molecules is fibroblast growth factor, but other growth factors (transforming growth factor, epidermal growth factor, etc.) may also be involved. The localization and expression of growth factors and of other signaling molecules (Sonic hedgehog, Gremlin, bone morphogenetic protein, MSx, etc.; see Sanz-Ezquerro and Tockle 2003) in the regenerating blastema of the tail compared with that of the limb should be determined. These growth factors can be administered or placed in specific areas of the amputated limbs to see whether they can stimulate regeneration, in particular whether they can induce the formation of the autopodium and digits. This can be done by the application of microbeads releasing the molecule being tested in the limited cases of limb outgrowths (Fig. 1.2).
3. The recovery from paraplegia observed in numerous cases of spinal cord injury in lizards (Raffaelli and Palladini 1969; Alibardi, unpublished observations) should also be analyzed for a possible medical follow-up. It is known that the lumbar or thoracic spinal cord allows the regeneration of axons when it is autotransplanted into the tail, in relation to the presence of a regenerative tail blastema (Simpson and Pollack 1985). The complete resection of the spinal cord at lumbar and thoracic levels in the lizard *Anolis carolinensis* produces a permanent paralysis (Simpson 1961, 1970, 1983; Simpson and Duffy 1994). However, other studies on the wall lizards *Podarcis muralis* and *Podarcis sicula*, species that regenerate a larger tail than *A. carolinensis*, have indicated that after the resection of the lumbar spinal cord, the initial paraplegia can recover in 25–45 days after the operation (Raffaelli and Palladini 1969; Alibardi, unpublished observations). In these cases, the microscopic analysis of the lesioned spinal cord has shown that some regenerating nerves can bypass the gap of the sectioned spinal cord. However, the careful histological control of the lizards operated on has also shown that in animals that recovered limb motility the spinal cord was not completely transected (Furieri 1957). Therefore, in these cases, it is likely that some of the bridge nerves have rebuilt the intrinsic local spinal locomotor circuits present in the spinal cord (Bernstein 1983; Schwab and Bartholdi 1996; Sharma and Peng 2001; Borgens 2003). The latter possibility, however, does not diminish the value of the lizard model, since most spinal cord injuries in humans do not completely transect the spinal cord, and therefore the lizard model can be of interest in the study of the reestablishment of local spinal locomotor circuits. This is interesting considering that after lumbar lesion of the spinal cord the tail was also amputated and it regenerates normally (Alibardi, unpublished observations). Present analysis is trying to evaluate whether the presence of regenerating tissues at 1–2 cm from the lumbar injury stimulates the regeneration of long spinal cord nerves to cross the gap and progress into the regenerating tail.

The ultrastructural analysis of the complete or largely transected spinal cord of the lumbar spinal cord has indicated that some axons cross the scarring gap in the presence of a nearby regenerating tail. The reactive proximal spinal cord (upstream of the lesion) remains viable, whereas the distal spinal cord (downstream of the lesion) is more affected and most neurons disappear and axons degenerate. However, after 20–30 days from the lesion, numerous, small glial cells of undetermined nature, and sparse neurons are still viable in the proximal and even in the distal spinal cord. The lizard model therefore represents a unique experimental case in which the influence of a target tissue (the regenerating blastema) may stimulate the regrowth of axons within some 1–2-cm distance from the lesioned lumbar spinal cord. The clarification of this issue using the lizard model may allow the discovery of trophic factors involved in the guidance of transected axons over a long distance within the spinal cord.

In conclusion, if the last issues presented in this review stimulate some researchers to adopt and exploit the lizard model of amniote regeneration, my effort in summarizing the topic will have largely achieved one of its main goals.