Multi-author Review
Molecular and cellular basis of regeneration and tissue repair

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Regeneration and tissue repair: themes and variations

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Introduction (Part of a Multi-author Review)

Regeneration and tissue repair are widely spread in the animal kingdom, and actually present in many phyla, although with surprisingly different efficiencies within a given phylum [1]. Moreover, even though most regenerative processes depend on blastema formation, i.e. proliferation of mesenchymal cells in the vicinity of the amputation plane, the ways to achieve this process are multiple (Fig. 1). In fact, the questions linked to the phenomena of regeneration and tissue repair are numerous, and each forms in itself one piece of this amazing puzzle for biologists to reconstitute. Some of those are listed here: Where is the boundary between regeneration and tissue repair? How much does tissue repair impinge on regeneration? What are the mechanisms that control cell proliferation in cases where a blastema is formed and where it does not? Are these mechanisms conserved across evolution? Where are the differences between the mechanisms that control cell proliferation in blastemas and those that control cell proliferation in oncogenic tumors? Are the regenerative processes leading to the replacement of amputated structures close to embryonic/fetal developmental processes, or do they rather represent distinct 'adult developmental processes'? What are the memory mechanisms that allow the reactivation of an embryonic/fetal/larval developmental program? What are the criteria that distinguish regeneration in closely related species such as in the neotenic axolotl or in the post-metamorphic newt? Is it possible to consider appendage regeneration in a larval-stage animal as a 'short' memory process, i.e. memory of a recently achieved developmental process, whereas that occurring in adult animals would involve a 'long' memory process? At the other extremity of the life cycle, what are the links between the regeneration potential and the ageing process in a given species? These questions are fundamental biological questions, and their understanding will open new avenues to regenerative medicine. An important prerequisite, however, is systematic comparative functional analyses of the various regenerative modes at hand in vertebrates as well as invertebrates. In this issue we propose to approach some of these questions through a variety of angles and model systems.

Crescenzi and his colleagues discuss the various regulations of the cell cycle exit and cell cycle re-entry at work during quiescence, terminal differentiation and proliferative senescence. They show that the classical view, where growth factor stimulation plays the major role in pushing mammalian cells from G0/G1 to S phase, is incomplete, as inhibitors of the cyclin-dependent kinases are also key players in that balance. Their review reports recent results that demonstrate that ablation of the CDK inhibitors can actually force cells to re-enter the S phase. The most striking finding is that this balance between growth factors and CDK inhibitors can be manipulated not only in quiescent cells but also in senescent cells as well as terminally differentiated cells [2]. This possibility of tightly

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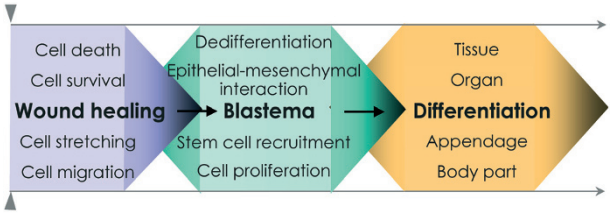

Figure 1. Schematic representation of the three main phases of regeneration in vertebrates and invertebrates. Arrowheads represent the regeneration starting point (injury, amputation, toxic shock and so on); horizontal arrows signal time after induction. The duration of each phase varies according to the regenerative context. Three main cellular mechanisms, which in most contexts require epithelial-mesenchymal interactions and cell migration, can be used to achieve blastema formation. **Dedifferentiation:** Terminally differentiated cells lose their differentiated state and re-enter the cell cycle, providing proliferating progenitors. **Stem cell recruitment:** Stem cells or multipotent progenitor cells that stay quiescent in homeostatic conditions re-enter the cell cycle. **Cell proliferation by differentiated cells:** those that are not terminally differentiated can re-enter the cell cycle, proliferate and regenerate the respective tissues without modifying their character. **Transdifferentiation**, i.e. the ability of differentiated cells to irreversibly change their identity with or without re-entering the cell cycle, is classically described in regenerative contexts that do not require blastema formation [see the reviews by Tsonis and Makarev, Eberhard and Tosh in this issue].

targeting cell cycle re-entry in terminally differentiated cells is still in its infancy but opens new windows for regenerative medicine.

The remarkable regenerative capacity of tripoblastic planaria has been known for more than 100 years. Rossi and his colleagues give an overview of the impressive development that has characterized planarian regeneration research during the past decade. In particular, the molecular and cellular description of the key stem cell population, the neoblasts, has progressed significantly. Neoblasts encompass a totipotent stem cell population, which as the authors explain, can be subdivided into at least two different groups. One is constantly cycling with normal cell cycle length, while the other is in a long-term self-renewing state by either dividing infrequently or having a very long cell cycle. Neoblasts participate in normal homeostatic cell replacement and also give rise to the regeneration blastema. Using RNA interference it is now possible to distinguish which genes are responsible for neoblast self-renewal and blastema formation, and which genes are important for homeostasis and regeneration. For example, work in two different species, *Schmidtea mediterranea* and *Dugesia japonica*, suggests that two genes, the *Pumilio* homologue *Djpum* and the bruno-like *bruli* are involved in neoblast maintainance but are not required for blastema formation [3, 4]. In contrast, stem cell maintainance is not dependent on the Piwi homologue *Smedwi-2*; instead, this gene is required for the production of neoblast progeny [5]. Rossi et al. also touch on their current efforts, combining of microarrays with knockdown approaches, which hold the promise of revealing novel aspects of stem cell biology.

In vitro manipulation of skin stem cells already allow for the reconstruction of the skin in clinics, although not a fully functional skin. Metcalfe and Ferguson highlight the importance of understanding wound repair both from the point of view of skin regeneration on its own, and also because studies in lower vertebrates showed that scar-free healing precedes regeneration of complex structures. With a few exception, such as healing of ear punches in the MRL/MpJ mouse [6], adult mammalian wound healing is not scar-free, differentiated structures do not regenerate, and a proper vasculature is absent. The authors underline the important task of understanding how inflammation, cell proliferation, cell migration, cellular signaling and cell recruitment are intertwined. When it comes to cell recruitment to the wound, an unusual cell type, so-called fibrocytes, receive considerable attention. These cells are unusual in the sense that, despite circulating in peripheral blood, they can deposit matrix. Since they can induce angiogenesis *in vivo* and have the ability to form tubelike structures *in vitro* [7 and A. D. Metcalfe, unpublished], fibrocytes represent an interesting alternative population for cell replacement in severed skin. Based on the fact that transition of fibrocytes to myofibroblasts occurs, the authors also suggest that further characterization of this cell type may reveal new ways of skin therapy and prevention of scarring.

The plasticity of differentiated cells either during development or in pathological conditions can be investigated in organs such as the liver and the pancreas as reported here by Eberhard and Tosh. The importance of metaplasia and transdifferentiation (i.e. cell- or tissue-type conversion) is well recognized in degenerative diseases and developing cancers. Interestingly, in adult organs, those two cellular processes occur spontaneously after injury, which provides an experimental framework for investigating the genes that promote transdifferentiation in adult tissues. The occurrence of metaplasia and transdifferentiation in injured adult tissues possibly reflects the developmental situation where two cell types differentiate from a common committed cell sheet. Indeed, in developing organs, the mislocalization of some cell types (heterotopia) can be observed [8]. As a consequence, understanding the molecular control of transdifferentiation would clearly highlight the content of a common toolbox used during develop-

mental but also regenerative and pathological processes, where cells are either programmed or reprogrammed. In this issue, Eberhardt and Tosh describe several pathological contexts where metaplasia leads to cancers, as in the oesophagus (Barret's metaplasia), the pancreas, the liver and the uterus. Interestingly, in several cases a similar process could be reproduced experimentally *in vivo* or *in vitro*, allowing the dissection of the molecular control of this cellular reprogramming. By acting on those pathways, Eberhardt and Tosh propose two distinct therapeutic approaches. The first one aims at preventing transdifferentiation processes that are pathogenic; the second, in contrast, aims at enhancing transdifferentiation to provide an alternative source to cells that are destroyed during degenerative processes, as in diabetes. In this latter case, targeted overexpression of the Pdx1 homeobox gene in mice or *Xenopus* was shown to reprogram hepatocytes to pancreatic β-cells [9, 10]. This last approach offers potential regenerative therapies that would circumvent the need for either embryonic or adult stem cells.

Lens regeneration in newts is a classical example of adult organ regeneration by transdifferentiation. Upon lens removal the pigmented epithelial cells (PECs) of the dorsal iris re-enter the cell cycle and transdifferentiate into lens. Notably, ventral PECs do not normally contribute to the new lens. Tsonis and Makarev address two main questions: (1) What makes dorsal PECs contribute to regeneration, while ventral cells do not? and (2) How do the molecular programs that determine dorsal/ventral identity during embryonic development relate to the mechanisms of lens regeneration in the adult newt? The authors present data suggesting that inhibition of BMP signaling is crucial for lens regeneration. By inhibiting BMP signaling, it is possible to induce ventral PEC transdifferentiation into lens. Since inhibition of the BMP pathway is also responsible for the dorsalization of embryos in different species, the authors propose that maintaining the embryonic mechanisms that control dorsal/ventral identity gives the dorsal iris an advantage over the ventral iris that results in lens regeneration. If this model is correct, one interesting question is to what extent "dorsalization" of the ventral iris leads to lens regeneration without further instructions. In other words: To what extent do dorsalization and lens regeneration share common molecular cues?

In the developing chick the spinal cord regenerates well up to a certain stage. Ferretti and Whalley discuss the requirements for successful regeneration of the neural tube, focusing on cell survival and cell replacement after injury. They consider these questions to be crucial in determining regenerative ability, not only the subsequent axonal regrowth, axonal path finding and synapse formation that have attracted most of the scientific attention up to now. They distinguish three phases in the developing chick spinal cord: the earliest days of development (up to day 5) when progenitors are numerous and re-growth of the neural tube is supported by regulation rather than regeneration; the mid-phase from day 5 to day 12, the only regeneration-permissive period, when the spinal cord is at an advanced stage of maturation but still lacks myelination; and finally the late phase, from day 13 onwards, when injury leads to extensive haemorrhage and cavitation that prevent efficient regeneration. These latter events correspond to what is observed in the injured mammalian spinal cord and actually correlate with massive apoptosis [11]. The identification of the blood factors that drive this process is certainly one path for future therapies to increase cell and axonal survival. Ferretti and Whalley have shown that in the chick embryo neurogenesis actually takes place far later than previously reported (up to day 12), likely contributing to the neuronal replacement observed during the regeneration-permissive period. Nevertheless, the origin of the progenitors remains unclear. For axonal regrowth and myelination, the role of inhibitory factors such as the Nogo proteins [12] appears predominant. The chick model system offers two advantages in investigating these inhibitors. First, it allows an accurate comparison of their behaviors in contexts that are either permissive or non-permissive for regeneration; second, they can be studied independent of the glia scar, which is never observed in the embryonic chick but always present in adult mammals.

Amphibians are powerful models of vertebrate regeneration, especially urodeles, which regenerate a wide variety of complex structures as adults. The ability to rebuild body parts during defined larval stages is, however, apparent also in the anuran *Xenopus*. Slack and his colleagues describe recent technical improvements, which makes *Xenopus* a very useful system in studies of stem cell activation, formation of a proliferation zone, which in many cases resembles a blastema, and regeneration of lost structures, for example the tail. As Slack et al. highlight, the regenerated tail in the tadpole is not quite the same as the original, as no spinal ganglia are formed, nor does the segmented myotome reappear. Nevertheless, elegant experiments using transgenic tadpoles reveal important aspects of cell lineage during tail regeneration and also identify key molecular pathways that control regeneration [13, 14]. An intriguing question that Slack et al. raise is whether both urodeles (such as the newt and axolotl) and

anurans (such as *Xenopus*) use the same mechanisms during regeneration. It appears that there are considerable differences but similarities as well. Recent data from the Slack laboratory show that cellular dedifferentiation as seen in urodeles does not occur in *Xenopus*, e.g. [14]. In the case of skeletal muscle, regeneration is fuelled by a Pax7+ stem cell population called satellite cells. However this mechanism seems also be used by urodeles in addition to cellular dedifferentiation [15]. In our view, both urodeles and anurans represent valid and important systems to address the questions how tissue repair and regeneration in larvae as well as in adults relate to each other, and to provide solid fundament for regenerative medicine.

Regeneration of an appendage according to the position of the amputation level is well evidenced by intercalation. Noji and his colleagues reproduced in the regenerating nymphal leg of the cricket some of the classical transplantation experiments that highlight the rules of intercalary regeneration. Interestingly, these rules, i.e. the missing values are intercalated within the regenerating appendage via the shortest route, are evolutionarily conserved from insects to urodeles. They also provide a detailed comparative analysis of the molecular signals driving leg development in *Drosophila* and leg regeneration in the cricket: in both contexts, the same signals, wnt, Hh, Dpp, EGF, appear to define the anterior-posterior, dorso-ventral and proximo-distal axes that obey the molecular boundary model. RNA interference proved to be efficient in both the developing and the regenerating cricket nymph [16], opening the possibility to compare the signaling pathways at work in those two contexts. Among them the EGF signaling pathway is a candidate to define the most distal position of the regenerating and developing leg. Therefore, the model of leg regeneration in the cricket offers a potent system to accurately compare the processes underlying appendage development and appendage regeneration and to dissect the mechanisms supporting the memory processes at work during regeneration.

The memory of positional information, i.e. the building of the proper structure according to the amputation level, is a puzzling question also in urodeles [17]. Campbell and Crews discuss here the role of the wound epidermis in blastema formation and its contribution to establishing the correct positional values in the regenerating limb. The epidermis surrounding the wound first migrates over the wound and, after several rounds of cell division, forms a thick regeneration-specific structure, the wound epidermis. Transplantation experiments performed on X-ray irradiated stumps showed that the epidermal cells forming the wound epidermis need to originate from distinct positions (anterior, posterior, dorsal, ventral) to initiate regeneration [18]. Using a different approach, the Crews' lab confirmed this positional discontinuity model, by inducing ectopic bumps (small blastemas that eventually regress) with skin grafts of different origins. Campbell and Crews postulate that the wound epidermis, which is usually considered a rather passive structure in the regeneration process, actually delivers essential signals to the underlying mesenchymal cells to trigger blastema formation with the appropriate positional memory. Moreover, close contact between epidermal cells of various origins causes blastema formation in planarians as well [19]. Once the signals in those species are identified, it will be of interest to see what is left from this information in amputated appendages of species that do not regenerate.

The field of regenerative medicine is still in its infancy. Clearly, a multitude of problems must be solved before significant progress can be made towards the ultimate goal: the functional regeneration of organs and complex structures in species in which it does not normally occur. The reviews in this issue accentuate the need to look closely at natural examples of functional regeneration. Research in these models, which represent most major phyla, has undergone impressive technical improvement. The models, ranging from hydra to mammals, are likely to create the well-needed interface between developmental biology, stem cell biology, regeneration biology and tissue engineering to move regenerative medicine forward.

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