

Pattern Formation in Regenerating Tissues

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1 Tissue Repair Versus Pattern Formation in Regenerating Tissues

To understand the concept of pattern formation and its role in regeneration, the basic differences between repair and regeneration need to be explained. Repair is defined as tissue restoration of a damaged tissue, without organized patterning. For example, vertebrates including humans are capable of reconstituting a functional liver following removal of up to 70 % of the original liver mass [1, 2]. Thus, liver repair includes reconstitution of the same volume but it lacks reconstruction of the same tissue pattern. Similarly, wound healing of the skin includes tissue repair by formation of a scar tissue that lacks some of the characteristic features of the original tissue. These are both examples for tissue repair that lack true regeneration or patterning. When looking at regeneration of whole body parts in salamanders, the whole limb is restored into its original form. In this case, the re-establishment of patterns is necessary because otherwise regeneration is meaningless.

2 Models of Pattern Formation in Regenerative Tissues

Traditionally regenerating tissues have been favorable models in the theoretical and experimental approaches to pattern formation. Issues of the exact restoration of patterns during regeneration have been explicitly discussed by researchers such as T. H. Morgan in the nineteenth and the beginning of the twentieth century [3]. However, it was not until the mid 1960s that formulation of ideas to explain mechanisms of pattern formation were presented. Lewis Wolpert established the

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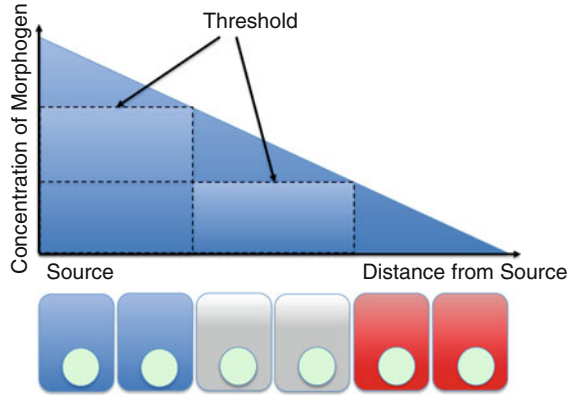


Fig. 1 The morphogen hypothesis (Adapted and modified from Jaeger et al. [43] according to Wolpert [4, 5]). Localized production of a morphogen by the blue source cells could be distributed in direction of the red cells by some form of active transport and other means according to a source and sink model. The concentration of the substance when produced at a fixed value would thus specify the cellular position when looking at the generated linear gradient. Cellular response is depending on a certain morphogen threshold that is defined by the distance of a cell towards the source

“Morphogen Hypothesis” by assuming production of different morphogens in a tissue system (Fig. 1) [4, 5]. He suggested that positional information along a certain axis can be provided by a gradient of a factor and that the cells will know their position because they will respond to a corresponding concentration of the morphogen. For instance, a suggested morphogen gradient that defines the proximal-distal axis in the regenerating newt limb could be one with higher concentration at the proximal regions and lower at the distal regions (Fig. 1).

A different set of ideas was presented by the “Polar Coordinate Model” for limb regeneration in the 1970s [6, 7]. The Polar Coordinate Model mainly emphasized that cell-to-cell communication is a necessary requirement for pattern formation and not morphogens. This model defines the site of the regenerating limb blastema using three-dimensional coordinates. By giving each position in the regenerating limb a coordinate Bryant et al. were able to successfully predict the outcome of regeneration as well as the polarity of limb following transplantation of blastemas from different positions of the limb.

In line with the Polar Coordinate Model and the Morphogen Hypothesis, Hans Meinhardt developed the “Boundary Model” in 1983 that applies the uni-dimensional Morphogen Hypothesis to all three-dimensions [8]. In detail, Meinhardt explains the establishment of coordinates/tissue identity within the embryonic growth axis by delineating distinct tissue boundaries between anterior primary competent cells (A) versus polarizing cells in the posterior (P), and cells of the dorsal (D) and ventral axis (V). Tissue identity is established by tissue area restricted morphogen production within well-defined borders that only allow unidirectional morphogen permeability. When two differently determined cell types meet at a certain tissue boundary, cooperative production of new morphogens in form of an area gradient can give information about the proximity of this cell and other cells of the tissue towards the tissue border.

Correspondingly, the established morphogen area gradient can provide directional information for tissue expansion in line with the embryonic growth axis. This model was also suggested to apply to limb regeneration when A/P and D/V area information is available to regenerating cells at the site of the limb blastema for production of a morphogen for induction of distalization.

These ideas eventually led to studies to identify such morphogens. The first candidate was retinoic acid [9–11]. During amphibian limb regeneration retinoic acid was found to reset the distal cell position to more proximal values resulting in proximalization of regeneration with additional humerus, radius and ulna. Similarly, during chick embryo development retinoic acid administration to the anterior limb bud results in duplication of all the distal limb structures along the antero-posterior axis [10, 12, 13]. Thus, the morphogenic effect of retinoic acid during development entails duplication along the antero-posterior axis compared to the proximalization effect of the proximal-distal axis during limb regeneration.

Subsequent investigations demonstrated the endogenous distribution of retinoic acid. While the group of Eichele et al. confirmed existence of a retinoic acid gradient that was higher in the posterior versus anterior chick limb bud [14, 15], Maden et al. also demonstrated existence of higher levels of the retinoic acid carrier protein CRABP in the anterior versus posterior chick limb bud suggesting a compensatory mechanism for gradient-established differences in retinoic acid levels [16, 17]. In addition, variable retinoic acid levels were found within regenerating amphibian limbs. For instance, in axolotls (*Ambystoma mexicanum*) higher retinoic acid levels were found in the posterior versus the anterior limb blastema, whereas the African clawed frog (*Xenopus laevis*) demonstrated no particular antero-posterior retinoic acid gradient [18, 19]. Also, analysis and cloning of retinoic acid receptor expression in newts did not demonstrate any kind of anterior-posterior gradient in receptor distribution throughout the limb blastema to explain the action of retinoic acid [20–25].

The actual existence of endogenous morphogen gradient molecules has recently gained again some momentum. In accordance with the “Source and Sink Model” that was originally created by Francis Crick and was recently reiterated by Schier et al. signalling proteins have been identified as the main morphogens during pattern formation in contrast to previously chemical molecules (Fig. 2) [26–28]. For example morphogens such as the signalling proteins FGF8 or DPP are either transported throughout the extracellular space by passive diffusion (Brownian motion) or active transport including transcytosis from cell to cell. It is now becoming more and more apparent that proteins diffuse as gradients in order to specify patterns.

3 Development Versus Regeneration

One of the major questions in regeneration research is how mechanisms of patterning compare with the ones during development. We will provide some insight into this question by considering the role and expression of key morphogenesis genes in limb and lens development and regeneration.

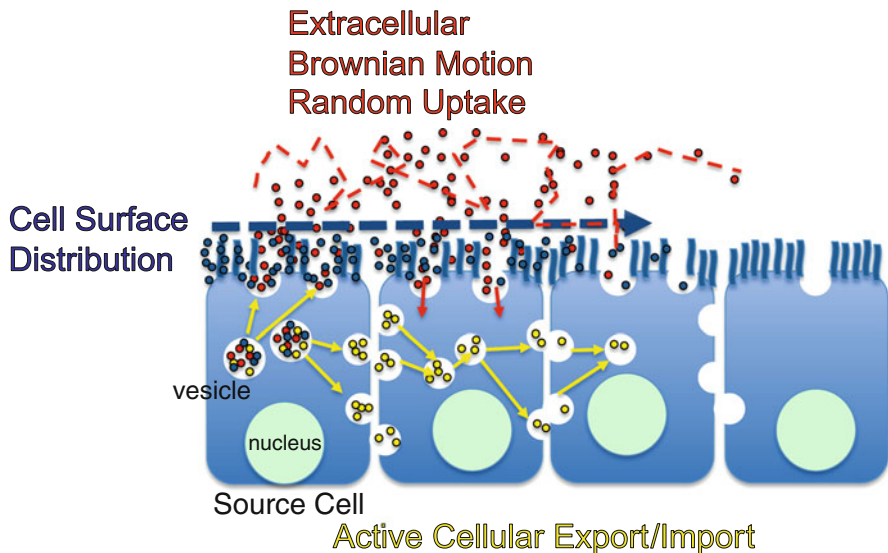


Fig. 2 Models of morphogen dispersal (Adapted and modified from Schier et al. [26]). Source cells harbor vesicles filled with morphogen molecules that fuse with the cell membrane and release their contents. Yu et al. [28] proposed that Brownian motion of molecules in the extracellular space leads to dispersal of the FGF8 morphogen as shown in *red*. Kicheva et al. suggested that repeated release and uptake by cells (transcytosis) leads to dispersal of the morphogen Dpp in the fly wing as delineated in *yellow* [44]. A few slowly diffusing FGF8 molecules are associated with carbohydrate cell surface distribution as delineated in *blue*. This cell-surface pool may contribute to long-range dispersal of FGF8

During limb regeneration in newts the regenerating blastema emerges from already patterned adult tissue. The adult limb of a newt, or a salamander has a defined skeletal pattern including humerus, ulna, radius, carpals and the fingers. Following surgical removal of the limb, the wound is covered by the wound epithelium within several hours. The differentiated tissue underneath the wound epithelium de-differentiates and forms the blastema by eliminating all previously established adult tissue characteristics. The de-differentiated blastema is then able to differentiate into the necessary cell types to reliably regenerate exactly the same structure [29]. With regards to gene expression pattern, research in axolotls clearly demonstrated existence of different patterns between amphibian limb regeneration and limb development. For instance the group of Gardiner et al. demonstrated that different members of the HoxA family, e.g., HoxA13 and HoxA9 that are expressed in cells of developing and regenerating limb buds do not follow the same spatial and temporal expression pattern during limb development and regeneration [30]. For instance, during limb development HoxA13 was suggested to specify the development of distal limb structures in comparison to HoxA9 that acts on proximal structure development. In contrast, HoxA13 and HoxA9 are both expressed at similar time points within the regenerating limb bud where they are important for the formation of distal limb structures.

Another interesting example is lens development and regeneration. To establish the very defined lens pattern with well arranged posterior-anterior polarity and fibers (Fig. 3A) lens development in vertebrates ensues by the contact of the surface ectoderm with the optic cup that leads to the formation of the lens placode (Fig. 3B). The lens placode then differentiates to form the distinct pattern of the lens. During vertebrate lens regeneration, the origin of the regenerating lens is different from the developing lens (Fig. 3C). For instance, in frogs the lens is regenerated from the inner layer of the cornea [31–33]. In contrast, lens regeneration in the newt is regenerated from dorsal iris pigmented epithelial cells that represent a completely different cell type [34, 35].

To explain these differences at the molecular level is challenging. The main regulatory pathway in the development of the eye is the *pax6/ey* subnetwork, which in vertebrates also includes the regulatory genes *six3* and *six6*. However, in some parts of the eye *six3* or *six6* depend on *pax6* and in the lens they do not [36, 37]. *Six3* regulation has been thought to be important for the induction of lens regeneration in newts [38]. Thus, it could be that differences in the regulatory role of the *pax6* subnetwork could account for the differences in the induction of lens regeneration.

In conclusion, both cell-to-cell communication and morphogens are likely to establish pattern formation during regeneration and development. The differences between differentiation and regeneration can be explained by the tissue origin, e.g., embryonic versus adult tissue patterning, and the corresponding differences in gene expression profiles between developing and regenerating tissues.

4 Role of Stem Cells in Regenerative Biology and Pattern Formation

Stem cells are defined as the cells that execute the repair of damaged tissue. To date, there remains the question if embryonic stem cells or induced reprogrammed stem cells are capable of building a structured organ or body part. Adult somatic fibroblasts cells of higher vertebrates, e.g., mouse, or humans were demonstrated to de-differentiate via a cocktail of four genes, *oct4*, *sox2*, *klf4*, and *c-myc* to become pluripotent stem cells [39]. When looking at the newt blastema, the newt somatic cells are capable of de-differentiation, similar to adult stem cells within higher vertebrates. However, blastema cells differ by being able to execute organ pattern formation that replaces only the part of limb that needs to be reproduced. When we examined expression of these four genes in newt limb blastema or lens regeneration only three of them, *sox2*, *klf4*, and *c-myc* were expressed but not *oct4* [40]. Oct4 has been heralded as the most important pluripotent stem cell maintaining factor. Correspondingly, the lack of *oct4* expression might be the difference between attaining the status of a stem cell and a de-differentiated cell in the newt blastema. In other words blastema cells (or any other regeneration-involved cell) cannot be pluripotent in order to ensure fidelity of regeneration. A recent study by Kragl et al. that used green fluorescence protein to trace axolotl

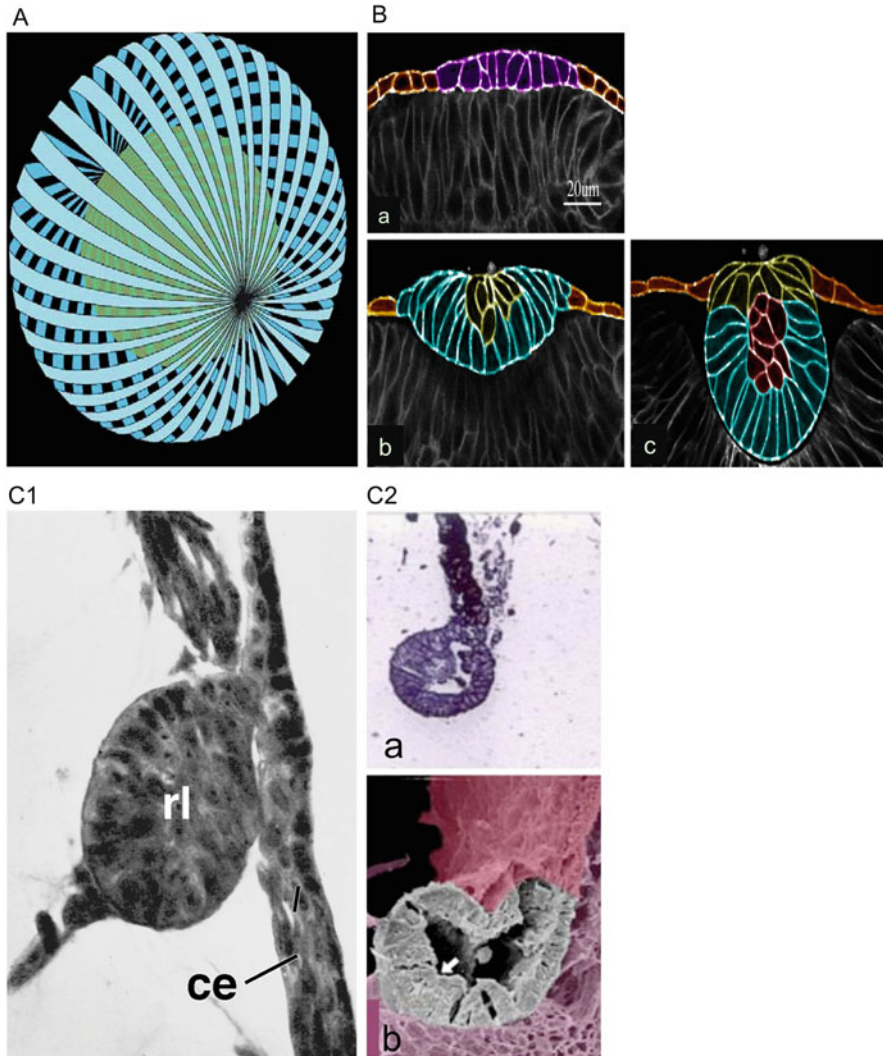


Fig. 3 Lens pattern formation during development and regeneration. (A) Complexity of Avian lens structure (umbilical suture type lens) (According to Kuszak et al. [45]). (B) Lens pattern formation during zebrafish development (According to Greiling and Clark [46]). Diagram representing the progressive stages of lens development in the zebrafish from the placode to spherical lens and the presumptive cellular differentiation at each stage. Cell membranes have been pseudocolored. *a* At approximately 16 h post-fertilization (hpf), the cells in the surface ectoderm are *orange*, and the cells in the lens placode are *purple*. *b* At 18 hpf, elongating fiber-like cells are *blue*. *c* At 20 hpf, the cells in the organizing center (*red*) of the delaminating and elongated lens mass are surrounded by columnar primary fiber cells (*blue*). (C) Pattern formation during lens regeneration in amphibians (According to Henry and Tsonis [47]). **C1:** cornea-lens trans-differentiation in *X. laevis*: *ce* corneal epithelium; *rl* regenerating lens vesicle, **C2:** iris-lens trans-differentiation in newt: *a* phase-contrast image and *b* electron microscopy of lens vesicle formation (in *grey*) at day 14 lens vesicle elongating from iris stem followed by differentiation of lens fibers at the posterior part of the lens vesicle (*arrow*). Reproduced with permissions.

limb blastema cells revealed a distinct lineage-restriction (de-differentiation and re-differentiation potential) of defined cell types, e.g., muscle cells, epidermal cells, cartilage cells and Schwann cells [41]. In other an epidermal cell will de-differentiate according to the embryonic tissue origin and will give rise again to epidermis, a Schwann cell will dedifferentiate but will give rise to Schwann cells etc. In contrast, due to the lack of specific markers for connective tissue fibroblasts, there seems to be no lineage-restriction for fibroblast cells.

However, an intriguing study by Eiraku et al. comes to support the existence of an intrinsic positional memory within embryonic stem cells [42]. Mouse embryonic stem cell aggregates were able to generate the complex three-dimensional pattern of an optic cup in a self-directed fashion within an *in vitro* cell culture model that matched the *in vivo* temporal order of optic cup development. Following distinct cell culture conditions for initial induction of a neuroepithelium, a distally inward folded cup-like structure together with formation of a proximal pigment epithelium could be observed suggesting existence of an ES cell intrinsic self-organizing program for spatial pattern formation. In addition, the study also demonstrated that besides the stepwise domain-specific intrinsic information, soluble growth factors of local epithelia contribute to the tissue differentiation process. This study further supports the idea that certain cell types such as mesenchymal stem cells, blastema cells or fibroblast cells store intrinsic information for self-organized tissue pattern formation, that lacks explanation with current models on pattern formation.

In conclusion, this brief report touches upon tissue patterning during regeneration and the possibility that even dissociated cells can have intrinsic information to form patterns is elucidated. This provides new insights into the possible mechanisms of pattern formation, that most likely calls for a re-assessment of the theoretical models that have been used to explain the process. However, we do suggest that further studies on differences/similarities between stem cells and newt cells contributing to regeneration should be paramount for understanding the mechanisms of pattern formation.

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