Beta-catenin and axis formation in planarians

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In three recent articles it was shown that β-catenin is crucial for the establishment and the maintenance of the overall polarity and especially for the character 'posterior' in planarians. If the transcription of the β-catenin gene was silenced by RNA interference, the overall polarity is lost, and in regenerating fragments a posterior blastema displays anterior characters by forming eyes and anterior ganglia. An attempt is made to integrate these new data, well-known older observations, and observations from other regenerating systems into an outline of a model that will clarify our current understanding as well as highlight areas still to be developed.

Keywords: beta-catenin; body axes; organizing regions; pattern formation; planarians; regeneration; tissue polarity

Introduction

Planarians are most famous for their almost unlimited capacity for regeneration.\(^{(1–4)}\) The underlying molecular network is largely unknown. By silencing β-catenin expression using RNA interference it was shown that β-catenin is crucial for the establishment and the maintenance of the overall polarity and especially for the character 'posterior'.\(^{(5–7)}\) The significance of these papers has been emphasized in two editorial articles.\(^{(8,9)}\) If the transcription of the β-catenin gene is blocked, posterior blastemas display anterior characters; eyes and anterior ganglia appear. Correspondingly, upregulation of β-catenin, accomplished by blocking APC (a degrading component in the β-catenin pathway), leads to tail structures in an anterior blastema.\(^{(10)}\) β-Catenin is not only required to re-establish posterior structures during regeneration. Blocking β-catenin transcription in intact animals leads to a slow disappearance of posterior structures and to the spread of anterior structures towards posterior, showing that it is also required for the maintenance of polarity in unperturbed animals. It is not only the most posterior structures that are affected. The posterior gut, easily recognizable by its characteristic bifurcation and the pharynx disappears as does the expression of HoxD, a gene characteristic for the trunk. Eventually, nearly radially symmetric cephalic structures remain with many anterior markers. In planarians, cell renewal occurs exclusively from multipotent stem cells, the neoblasts.\(^{(11)}\) This suggests that after β-catenin silencing, the position-specific differentiation of the stem cells is no longer functional.

Assuming that the axial pattern is set-up by an anterior and a posterior organizer, evidence in the three papers\(^{(6–7)}\) indicates that substantial differences exist between these terminal organizers. After β-catenin silencing, many anterior structures are formed with a close spacing. In contrast, after β-catenin upregulation, only one supernumerary posterior organizer is formed at the largest possible distance from the normal posterior organizer.\(^{(5)}\) Most interestingly, the supernumerary anterior markers appear only at lateral, and neither at dorsal or ventral positions, indicating a link between the anteroposterior and the dorsoventral (DV) patterning that will be discussed further below.

β-Catenin is ubiquitously transcribed

A surprising observation is reported only briefly in these papers. Although clearly required for the formation of posterior structures, β-catenin is transcribed ubiquitously in the animal. In a regenerating central fragment, β-catenin is upregulated in both the anterior and the posterior blastema. Thus, local upregulation does not necessarily lead to posterior structures. Clearly, other and as yet unknown components or β-catenin modifications are involved to accomplish its position-specific action, for instance, to specify anterior structures in anterior blastemas in spite of the β-catenin upregulation. The in situ data show only the transcription, not the nuclear localization of β-catenin, which is required for gene activation. Also it is still unknown whether typical downstream components such as Tcf are activated in a position-specific way. β-catenin is a part of the canonical Wnt pathway but is also involved in forming cell–cell contacts.\(^{(10)}\) However, silencing Wnt or components of the Wnt pathway (Frizzled or porcupine) has no effect.\(^{(5)}\) This could indicate that the action of β-catenin is at least, in part, independent of the Wnt pathway.\(^{(10)}\) In a related species, Dugesia japonica, a member of the Wnt family (DjwntA) has been shown to be involved in the patterning of the posterior part of the brain,\(^{(11)}\) i.e. a very anterior structure. Thus, even with these new results, our knowledge about the underlying network that regulates planarian patterning is still fragmentary. I will attempt to utilize the recent data, older observations and findings from other regenerating systems to put together a
coherent picture of our current knowledge though many pieces of the puzzle are still missing.

Parallels and differences of β-catenin expression in planarians and hydra

The expression and function of β-catenin shows interesting parallels and differences to that observed in hydra, another well-investigated model system with a nearly unlimited capacity for regeneration. The so-called head in hydra is in fact the posterior terminal structure. In hydra β-catenin is expressed in a graded manner with a high point in the head region, i.e. posterior. Thus, the actual expression of β-catenin in hydra corresponds to its functional requirement for ‘posterior’ in planarians, although, as mentioned, β-catenin is ubiquitously transcribed in planarians. In hydra, Wnt3a expression occurs only in a much smaller region, at the very tip of the hypostome, i.e. at the opening of the gastric column, and thus at the highest β-catenin level. Only this very tip has a strong axis-inducing capacity. In other words, the link between β-catenin expression and Wnt signalling, which is missing in planarians, exists in hydra (reviewed in ref.16). However, in hydra, β-catenin and Wnt seem not to be components of a direct loop. In re-aggregating hydra cells, first a ubiquitous expression of β-catenin appears that becomes subsequently confined to relatively broad peaks. Somewhat later, Wnt3a-peaks appear rapidly at the regions of highest β-catenin concentration as small sharp patches. This suggests that organizer formation in hydra occurs in several steps, and that β-catenin plays therein an important, but not the only, role. The experiments using β-catenin stabilization by APC in planarians mentioned above5 have close parallels to observations in hydra. Treatment of hydra with DAG (diacylglycerol), a drug known to block β-catenin degradation, can lead to regenerating animals with two heads. Likewise, ectopic heads and giant buds (i.e. posterior organizing centres) are induced by gsk-3β inhibitors, i.e. by β-catenin stabilization. A direct ubiquitous elevation of β-catenin (by the drug Alsterpaullone) leads in hydra to posterior structures all over, namely to a dense lawn of tentacles. However, a supernumerary hypostome, the most posterior structure, is formed only occasionally and long after removal of the drug.

The problem of maintaining polarity during growth

Characteristic for planarians and for hydra is that regeneration occurs with the original polarity maintained. This is not a universal feature of axial patterning. For instance, after separation of a sea urchin embryo along the animal-vegetal axis, both fragments regenerate the oral–aboral axis. This, however, occurs with mirror-symmetric symmetry. In other words, one of the fragments has changed its polarity. This process is well understood from a theoretical point of view. Why is the polarity maintained in some systems but not in others? In contrast to the situation in sea urchins, patterning in planarians (and in hydra) works over a wide range of sizes. Modelling revealed that additional components are required to maintain unique terminal organizers in strongly growing systems.

The regeneration of a complete animal from a tiny, nearly uniform piece of tissue indicates that molecular mechanisms are involved that are able to generate patterns de novo. Pattern formation from nearly homogeneous initial situations can be explained by reactions based on local self-activation and long-range inhibition. The de novo pattern formation in planarians is supported by the finding that downregulation of β-catenin leads to multiple anterior markers that emerge with a somewhat regular spacing. The patterns observed after β-catenin over-expression5 argue into the same direction. Formed are two clearly defined terminal poles with posterior character and not a structure-less tissue with posterior character. These observations suggest that planarians are organized by an anterior and a posterior pattern-forming system that create local organizing regions. A possible realization of a pattern-forming reaction consists of a short-ranging autocatalytic activator and a long-ranging inhibitor. Direct evidence for such mechanism exists for the Nodal/Lef/Lefty interaction involved in the oral–aboral patterning of sea urchins mentioned above (see ref.17). This model also describes elementary steps in regeneration: after removal of the activated region, the inhibitor fades away and a new self-enhancing activation is triggered. At least two such systems would be required for planarians, one for the posterior and one for the anterior organizer. So far we do not know their molecular basis and we do not know whether any of the markers used5 to identify anterior or posterior character are directly involved in the formation of these organizing regions. However, the fact that anterior and posterior organizing centres can be formed even in small fragments suggests that these organizing regions can be small in comparison of the total extension of an adult animal.

As mentioned a single supernumerary posterior organizer is formed after upregulation5 while multiple anterior organizers appear after downregulation of β-catenin. This indicates that the long-range inhibition of both terminal organizers have a very different range; it is large for the posterior and small for the anterior organizer. This accounts for the classical observation of Morgan25 that very small fragments regenerate only symmetrical double heads but not double tails. In terms of the model, there would be no space to accommodate the more space-filling loop for ‘posterior’ in which β-catenin is involved. Symmetrical regeneration of small fragments occurs also in hydroids.
A graded competence as a possible source of polarity

Since the terminal organizing regions are presumably small, the question arises of how the tissue at large acquires and maintains its polarity. How can a piece of central tissue, located far away from the terminal organizers, ‘remember’ its original anteroposterior polarity after separation from the remaining tissue? Presumably a systematically graded difference in the ability to generate the anterior or posterior organizer exists along the main body axis. This graded property can be regarded as the competence of the tissue to form the organizers – a feature termed the ‘head activation gradient’ in the hydra literature and ‘source density’ in our original theory.\(^{(23)}\) Then, in any fragment, independent of its original position and size, small differences in this competence exists between both ends. Due to the graded competence, the region with the relatively highest competence within the fragment will win the competition. This is the region of the fragment that was before cutting closest to the original organizer. Thus, the graded competence is decisive for the orientation of the regenerating pattern while the final pattern is over a wide range more or less independent of it. This model, originally worked out for hydra patterning, describes the maintenance of polarity in spite of growth and the regeneration of small fragments with correct polarity.\(^{(27)}\)

Thus, in terms of the model for systems like planarians or hydra that maintain polarity over a wide range of size, it is crucial to make a distinction between the organizer(s) on the one hand, and the graded competence to form the organizer on the other. For hydra, there is firm evidence that the overall polarity is not based on some sort of planar cell polarity but on a graded distribution of tissue compounds. The position-specific competence is preserved even after dissociation into single cells, a procedure that certainly wipes out any alignment of polar cells.\(^{(28)}\) In contrast, in systems like sea urchins, in which regeneration can occur with polarity reversal, the competence is assumed to be uniform.

To maintain this graded competence within the growing tissue, a permanent feedback is required between the terminal organizers on the one hand and the long-ranging and long-lasting competence on the other. Obviously, this feedback is corrupted after β-catenin silencing. However, due to the mutual dependence of both processes, it is difficult to decide whether β-catenin is involved in organizer formation or in setting up the competence. One criterion would be the time constraints. The formation of a new organizer is expected to be a fast process. In contrast, the competence should be a long-lasting tissue property that remains essentially unchanged during early steps of regeneration. In hydra, β-catenin reappears in less than 2 hours after head removal,\(^{(13)}\) suggesting that it a fast process and thus involved in organizer formation. In contrast, tissue polarity is a very stable feature. As determined by grafting experiments, polarity reversal of the axis takes 1–2 days.\(^{(29)}\)

In simple pattern-forming reactions, multiple peaks are expected to appear during growth. The graded competence is proposed to play an important role in suppressing supernumerary organizers and thus in the maintenance of the polar nature of the body axes during growth. With increase in distance of a cell from a terminal organizer, the long-ranging inhibition from the terminal organizer becomes lower and lower. In the same way, however, also the competence of a cell to trigger a secondary organizing region declines. Even if the inhibition that spreads from the existing organizer becomes low, the low competence hinders the trigger of a supernumerary organizer. In this way, the range of dominance of an existing organizing region increases dramatically. This scheme accounts for the finding that, if the overall polarity is lost due to a loss of the posterior high point in the competence, multiple anterior organizers appear.\(^{(5–7)}\) Thus, the ability of fragments to regenerate with the correct polarity is proposed to have its origin not in any evolutionary advantage of this feature in itself, but it is a straightforward requirement in systems that combine the maintenance of unique organizing regions, and thus of axial polarity, during growth with the ability to form new organizing regions. The latter is necessary in planarians and in hydra for asexual reproduction.

How to keep the two main body axes perpendicular to each other

A very important aspect of axes formation is that the two main body axes reliably obtain an orthogonal orientation relative to each other. The papers\(^{(5–7)}\) agree in the observation that after β-catenin silencing, supernumerary anterior organizers appear always laterally and never at the dorsal or the ventral side. This finding supports a recently proposed model that explains how this axes alignment is achieved in planarians.\(^{(30)}\)

Although almost any fragment of a planarian can regenerate, regeneration occurs only if dorsal and ventral tissue becomes juxtaposed during wound closure.\(^{(31,32)}\) This is usually the case after longitudinal or transversal cuts. However, after grafting together, e.g., two dorsal halves and subsequent fragmentation, no regeneration occurs. To account for this feature I proposed that in planarians the DV patterning is hierarchically the primary event. The common boundary between dorsal and ventral tissue obtains organizing functions. Anterior and posterior organizing regions can only appear close to this organizing border, similar as the most distal parts of insect appendages can only appear on the anteroposterior compartment border (for modelling see ref. \(^{(33)}\)). In this way, the two axes are oriented necessarily perpendicular to each other. After β-catenin silencing, the DV
pattern is maintained.\(^{(5)}\) The restriction of the supernumerary anterior organizers only along this DV border provides strong support for this model, postulating a dependence of the anteroposterior axis formation on the DV patterning, which is unusual in other systems. During longitudinal fragmentation, this DV border is restored and Noggin becomes transiently expressed at this border.\(^{(34)}\) Noggin, however, is expressed in vertebrates along the single midline, that gives rise to the future single CNS that forms all along the AP axis. In planarians, however, there are two organizing DV border that stretch from the anterior to the posterior pole, one at each side. This is proposed to be the reason for the two nerve cords in planarians, again one on each side.

Many open questions remain

The three papers\(^{(6–7)}\) have convincingly shown that β-catenin is crucial for the overall polarity of planarians. As usual, with new information new questions arise. An urgent one is the functioning of the network that allows the formation of the posterior organizer. This means in terms of the models: What is the self-enhancing reaction? What is the basis of the long-range inhibition? The same questions are still open for hydra and its relatives. Hopefully we will have soon an answer for both model systems that are at the base of the evolutionary tree of higher organisms.

Secondly, for an organism as a planarian a wealth of differently determined cells have to be produced. These papers have shown that much of this position-specific differentiation breaks down if β-catenin is no longer available, suggesting a gradient-based mechanism. However, planarians are well known for their capacity of intercalary regeneration, suggesting that cells check whether they have the correct neighbours. If not, the missing cell type will be specified and inserted at the correct position.\(^{(35,36)}\) What is the relation between β-catenin and this fine-grained cell specification? The maintenance of a particular cell type seems to depend on the correct neighbours, a phenomenon that has been called ‘cell sociology’ in planarians\(^{(37)}\) (for modelling see ref.\(^{(17)}\)). Is the loss of so many structures after β-catenin silencing a chain reaction in that the loss of one crucial structure leads to the loss of its neighbours, causing the loss of the neighbours of the neighbours, and so on?

As mentioned, in planarians, cell renewal comes exclusively from stem cells.\(^{(1)}\) For blastema formation, these stem cells can move through the entire organism to form the correct cell types at the correct positions.\(^{(38)}\) Formation and shaping of gradients by moving cells that come to rest at precise positions is a well-known phenomenon also in very different developmental systems.\(^{(39)}\) Its molecular and mechanistic base is still elusive. Thus, the discovery of the loss of structures after β-catenin silencing, very interesting in itself, also remind us of the many basic problems that are still open in pattern formation.

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References