by using a combination of *in vivo* and *in vitro* experiments, the authors demonstrated that AKR2 (A and B) could improve the targeting and insertion of chloroplast OMPs. Binding affinity of AKR2 for chloroplasts is mediated by its C-terminal ankyrin repeats and can be competed, suggesting that association occurs at specific sites — that is, at a membrane-bound receptor (Fig. 1).

Finally, to investigate the role of AKR2 *in planta*, the authors generated plants in which the total pool of AKR2 was severely depleted[1]. Plants with very low levels of AKR2 protein displayed a severe chlorotic phenotype, indicating a defect in chloroplast biogenesis; the organelles lacked a discernable thylakoid system and contained a large number of vesicles. The mutant plants were not only severely depleted in chloroplast OMPs, but also in internal, stromal and thylakoidal proteins.

The severe phenotype of the mutant *Arabidopsis* plants was most likely due mainly to a defect in TOC complex assembly. The atToc33 and atToc34 receptors were depleted, probably because AKR2 is required to assist their targeting (as suggested by the fact that AKR2A binds to their C-terminal targeting signals). In turn, the Toc34 proteins are known to be involved in the targeting of Toc159 receptors, and together, both receptor components are required for the insertion of the Toc75 protein import channel[3]. As the TOC machinery is required for the import of precursor proteins with classical transit peptides, significant depletion of TOC complexes would be expected to have a deleterious effect on organellar development.

Interestingly, AKR2A was identified in an earlier study by its interaction with a 14-3-3 adaptor protein, and proposed to be important for disease resistance and oxidation metabolism[14]. Plants in which the expression of AKR2 was reduced, produced elevated levels of H_2O_2 and displayed a leaf necrosis phenotype typical of the hypersensitive response to pathogen infection. The plants also showed a mild chlorotic phenotype. It is possible that the production of reactive oxygen species in these plants was an indirect consequence of a defect in chloroplast biogenesis. Nonetheless, the fact that AKR2 binds to a 14-3-3 protein raises the possibility that AKR2 also targets precursor proteins with classical transit peptides, as an unidentified 14-3-3 protein was previously proposed to be part of a precursor protein guidance complex[15].

The work of Bae *et al.*[1] raises many questions. First, is AKR2 specific for chloroplast OMPs with TMD/CPR-type targeting signals, or does it also recognize other, as yet undefined signals in different types of chloroplast OMP (for example, putative internal signals in β-barrel proteins)? Second, what is the receptor for AKR2 at the chloroplast surface, and what other factors are involved? Third, once AKR2 docks at the chloroplast OM, how is the substrate transferred to the membrane protein(s) that mediate insertion, and how does insertion occur?

Remarkably, AKR2 recognizes targeting signals that are similar not only to those of type-I membrane proteins of the ER, but even more so to those found in mitochondrial OM proteins[16]. Perhaps the N-termini of nascent chains are first screened by SRP, and then those that evade SRP-binding are recognized post-translationally by distinct, soluble targeting factors for the other compartments. However, such a targeting factor has yet to be identified for mitochondrial OMPs.

Other types of chloroplast OMP have been proposed to use different targeting pathways with distinct requirements[4], and so it will be interesting to determine whether these are also assisted by AKR2, or by other factors. Perhaps there is still room for 'spontaneous insertion'.

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Heads or tails: can Wnt tell which one is up?

Elly M. Tanaka and Gilbert Weidinger

Planarian flatworms regenerate their heads and tails after amputation. It turns out that they use Wnt–β-catenin signalling to determine where the head and the tail should form.

Hercules had a tough time killing the mythic Lernean hydra, a nine-headed monster whose heads kept growing back once cut. The cnidarian *Hydra*, aptly named after that Greek beast, as well as the plathelminth flatworm *Planaria*, can indeed re-grow their heads; and all other parts of their bodies, for that matter. If you cut off both the head and tail from a planarian, the trunk will grow a head at the anterior wound and a tail at the posterior wound (Fig. 1a). Thus, the central body fragment retains polarity and a memory of what is missing[1]. Interestingly, over a century ago, T. H. Morgan observed that very thin trunk slices will occasionally regenerate a head on both ends[1]. This led him to suggest that a gradient of material along the body determines anterior-posterior polarity and that a minimal length is required for a robust gradient to form and normal polarity to be achieved. The molecular mechanisms establishing anterior-posterior polarity have thus far been elusive. Now, two new studies have shown that Wnt–β-catenin signalling determines where

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heads and tails will form[2,3]. This finding is particularly striking in light of the known role this pathway plays in setting up the head-to-tail axis during vertebrate embryogenesis.

Both studies showed that when *β-catenin* is knocked down by RNA interference (RNAi) in regenerating trunk fragments, the posterior wound forms a head instead of a tail, whereas the anterior wound regenerates a head as normal, resulting in two-headed animals (Fig. 1b). In addition, whereas animals sliced into left or right halves normally regenerate head, trunk and tail sideways, after knockdown of *β-catenin* they instead produced multiple sideways facing heads all along the anterior-posterior axis. Thus, *β-catenin* is required for regeneration of posterior structures and in its absence, all regenerating tissue appears to be of anterior character by default. Consistent with this hypothesis, Gurley *et al.*[2] found that ectopic Wnt signalling triggered by knockdown of *APC*, an inhibitor of β-catenin, was sufficient to transform the default anterior head structures into more posterior fates and thus caused animals to regenerate a tail instead of a head from an anterior wound, resulting in two-tailed worms (Fig. 1c). In other words, Wnt–β-catenin signalling needs to be switched off for the head to form and switched on for the tail to regenerate.

What activates or inhibits β-catenin signalling during regeneration? Although Gurley *et al.*[2] showed that knockdown of *Dishevelled* orthologues (positive regulators of β-catenin), also caused ectopic head formation, it remains unknown whether tail formation is actually triggered by extracellular Wnt ligands during regeneration. Both studies characterized several planarian Wnt proteins, and Petersen and Reddien show that some of them were expressed near posterior-facing wounds after amputation[3]. However, genetic evidence for a role of Wnts or their Frizzled receptors in planarian regeneration is missing, as knockdown of these proteins did not produce phenotypes. Although it cannot be excluded that the Dishevelled–β-catenin pathway is activated independently of Wnt ligands, the knockdown analysis might be complicated by extensive redundancy between Wnts and Frizzleds, as is commonly observed in other systems.

Wnt–β-catenin signalling clearly determines head–tail polarity during planarian regeneration, but it is currently not known whether the pathway also sets up the anterior-posterior body axis during planarian embryonic development. Intriguingly, however, this is exactly the role

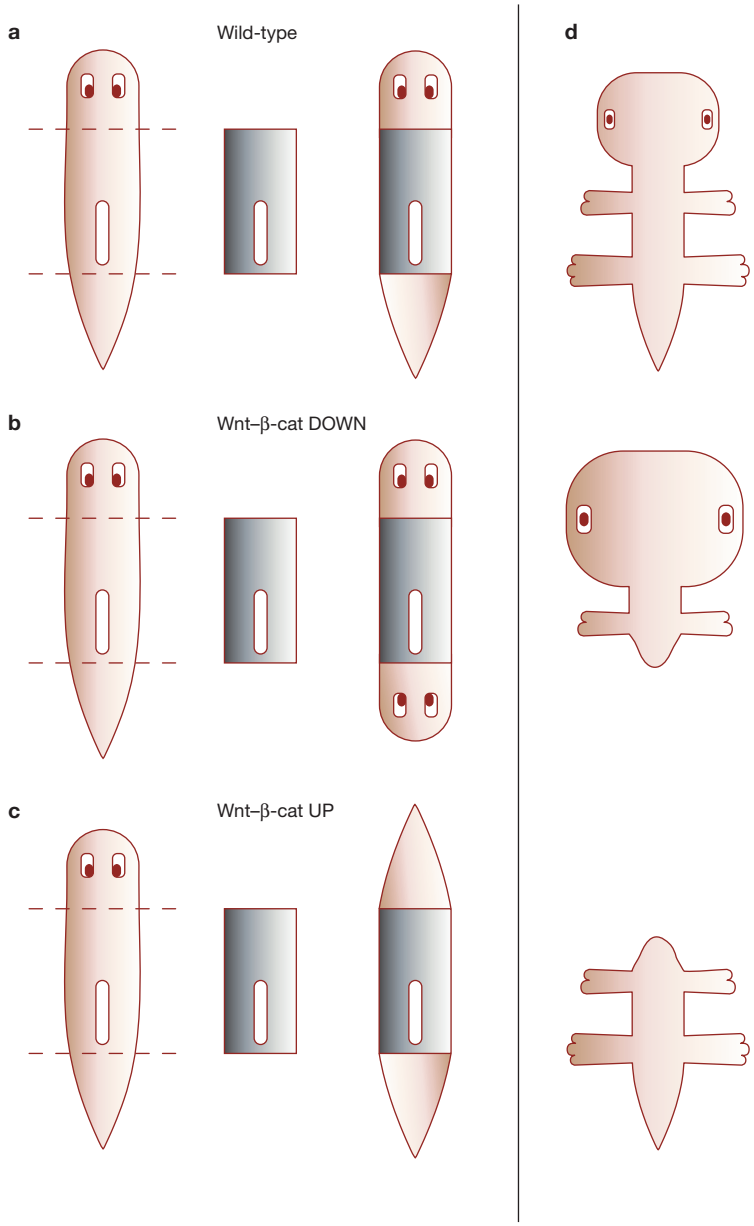

Figure 1 Wnt–β-catenin signalling regulates head and tail formation during planarian regeneration and vertebrate embryogenesis. (**a**) Wild-type planarian trunk fragments regenerate a head from the anterior wound and a tail from the posterior wound. (**b**) Knockdown of the Wnt–β-catenin signalling pathway by RNAi in the whole animal results in formation of an ectopic head from the posterior wound. (**c**) Upregulation of the Wnt–β-catenin signalling after knockdown of the negative regulator *APC* causes regeneration of two tails. (**d**) During vertebrate embryogenesis, Wnt–β-catenin signalling also specifies the posterior structures and needs to be off for the head to form. Embryos in which the pathway is suppressed during gastrulation develop big heads and lack posterior trunk and tail (middle), whereas overactivation of Wnt–β-catenin signalling causes loss of head structures (bottom).

Wnt–β-catenin signalling has during vertebrate embryonic development[4,5]; inhibition of Wnt signalling during gastrulation, when the anterior-posterior axis is set up, causes loss of trunk and tail structures, whereas overactivation of Wnt–β-catenin signalling results in embryos that form no head (Fig. 1d). Wnt–β-catenin signalling instructs cells to develop into posterior structures, whereas head formation requires that Wnt signalling is suppressed by the action of several extracellular inhibitors.

Does this mean that specifying the anterior-posterior axis is an ancient function of Wnt–β-catenin signalling already present in

the ancestor of all bilaterally symmetric animals? Modern bilaterians fall into three major branches: deuteromes, including sea urchins and vertebrates; ecdysozoa, such as the nematode *Caenorhabdititis elegans*; and lophotrochozoa, to which planaria belong. Despite the different embryology in these phyla, it is intriguing that Wnt–β-catenin signalling seems to specify posterior cell fates in members of all three branches[6–8]. In fact, a role for Wnt signalling in specifying head-tail polarity might even be more ancient. *Hydra vulgaris*, which regenerates from very small pieces, similarly to the planarian, is a member of the more primitive phylum *Cnidaria*. Amazingly, when hydra cells are disaggregated and reaggregated into a ball, they spontaneously polarize to make a complete hydra [9]. What organizes or determines where the head and foot form in this animal? It was previously shown that a drug that activates the Wnt pathway, alsterpaullone, causes ectopic head tentacles to form along the body of the *Hydra*[10].

However, in bilateria, Wnt signalling specifies the posterior and inhibition of the Wnt pathway leads to head formation. Are the phenomena in hydra and vertebrates related at all, or do they represent independent implementations of the pathway for specifying body structures? In *Hydra*, activation, but not inhibition, of the Wnt pathway has been reported so far. It would be interesting to determine whether *β-catenin* knockdown in *Hydra* results in foot formation. This would indicate that the β-catenin pathway indeed functions as a head versus tail switch in the *Hydra* system as well. The seemingly reversed polarity may stem from the embryology of cnidarians. As in other animals, Wnt-pathway components are localized to the posterior of several cnidarian embryos[11,12]. However, in a relative of *Hydra*, *Hydractinia*, where the transition from embryo to adult has been studied, the posterior end of the larva gives rise to the head in the adult[11]. Taken together, these results suggest that the Wnt pathway may have had an ancient role in directing anterior-posterior axis formation.

Although animals such as *Planaria* and *Hydra* undergo regeneration of the whole body, vertebrates are more limited in their regenerative capacities. Nonetheless, some vertebrates (such as fish and amphibians) do regenerate entire appendages and Wnt signalling has been shown to have an essential role in this process[13]. When Wnt signalling is blocked by overexpression of the extracellular Wnt inhibitor, Dickkopf, zebrafish fin and amphibian limb regeneration are blocked at an early step — essentially no or few proliferating progenitors accumulate at the wound site. Could this mean that during appendage regeneration, Wnt signalling is implemented in a distinct way compared with regeneration of the whole body axis? It is clear that the Wnt pathway is used in a large multitude of contexts beyond anterior-posterior specification during development, organogenesis and tissue homeostasis[14]. During vertebrate appendage regeneration, a main role of Wnt is to control progenitor-cell proliferation[13]. In contrast, neither study discussed here reported a role for the Wnt pathway in proliferation at the wound site in planaria[2,3]. Perhaps as the various members of the planarian Wnt pathway are studied in more detail, a phenotype in this direction will be revealed. On the other hand, we should perhaps reconsider our interpretation of Wnt signalling in the vertebrate appendage, where it may be important for providing directionality by telling the appendage where the tip should be regenerated. Vertebrate appendages do not regenerate bidirectionally — in other words, you cannot grow a shoulder out of a hand — and by inhibiting Wnt signalling, we may be disturbing the polarity of regeneration, but the only interpretable outcome of this perturbation is a block in growth.

A surprising aspect of the planaria is the presence of the β-catenin anterior-posterior specification system within adult tissue: both studies showed that *β-catenin* knockdown of intact, non-regenerating worms resulted in the slow transformation of posterior body parts into head structures[2,3]. It is likely that the use of embryonic signalling profiles during the homeostasis of adult tissues is one secret as to why the planaria can forever stay young and regenerate so well. These insights into the implementation of signalling pathways may one day help us to rejuvenate ourselves.

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