REGENERATIVE BIOLOGY

# On with their heads

**Data from three teams show that alteration of a single cell-signalling mechanism can unlock the latent head-regeneration potential in normally regeneration-deficient species of flatworm.** SEE LETTERS P.73, P.77 & P.81

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Voltaire was not only a leading philosopher of his time but, inspired by natural scientists, he also carried out intriguing experiments that showed that decapitated snails could regenerate their heads. On the basis of these observations, Voltaire was said to be optimistic about humans being able to do the same one day[1]. Although the arrival of that day is apparently not imminent, clues to how to unlock a latent potential for head regrowth are presented in three papers* in this issue[2–4].

Some animals regenerate organs and even body parts following major injuries. The distribution of species with these abilities is seemingly random: representatives range from relatively simple organisms, such as hydra, to the more complex flatworms, and on to vertebrates such as salamanders[5]. The regenerative capacity of these organisms is quite remarkable. For example, some flatworms can be sectioned into small pieces, each of which will give rise to a new, complete worm[6]. And salamanders, after amputation, regrow entire limbs that are fully functional, perfect copies of the original extremity[7].

Why some animals but not others respond to injury with such extensive regeneration is an outstanding question both from an evolutionary perspective and when considering possible interventions to enhance regenerative capacity. One way to address this question is to compare closely related species with differing injury responses. The three new papers all take this approach, by studying highly regenerative and regeneration-deficient flatworms. Two flatworm species, *Dugesia japonica* and *Schmidtea mediterranea*, can regenerate all missing body parts after transection, irrespective of how many times or where they are cut along the head-to-tail (anterior-to-posterior) axis. By contrast, the three non-regenerative species investigated in the studies reported here — *Phagocata kawakatsui* by Umesono *et al.*[2] (page 73), *Procotyla fluviatilis* by Sikes and Newmark[3] (page 77) and *Dendrocoelum lacteum* by Liu *et al.*[4] (page 81) — all fail to regenerate heads from the tail fragment left after transection through the most-posterior third of their bodies (Fig. 1).

*This article and the papers under discussion[2–4] were published online on 24 July 2013.

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**Figure 1 | *Dendrocoelum lacteum*.** These flatworms, the subjects of Liu and colleagues' study[4], are unable to regenerate a head from a tail fragment.

What mechanisms underlie these differences? Regeneration normally starts by wound healing and involves the proliferation of stem cells called neoblasts. These cells give rise to a cell mass called a blastema, which subsequently grows and differentiates into a fully patterned new body part. It seems that regeneration-deficient species respond to an injury with normal wound healing and cell-proliferation dynamics. This extended to blastema formation in the tail fragments of *D. lacteum*[4], but this was not observed in *P. fluviatilis*[3]. Thus, the regenerative process seems to stop at or just before blastema formation. However, given the paucity of biological markers for blastemas, some caution about these conclusions may be warranted.

Importantly, the papers converge on the identity of a molecular signalling pathway that should be targeted to evoke head regeneration from tail fragments in all three regeneration-deficient species — the Wnt/β-catenin pathway, which plays a key part in several processes during embryonic development and adult homeostasis in multicellular organisms. Wnt proteins cause accumulation of β-catenin, which in turn regulates gene expression[8], and perturbation of Wnt/β-catenin signalling is associated with developmental malformations, inappropriate cell turnover during adult homeostasis and tumour formation[9]. The common conclusion of the present studies is that downregulating the activity of this pathway by reducing β-catenin levels leads to head regeneration from otherwise regeneration-deficient tail fragments. Heads regenerated through this approach had morphologically normal brains and contained photoreceptors — the 'simple eyes' of these animals. Furthermore, regenerated animals displayed normal behavioural responses to light and reacquired feeding ability.

The significance of Wnt/β-catenin signalling in regeneration has previously been highlighted in regeneration-competent flatworms. In these animals, reduced activity of this pathway leads to outgrowth of a head instead of a tail from posterior-facing wounds (resulting in a two-headed animal) and, conversely, enhanced activation of the pathway by stabilization of β-catenin leads to tail instead of head outgrowth from anterior-facing wounds (giving a two-tailed animal)[10]. In agreement with these observations, two of the present studies[3,4] found that stabilization of β-catenin also results in tail outgrowth from tail fragments in the normally non-regenerative *P. fluviatilis* and *D. lacteum*.

A possible interpretation of these combined findings is that achieving polarity along the head-to-tail axis is required for injury responses to proceed to the creation of a new body part, and that the inability to progress past blastema formation to regenerate a head is the result of insufficient polarity in Wnt/β-catenin signalling (Table 1). The next question is how Wnt/β-catenin signalling creates axial polarity. Data from Umesono and colleagues' study[2] indicate that another pathway, ERK signalling, normally forms an activity gradient that decreases from head to tail, and the authors suggest that the Wnt/β-catenin pathway exerts its effects by suppressing ERK signalling. According to their

**TABLE 1 | SIGNALLING AND REGENERATION**
Effects of altered Wnt/β-catenin signalling in regeneration-deficient flatworms

| Wnt/β-catenin signalling | Axial polarity | Regeneration from tail fragment |
|---|---|---|
| Natural (deficient) regulation | Insufficient | None |
| Downregulated signalling | Correct | Head regeneration |
| Upregulated signalling | Reversed | Tail regeneration |

model, *P. kawakatsui* fails to regenerate a head from tail fragments because head regeneration requires high levels of ERK signalling and this is prevented by Wnt/β-catenin activity. It will be interesting to further dissect these putative molecular interactions and determine whether the model also holds true for other regeneration-deficient worms.

How should we consider the evolutionarily uneven distribution of regenerative ability, and what are the implications of the current findings for regeneration in mammals? Is it possible, as these studies might indicate, that regeneration is an inherent trait that is manifest in some organisms but silent in others, such that reshuffling the interactions of existing molecular components is sufficient for its awakening? Alternatively, is regeneration a capability that has been acquired by some species, possibly in a process that requires species-specific molecular components[11]?

Inducing regeneration of a body part in a species that cannot undergo full regeneration but is in many aspects regeneration-competent is quite different from achieving something comparable in largely non-regenerative organisms, which include most mammals. Nevertheless, the new studies highlight the importance of cross-species comparisons for understanding the molecular mechanisms of regeneration and identifying processes that prevent it from occurring. Moreover, the finding that manipulation of a single major signalling pathway is sufficient to induce regeneration of a body part gives hope for the future that we will be able at least to improve regenerative responses in humans. ■

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CLIMATE SCIENCE

# Unequal equinoxes

**Innovative measurements of ocean turbulence show that mixing of cold water from below makes the surface of the equatorial Pacific much colder in September than in March.** SEE LETTER P.64

SHANG-PING XIE

Equatorial countries such as Indonesia and Ecuador are associated with thunder showers and lush rainforests. In September 1835, when Charles Darwin set foot on the Galapagos Islands, an archipelago on the Equator some 1,000 kilometres west of the Ecuadorean coast, he found none of these. Instead, he saw very dry land and leafless trees "in an intertropical region, but without the beauty which generally accompanies such a position"[1]. The cold surface sea water that keeps the Galapagos dry is part of a basin-scale structure called the equatorial cold tongue, and is known to originate from beneath the surface. But how the cold deep water reaches the surface has never been satisfactorily measured through a seasonal cycle, because of the prohibitive costs of operating ships in this remote ocean.

On page 64 of this issue, Moum *et al.*[2] report multi-year measurements of ocean turbulence using a revolutionary instrument called an χ-pod, which is moored in the ocean (Fig. 1)*. They show that small ocean eddies of diameter less than 1 metre bring cold water from the thermocline layer (the ocean layer that separates the warm upper part of the ocean from the cold deeper part) to the sea surface. The new instrument has finally clinched the connection between turbulence and climate, and holds the promise of improving global climate models.

In explaining the absence of coral reefs around the Galapagos Islands, Darwin calculated that sea surface temperature (SST) was 20 °C during his 35-day stay[3], close to the modern September climatology (21° C) for this region, but much lower than in the western Pacific (29 °C) at this time of the year. Had Darwin come to the islands in March, he would have been welcomed by trees covered with green leaves. SST in March reaches nearly 27 °C, warm enough to permit rain showers. The pronounced annual cycle in SST is a mystery given our experience that the seasonal change in temperature is caused by the local change in solar radiation. The Sun marches across the Equator twice a year, at the March and September equinoxes, yet the SSTs between the two months differ by a whopping 6 °C. What makes September cooler than March in the Galapagos?

Because of Earth's rotation, easterly winds force upward motion in the Pacific Ocean on the Equator that brings the cold water from the thermocline close to the surface, a process called upwelling. Likewise, southerly winds cause upwelling slightly (100–200 km) south of the Equator[4]. The climatic axis of symmetry turns out not to be on the geographic Equator, but 1,000 km to the north in the intertropical convergence zone where the southern and northern trade-wind systems converge to produce frequent thunder showers. Because the intertropical convergence zone is displaced north of the Equator, the Equator belongs to the Southern Hemisphere from a climatic perspective, being warm in March and cold in September[5]. In September, the seasonal warming of the Northern Hemisphere and cooling of the Southern Hemisphere accelerate the southeast trade winds, intensifying ocean upwelling. In March, the seasonal warming of the Southern Hemisphere weakens, but does not reverse the direction of the southeasterly cross-equatorial winds, suppressing ocean upwelling. Thus, the southeast trade winds attain an annual cycle in speed that drives annual variations in ocean upwelling and SST near the Equator[6].

Upwelling pushes the thermocline close to the surface but it takes turbulence at sub-metre scales to mix the cold thermocline water into the surface layer. Conventionally, measurements of such ocean mixing are made by dropping microstructure profilers from ships, but nobody can afford to station a research vessel on the Equator for a year — the minimum length of time needed to unlock the mystery of the equatorial annual cycle. Moum and colleagues invented χ-pods for deployment on moored buoys[7] to measure the dissipation rate of turbulent temperature variance ($\chi_T$) for up to a year at a time. $\chi_T$ is a measure of turbulent

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