Germline Regeneration: The Worms' Turn

Asexual reproduction in the annelid *Enchytraeus japonensis* entails the regeneration of primordial germ cells from body parts that lack gonads. New primordial germ cells arise from *piwi*-expressing germline stem cells that are distinct from somatic stem cells.

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We're members of a strange species, you and I. A rational scientific establishment would be working hell bent for leather on ways to bring the size and impact of the human population into line with the carrying capacity of the planet, yet so many of us are engrossed with stem cell biology, tissue regeneration and other high-tech measures aimed at prolonging life. Is this connected to the graying of the baby boomers? In any case, these interests have unquestionably fueled resurgence in some formerly obscure areas of comparative development and evolution, because our fascination with regeneration is in effect an attempt to recapture our phylogenetic youth.

In humans and in the 'model organisms' commonly used for laboratory studies of developmental biology, reproduction is almost entirely restricted to embryonic development, initiated by gametic fusion. But model organisms represent only a small sampling from just two of the three super-phyla of bilaterally symmetric metazoans, namely Deuterostomia — vertebrate species such as fish, frog and mouse — and Ecdysozoa — the fruitfly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. In contrast, species descendent from branches nearer the base of the metazoan tree, such as cnidarians and acoelomorphs, typically reproduce both sexually and asexually (by fission or budding), and also exhibit a striking capacity for regeneration. Such developmental plasticity is also seen among major bilaterian taxa, including many annelids and flatworms — from the third and least-studied bilaterian super-phylum, Lophotrochozoa — and echinoderms, from the super-phylum Deuterostomia.

The haploid gametes that fuse to initiate embryonic development arise as the exclusive progeny of 'primordial germ cells', an evolutionarily ancient cell type that is homologous at least throughout metazoans, as judged by cytoarchitecture and the shared expression of genes such as homologs of *nanos*, *vasa* [1] and *piwi* [2,3]. It is argued that germ cell and somatic lineages must be separate to avoid evolutionarily unsustainable competition between different cell lineages within the organism [4]. Segregating the primordial germ cell and somatic lineages early in development also offers the advantages of minimizing the possibilities for passing on any somatic mutations into the germline, and would also minimize the need to undo any chromatin modifications that are imposed as part of the normal developmental program. Thus, notwithstanding the fact that the embryonic origins of primordial germ cells vary widely even within phyla [1], the conventional wisdom is that "primordial germ cell formation is a one time thing", with no interconversion between primordial germ cell and somatic cell lineages, except as a consequence of fertilization and embryogenesis.

In light of this, the developmental plasticity observed in non-model organisms poses this conundrum: during asexual reproduction or regeneration, is the germline reconstituted from parts of the body that lack gonads? And if so, what is the source of the new primordial germ cells? The answer to the former question must certainly be "yes", or else asexual reproduction would be somewhat of a dead-end proposition. But the origins of primordial germ cells in asexual reproduction or regeneration are problematic. For one thing, cell lineage tracing is far more difficult in adult tissues than in embryos because the former have more and smaller cells. Moreover, the non-model systems where these phenomena occur have been almost by definition refractory to experimental interrogation.

For example, the flatworm *Schmidtea mediterranea* is a lophotrochozoan species (phylum Platyhelminthes) which is now being used to study stem-cell processes in regeneration [5]. In *S. mediterranea*, tissue homeostasis and regeneration are achieved via stem cells called neoblasts. It appears that all neoblasts express two *piwi* homologs and RNA interference (RNAi) knockdowns of one of these, *smedwi-2*, eliminates the ability of the planaria to maintain its tissues or regenerate [6]. Thus, it appears that, in *S. mediterranea*, somatic stem cells express *piwi* genes as an intrinsic part of their identity.

Notwithstanding the evolutionary arguments, available evidence suggests that the neoblasts are a homogeneous population of stem cells, from which it would follow that they must give rise to both somatic and primordial germ cell lineages. But in fact, this question remains open. The strain of *Schmidtea* being used for regeneration studies has lost the capacity for sexual reproduction, apparently as a result of a chromosomal translocation [7]. But whether this

mutation wipes out a cryptic but distinct primordial germ cell lineage or renders a single neoblast lineage incapable of generating germline precursors remains to be determined.

In work published recently in *Current Biology*, Tadakoro *et al.* [2] addressed this problem using expression of a *piwi* homolog as a marker for primordial germ cells and their precursors in a second lophotrochozoan species, *Enchytraeus japonensis*, an oligochaete annelid akin to earthworms and leeches (subphylum Clitellata [8]). Like all clitellates, *E. japonensis* is hermaphroditic; it reproduces asexually (by fission) under conditions of good nutrition, but maintains rudimentary male and female gonadal tissues, as judged by small clusters of cells expressing *Ej-piwi* in segments seven and eight. Upon starvation, the worms undergo sexual maturation and the clusters of cells expressing *Ej-piwi* expand within the enlarging gonads.

E. japonensis also regenerates after amputation. Tadakoro *et al.* [2] trisected animals, then allowed separate pools of anterior, middle and posterior fragments to regenerate. Upon starvation, the worms arising from each pool differentiated gonads with equally high efficiency (>80%), validating the assumption that the germline does regenerate from portions of the worm that would not normally form gonads.

So what is the source of these new primordial germ cells? In well-fed, asexually reproducing *E. japonensis*, Tadakoro *et al.* [2] observed a second population of *Ej-piwi* positive cells, distributed sparsely and irregularly along the body axis. To reconstruct the fate of these cells during regeneration, the number and position of *Ej-piwi* positive cells was noted in specimens fixed at a selected time point following amputation of the head and gonadal segments. Notwithstanding the caveats imposed by the inability to make real time observations, it appears that cells expressing *Ej-piwi* proliferate selectively in the front most mid-body segment, just

posterior to the site of amputation, between 24–48 hours following amputation, then migrate into the prospective segments seven and eight of the regenerating head between 48–72 hours after amputation.

Intriguingly, by the time the presumptive primordial germ cells migrate, the regeneration of the somatic tissues is well underway. Tadakoro *et al.* [2] used an ingenious combination of amputation, BrdU labeling and re-amputation to demonstrate that a previously described, segmentally iterated population of cells (also called neoblasts) is a primary if not the exclusive source of the somatic cells within the regenerated tissue. Thus, these experiments provide compelling evidence that, in *E. japonensis*, there are distinct germline and somatic stem cell lineages, each of which undergoes its own program of proliferation migration and differentiation during asexual reproduction and regeneration. In light of these results, it will be interesting to see if *S. mediterranea* is really an exception to the generalization concerning the segregation of germline and somatic lineages.

Questions remain. What are the embryonic origins of the primordial germ cells and when during embryogenesis does the primordial germ cell lineage segregate from somatic lineages in *E. japonensis*? Lineage tracing experiments in other clitellate annelids, a leech and another oligochaete, revealed that the primordial germ cells arise from the mesodermal lineage founded by the 4d micromere [9,10], a cell regarded as a homologous mesodermal precursor throughout the spirally cleaving taxa [11,12]. Diverse clitellate annelids exhibit well-conserved, highly determinate embryonic cell lineages [13–17], so these findings may well extrapolate to *E. japonensis*. Curiously, cell lineage studies in the leech *Helobdella robusta* [10] reveal that that the primordial germ cells separate from somatic mesoderm relatively late in development (after more than 20 rounds of zygotic mitosis, compared to four in

C. elegans, for example). We can hope that further comparisons between *Enchytraeus* and other clitellates such as *H. robusta* will yield mechanistic insights into how the capacity for asexual reproduction and robust regeneration was lost in the latter, but what evolutionary tradeoff could sustain such a loss?

Asexual reproduction permits an organism to promulgate its genome 'selfishly' undiluted, so long as conditions are compatible with growth. The evolutionary rationale(s) for sexual reproduction remain unresolved [18], but one possibility is that it provides a 'plan B' for when conditions change — meiotic recombination and fusion of haploid gametes, from the same or different individuals, permits the exploration of different genotypes that may be better able to survive an altered environment. This reasoning is consistent with the observations that in *E. japonensis* and many other taxa capable of both sexual and asexual reproduction, sexualization is induced by stressful conditions, in contrast to humans (and flies).

Just as it is more efficient to build from scratch rather than renovate an existing structure (especially if the building must remain in use during renovation), we speculate that the process of embryonic development (a necessary correlate of sexual reproduction) permitted the elaboration of larger and more complex body plans in various bilaterian lineages, until finally a tipping point was reached, beyond which asexual reproduction was no longer possible. Thus it could be that losing the ability to reproduce asexually and regenerate efficiently was a landmark on the road to evolving the mental and physical wherewithal to determine how it happened and to wonder why.

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Animal Behavior: Who Will Croak Next?

A recent study with the predatory bat *Trachops cirrhosus* has shown the importance for this species of social learning about novel prey using auditory, rather than visual or olfactory, cues.

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Observations of social learning of foraging behaviors in non-human animals have long fascinated naturalists. Among the most celebrated documentations of innovation and social learning in animals involve washing of sweet potatoes and wheat by free-ranging Japanese macaques (*Macaca fuscata*) and opening milk bottles by titmice (*Parus* spp.). These beneficial behaviors were first observed in one or a small number of individuals, but then they spread through populations [1]. These and other studies have inspired careful experimental designs to determine the sensory cues and cognitive mechanisms underlying innovation and social learning in non-human animals. Visual and sometimes olfactory cues have been shown to provide the basis for information transfer between individuals in many species [2].

In this issue of *Current Biology*, Page and Ryan [3] report how fringe-lipped bats, *Trachops cirrhosus* (Figure 1), learn from one another by attending to prey-generated acoustic cues and bat feeding sounds (sounds of lip smacking and chewing). Earlier, Page and Ryan [4] had shown that fringe-lipped bats make and reverse novel cue–consequence

Figure 1. Fringe-lipped bats are best known for preying on frogs but also eat a variety of other animal prey. Fringe-lipped bats use male frog calls to locate and identify potential prey. Page and Ryan [3] have demonstrated that these bats learn to associate toad calls with food rewards, behavior that is learned through observation of conspecifics. (Photo courtesy of Sandra Peters.)

associations between the mating songs of anurans (frogs and toads) and their relative profitability as prey. Their new work used this behavioral flexibility in cue–consequence association as a vehicle for training some bats — 'tutors' — to approach speakers playing mating calls of adult male cane toads (*Bufo marinus*) and then receive a nutritious reward. *Bufo marinus* is poisonous and adults are much too large for the bats to eat.

Bats inexperienced with the toad call–profitable resource association were allowed to observe tutors taking pieces of fish placed on a horizontal screen above microphones broadcasting