Features

Breakthroughs and blind ends

Evolution of a through gut with an oral and an anal opening conceivably marked a breakthrough in early animal history, as basal animals have a blind ending gut with only one opening. How this occurred, and whether a through gut evolved only once or several times is a matter of debate. Perhaps the different modes by which guts develop in animal embryos that are used for the classification of animals can shed some light on this question. **Florian Maderspacher** examines.

The grouping of organisms based on shared characters is one of the oldest exercises in biology. Based on sets of shared characters, organisms are categorised into larger systematic units - families, orders, classes and phyla. A great deal of systematic biology is concerned with finding out which criteria are useful for grouping animals and which aren't - why is the number of neck vertebrae a reliable criterion for grouping animals, but body colour isn't? Useful in this sense, the cladistic sense, means that the relationships defined on the basis of shared characters or traits actually reflect an evolutionary relationship of shared descent.

If the relationships inferred by shared characters are to reflect the course of evolutionary diversification, then some of the diagnostic characters that allow classification should also reflect key evolutionary transitions that may have underlied the emergence of new forms. While many other factors, such as geographic isolation and genetic drift, may influence the emergence of new forms, some of these diagnostic characters are expected to shed light on how key adaptations arose.

One such key feature, one that at first sight may seem all too obvious, is the presence of a through gut in nearly all animals that are bilaterally symmetrical, as opposed to animals, such as sponges or corals, that lack a clear bilateral symmetry. Having a through gut confers obvious advantages in terms of food acquisition and processing as it entails the food moving in at the mouth and out at the other end, the anus; it is thus reasonable to assume that the invention of a through gut must have been quite a breakthrough in the evolution of animal body plans.

Bilaterian animals are organised taxonomically depending on the way their guts form during embryonic development. This begs the question of whether this taxonomic criterion actually informs our view of how through guts evolved in the first place.

Phylogenetic blind ends

There are two major divisions in the animal tree of life: animals with a clear bilateral symmetry and an anterior-posterior and dorsal-ventral axis - the bilaterians - are separated from a number of groups that evolved early and lack clear bilateral symmetry - the sponges, comb jellies, Placozoa and cnidarians. The second big division lies within the bilaterian animals, between protostomes and deuterostomes (Figure 1). Deuterostomes are the minority of phyla, comprising as its most prominent representatives the chordates (vertebrates, sea squirts and lancelets), hemichordates and echinoderms (sea urchin, starfish and the like), while all the remaining phyla are protostomes (Figure 2). This distinction, which is taxonomically quite robust - all bilaterians can be safely grouped into one or the other - is based on the embryonic development of a key feature of nearly all bilaterians, that of a through gut. All animals develop from a zygote into an usually hollow ball of cells called the 'blastula'. This ball later folds in at a given site, the so-called blastopore, such that part of the outer layer moves inside the embryo and forms the endodermal tissue layer from which the gut will arise. In 1908, the German zoologist Karl Grobben postulated that there are two principal ways in which the two openings can arise during embryonic development: either the first site of invagination, the blastopore, becomes the anus, or the site of invagination becomes the mouth. Grobben called animals in which the blastopore gives rise to the future mouth 'protostomes' (literally 'old-mouths') and animals in which the blastopore becomes the anus 'deuterostomes' ('new-mouths').

At first sight, this seems an odd criterion for grouping animals: for one, features of the adult body were traditionally used for classification, but in the mid-19th century van Baer, Darwin and others had realised that shared features of embryonic development may sometimes reveal relationships between animal groups, which, in terms of their adult morphology. look unrelated. A classic instance is the case of the musselresembling barnacles, which based on their larval and embryo stages could be identified as relatives of lobsters and shrimp.

One aspect that makes the protostome-deuterostome dichotomy interesting is that, despite its seeming oddity, it is very robust in phylogenetic terms: it indeed holds up well in the light of the molecular data that over the past two decades have been used to infer phylogenetic relationships, in particular the pattern of nucleotide divergence in shared genes. While significant uncertainty remains with regard to the position of individual phyla in the tree of animal life, the deuterostome-protostome divide much like the bilaterian-non-bilaterian divide - is now solidly accepted. The deuterostomes, the dwindling minority of phyla, are a monophyletic group, which by inference means there must have been a single ancestral deuterostome species in the deep evolutionary past from which all living deuterostomes are derived. All animals that are not deuterostomes, the vast majority of animal phyla, are protostomes; and, despite their diversity, molecular phylogeny indicates that they too are a monophyletic group. However, molecular phylogeny also revealed that some animal groups that were traditionally classed as deuterostomes based on the way their body openings form - such as the arrow worms and lophophorates - are actually protostomes.

To complicate things further, in yet other protostomes, mouth and anus form in a completely different way that is neither protostome- nor deuterostome-like, via a process called 'amphistomy'. In this mode, the two openings form simultaneously, as the blastula opening closes in the middle to give rise to both the anus and the mouth. This creates a paradoxical situation: on the one hand protostomes are a real phylogenetic unit, as evidenced by molecular analyses, yet the initial criterion by which they were recognised, development of the mouth from the blastopore, is no longer of value as a defining characteristic; in fact, as of now there is no such shared derived trait that could serve to unequivocally define protostomes. In a sense, they are a 'clade without qualities'. At the same time, the presence of phyla with deuterostome development within the protostomes makes deuterostome development also no longer an exclusive trait of the deuterostomes, although there are other traits, such as presence of gill slits that help reconcile molecular and morphological phylogeny.

Ontogenetic breakthroughs

So, if the protostome-deuterostome distinction is per se not that useful for phylogenetic grouping, why bother? There is a second reason why this criterion is of interest: the origin of a through gut with mouth and anus is an important evolutionary transition that is presumed to have occurred early in animal evolution. Given that the most early evolved animals, such as corals and medusae, have a dead end gut with a single opening, at some point in evolution, guite literally, a breakthrough must have occurred that led to an oral and anal opening. As there are fundamentally different ways to make a gut ontogenetically - the protostome way, the deuterostome way and other ways, such as amphistomy - the question is whether the ancestor of all the bilateral animals had a through gut already or whether through guts evolved independently multiple times. And if this ancestor had a through gut (OK?), how did it make it, the protostome way or the deuterostome way?

Evolutionary biology is in essence a historic science; the evolutionary transitions in question are often buried deep in time, fossil evidence is scarce and critical intermediates may have been lost. Thus, the reconstruction of evolutionary intermediates, such as the ancestor of all bilateral animals, the so-called 'urbilaterian', relies on inferences drawn from existing life forms. Modern comparative molecular





A highly simplified and schematic bilaterian tree: in deuterostomes, (right), the anus forms from the blastopore (red) and the mouth is formed secondarily (blue). In many protostomes (left), the mouth develops from the blastopore (red) and the anus is formed secondarily (blue) (protostomy). However, some protostome taxa show deuterostomy, some develop mouth and anus simultaneously from the blastopore (amphistomy, purple) and some have a blind ending gut. The blastulae shown at the bottom right illustrate the different fates of the blastopore, not actual developmental events.

embryology seems to offer a way out as it not only compares embryonic stages morphologically, but also considers the genes that are used to generate them. It thus rests on a seemingly more solid base, less prone to mistake similarities as indicative of descent when they are similar only by convergent evolution. Gut development is a case in point, as several genes involved in gut development are evolutionarily conserved across animals.

One such gene is brachyury, a transcription factor. At first sight, brachyury expression seemed to fit the predictions of the protostome and deuterostome distinction perfectly: in protostomes, such as flies and nematodes, it is expressed in the hindgut, while in deuterostomes it is expressed in the fore- and hindgut. When, a few years ago, another canonical protostome, an annelid worm, was examined, it was found that brachyury is in fact expressed in both the hind- and foregut (Arendt, D., Technau, U., and Wittbrodt, J., (2001) Evolution of the bilaterian larval foregut. Nature 409, 81-85). Moreover, other genes whose expression marks the developing foregut in deuterostomes are likewise expressed in the foregut of this species. This high degree of similarity between protostomes and deuterostomes in terms of gut gene expression came as a surprise as brachyury expression had not been found in the foreguts of prominent protostome model species, such as fruit flies and nematode worms. But, in many ways, these species may represent a highly derived mode of development and the annelid studied may possess many more of the original developmental features of the urbilaterian.

Given the highly similar molecular architecture of gut development, it seemed unlikely that the guts of protostomes and deuterostomes could have evolved independently. Instead, at the beginning of the success story of bilateral animals, there must have been an ancestor with a through gut. From this ancestral form then, the current forms evolved, and in the course of evolution the ontogeny of the gut changed, leading to the present



Figure 2. Deuterostome and protostome diversity.

From top to bottom: a sea urchin, a typical deuterostome (http://en.wikipedia.org/wiki/ File:SeaDSC01286.JPG); the larva of a lobster, a typical protostome (http://en.wikipedia. org/wiki/File:Homarus_gammarus_zoea.jpg); a chaetognath or arrow worm, a protostome with deuterostome-like development (http:// it.wikipedia.org/wiki/File:MEB_back.png); and a trematode, member of the plathelminths, protostomes that lack a through gut (Photograph by Richard Ling, http://filckr.com/photos/82825649@N00/438039243).

diversity of protostome, deuterostome and intermediate types. Perhaps protostome development was the original mode, as the phylogenetic distribution of the deuterostome type — in some groups phylogenetically defined as protostomes and the classical deuterostomes — seems to suggest that it evolved several times.

But things aren't quite as straightforward. The protostomes are a messy bunch when it comes to gut development, and to complicate things further, there are a number of protostomes that actually do not have a through gut at all. The most prominent of these are the 'flatworms', the platyhelminths, and the question is whether their lack of a through gut is the remnant of an early stage in animal evolution or whether the gut got lost at some point. The former would be more plausible, if indeed the platyhelminths occupied a basal position in the bilaterian animals, which is not clear yet. However, it is clear that the majority of flatworms are protostomes. Thus, their lack of a through gut may indeed represent an evolutionary loss. This is generally somewhat of an old chestnut in evolutionary biology; it is often difficult to decide whether the seemingly primitive features of a group really are the remnants of an earlier evolutionary stage or whether they are the result of a tragic loss of features that occurred later in evolution.

In terms of gut development, there is perhaps a more informative group of animals, the Acoela (see also the Quick quide on accels in this issue). These tiny. worm-like animals, which live in marine habitats, in particular in the spaces between sand grains, also lack a through gut and were thus initially lumped with the platyhelminths. New molecular phylogenies indicate that the acoels branch earlier than all the other bilateral animals and may thus reflect an earlier evolutionary stage. When researchers recently looked at the expression of gut genes in developing acoels, they found that brachyury and other foregut genes are expressed around the future mouth of these animals (Hejnol, A. and Martindale, M.Q. (2008). Acoel development indicates the independent evolution of the bilaterian mouth and anus. Nature 456, 382-386). This can be interpreted as a similarity of the acoel mouth to the mouths of other animals and thus lends credence to the idea that the Acoela might indeed represent the living remnants of a primitive bilaterian ancestor. Interestingly, several known hindgut genes are also expressed in acoels, at the posterior, even though there is no anus. Instead they surround a genital opening. This raises the possibility that a gonadal opening may

have been co-opted as a second gut opening during evolution of a through gut and that this may have occurred several times independently.

Clearly, the jury is still out on whether a through gut evolved once early in the history of bilaterian animals or whether it was invented several times independently along the way. Given the epistemic limitations this kind of question ultimately suffers from - as ancestral stages are irretrievably lost, such analyses are limited to inferences drawn from extant forms - it may even be impossible to answer. But it is also a tale about the relationship between ontogeny and phylogeny, between individual development and evolutionary change, which has had a chequered past in biology. On the one hand, ontogenetic criteria, such as patterns of embryonic development, have been successfully used as criteria for phylogenetic classification and, thus, the inference of evolutionary relationships; but, as the case of protostomy versus deuterostomy shows, such relationships may be deceiving. On the other hand, as some of these criteria may well be indicative of key evolutionary transitions, such as the formation of a through gut, it is all too tempting to use ontogenetic stages to infer what earlier evolutionary stages may have looked like.

This has misled biological thinking - most notoriously perhaps in the case of Ernst Haeckel's famous phylogenetic law, which in its strictest formulation states that stages of ontogeny recapitulate stages of phylogeny and which now has been famously debunked. Modern comparative embryology, which relies on the genes that guide development, offers a way out of this quagmire as long as it avoids directly inferring the patterns of evolutionary transitions from patterns of development. Its success lies in simply providing more data points for comparison than the morphological analyses the likes of Haeckel were confined to. It thus has the potential to provide links indicative of the evolutionary past that the naked eye would miss. So far, only a handful of species has been analysed with regard to the question of the origin of a through gut, and there are many more in the sea - with more breakthroughs and blind ends to be expected.

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