Amphioxus regeneration: evolutionary and biomedical implications

ILDIKÓ M.L. SOMORJAI*1,2

1Biomedical Sciences Research Complex, North Haugh and
2Scottish Oceans Institute, East Sands, University of St Andrews, St Andrews, Scotland, UK

ABSTRACT  Regeneration is a variable trait in chordates, with some species capable of impressive abilities, and others of only wound healing with scarring. Regenerative capacity has been reported in the literature for 5 species from two cephalochordate genera, Branchiostoma and Asymmetron. Its cellular and molecular bases have been studied in some detail in only two species: tail regeneration in the European amphioxus B. lanceolatum; and oral cirrus regeneration in the Asian species B. japonicum. Gene expression analyses of germline formation and posterior elongation in cephalochordate embryos provide some insight into regulation of progenitor and stem cell function. When combined with functional studies of gene function, including overexpression and knockdown, these will open the door to amphioxus as a good model not only for understanding the evolution of regeneration, but also for biomedical purposes.

KEY WORDS: amphioxus, cephalochordate, regeneration, chordate, stem cell

Preamble

When I first began to search for literature describing cephalochordate regeneration a decade ago, I came across Asexual propagation and regeneration (1960), by Vorontsova and Liosner. Chapter XVII was devoted to reparative regeneration in the Echinodermata and the Lower Chordata. On page 283, under the heading “Acrania”, they wrote:

“There are only a few studies devoted to regeneration in amphioxus. Biberhoffer (sic) (1906) discovered regenerative phenomena in the most anterior parts of amphioxus containing portions of the notochord (Fig. 149). Regeneration of the posterior part of the body is doubtful (Probst, 1930)”.

The latter is accompanied by a line drawing, reproduced from Biberhofer (1906) showing at best limited regeneration of the anterior end of an amphioxus. This is a faithful account of the poverty of research into amphioxus regeneration until the end of the 20th century.

Following a brief introduction to the deuterostome regeneration context, I review here the literature on cephalochordate regeneration, as well as recent advances that have informed us on how amphioxus regeneration fits into the wider picture of chordate regeneration and stem cell biology.

The chordate regeneration context

Most organisms are subject to injuries at some stage of their lives that require a physiological and cellular response in order to ensure survival. This can range from daily wear-and-tear associated with normal ageing and cellular turnover, to traumatic events such as amputation. While wound healing and tissue replacement are common, the regrowth of lost body parts has a real energetic cost. The ability to regenerate as a trait is therefore extremely variable in the animal kingdom both in terms of frequency as well as penetrance, depending both on the ontogenetic stage of the organism as well as on the damaged tissue (Bely and Nyberg 2010; Seifert and Voss 2013).

Deuterostomes, which include ambulacrarians (echinoderms + hemichordates) and chordates (Fig. 1), are a good example of the regenerative spectrum. Asteroid echinoderms such as brittlestars can regenerate an entirely new organism from a discarded arm; in contrast, sea urchins have more limited abilities as adults, but...
regenerate well as larvae (reviewed in Carnevali 2006). The hemichordate Ptychodera flava is also emerging as a good system in which to study axial regenerative processes (Luttrel et al., 2016). A similar diversity in regenerative ability is apparent in the chordate lineages (Fig. 1). At one extreme, regeneration of the whole body from small pieces or circulating cells can be achieved, as seen in some colonial ascidians (Voskoboynik et al., 2008). This may be attributable to their use of asexual budding as their primary reproductive strategy (Kürn et al., 2011). Solitary ascidians also regenerate, but comparatively less well (Jeffery 2015). At the other end of the spectrum, many birds and mammals regenerate poorly as adults. For instance, laboratory mice can regenerate their digit tips, but only providing the amputation occurs within the nail bed (Lehoczky et al., 2011) in a process that relies on resident stem cells (Rinkevich et al., 2011). Many salamanders on the other hand can regenerate their limbs, tails, eyes and even jaws (e.g. Henry and Tsonis 2010; Haas and Whited 2017), reflecting the generally high regenerative capacities of anamniotes.

The existence of poor regenerators alongside sometimes closely-related species with more extensive regenerative capacity suggests that as a trait, regeneration may be under selection. For instance, African spiny mice of the Acomys genus are capable of true epimorphic tissue regeneration of ear hole pinnae, an ability lacking in standard laboratory mice (Gawriluk et al., 2011). Many salamanders on the other hand can regenerate their limbs, tails, eyes and even jaws (e.g. Henry and Tsonis 2010; Haas and Whited 2017), reflecting the generally high regenerative capacities of anamniotes.

At a deeper level, comparison of appendage regeneration in lungfish and salamanders suggests that the cellular and molecular mechanisms for appendage regeneration evolved in sarcopterygians (Nogueira et al., 2016). Combined with the observation that endochondral elements regenerate in Polypterus, an Actinopterygian fish, following pectoral fin amputation, this might indicate that regenerative abilities shared by living vertebrate groups arose at the base of bony fish. However, it is also argued that regeneration has evolved de novo in many lineages, particularly in relation to vertebrate appendages (Brockes and Kumar 2008; Slack 2017), underscoring the complexity of the problem.

In order to address the question of whether regenerative ability is ancestral, or rather independently derived, a good comparative framework assessing homologous structures is needed. Given that urochordates are morphologically, developmentally and genomically derived relative to vertebrates, cephalochordates (commonly referred to as “amphioxus” or “lancelets”) represent the best system in which to assess the presence of any shared regenerative mechanisms that might have existed in the chordate ancestor (Fig. 1A, B). In particular, if true limbs are only shared among tetrapods,
the post-anal tail in contrast is particularly suited for comparative studies of complex regeneration between vertebrates and amphioxus. It consists of several defining chordate characters, including notochord, a dorsal hollow nerve cord and segmented musculature (Fig. 1B). In addition, lancelets diverged prior to the two whole duplication events characteristic of vertebrates (Fig. 1A, "2R"), and have remarkably well conserved gene order (Putnam et al., 2008). The availability of genomic and transcriptomic resources for a number of species, coupled with the ability to perform functional studies in embryos (reviewed in Kozmikova and Kozmik 2015; Fig. 1C and associated references), set the stage for a new molecular era in cephalochordate regeneration studies.

Regenerative ability in cephalochordates

The first studies of regeneration in cephalochordates assessed regenerative ability primarily in the European species, Branchiostoma lanceolatum (Bert 1867; Nusbaum 1905; Biberhofer 1906; Probst 1930). Bert (1867) and then Nusbaum (1905) considered this species devoid of regenerative ability, stating that amputation of the tail resulted in the wound taking on a rosy colour (presumably the "red disease"), followed by gradual disintegration of the animal (Bert 1867). Franz (1925) observed that two amphioxus bisected through the atrium upon collection healed, but had not regenerated 8 weeks post-trauma. Neither Biberhofer (1906), nor Probst (1930) had much better luck with adult animals; young juveniles (6-9mm) showed some caudal regeneration after 13 days, but died within three weeks (Probst 1930).

The broadly negative reports in Branchiostoma around the turn of the 20th century contrast with Andrews' (1993) observations of Asymmetron lucayanum when he first described the species. He documents the caudal regenerative ability in this species, indicating a full recovery of all structures including the notochord and nerve cord (Andrews 1893). Direct or indirect evidence of posterior regeneration in Branchiostoma has also been shown in recent years in B. lanceolatum (Bone 1992; Pegeta 1992; Somorjai et al., 2012a), B. platae (Silva et al., 1998), B. japonicum (previously conflated with B. belcheri) and B. belcheri (Zhang et al., 2009). Zhang and colleagues (2009) use posterior blastemas in lieu of embryos to successfully make metaphase spreads for chromosome counts, but otherwise do not discuss regeneration in these species. In contrast, the recent, more detailed analyses of B. lanceolatum show that it has considerable tail regeneration capacity (Bone 1992; Pegeta 1992; Somorjai et al., 2012a), even after multiple rounds of amputation (Somorjai et al., 2012b). Interestingly, Bone (1992) states that animals allowed to bury in the gravel or maintained in the dark do not regenerate, in stark contrast to the successful regenerates observed by Pegeta (1992) and Somorjai et al., 2012a, b) under the aforementioned conditions. Together, the studies of caudal regeneration highlight anterior-posterior site of amputation, age, size, nutritional and disease status as important factors in the success and speed of the regenerative response.

Anterior regeneration is less well studied (Fig. 1C), but appears to be much more limited. Of the small B. lanceolatum (2.3 cm) amputated anteriorly, wound healing was observed in only a single small adult lancelet, and none at all in larger (2.8 cm) animals (Biberhofer 1906). Recent work confirms that only extreme anterior axial amputations result in any appreciable regeneration, even under prime conditions that promote complete tail regeneration (Somorjai et al., 2012a), suggesting this is not an artefact of environmental or physiological conditions. Exceptions to this are the oral cirri, the non-mineralised skeletal rods surrounding the mouth opening, which regenerate well in both B. japonicum and B. lanceolatum (Kaneto and Wada 2011; Somorjai et al., 2012a). Data are lacking on anterior regeneration outside Branchiostoma, and no regenerative abilities have been reported for the third cephalochordate genus, Epigonichthys. Thus, of the approximately 30 currently recognised species of cephalochordate (Poss and Boschung 1996), adult regenerative ability has so far been reported in 5 species from two genera, although comparative data indicate that B. floridae regenerates similarly well to B. lanceolatum (Somorjai, manuscript in preparation). Taken together, these studies suggest that the cephalochordate ancestor would have been able to regenerate the post-anal tail to a respectable degree, but most likely had limited anterior regeneration capacity.

The regenerative process

Tail regeneration

The cellular events occurring during regeneration have been described in most detail during post-anal tail regeneration in the European species, B. lanceolatum (Somorjai et al., 2012a). After posterior amputation, humoral fluids are released, and cellular debris around the amputation plane, including fragments of transected muscle fibres, are sloughed away (stage 0). Wound healing immediately follows (stage 1), with epidermal cells closing over the stump. This is occasionally accompanied by formation of haematoma-like swelling, which normally regresses. The timing of these events is variable, taking 1-2 days in adult animals in a size- and age-dependent manner (Somorjai et al., 2012a). In B. platae, wounds caused by sectioning the posterior third of the animal healed within 12h (Silva et al., 1995), and necrotic muscle cells were observed beneath the newly formed epithelium. However, the authors report that this was not accompanied by phagocytosis, although elongated endothelial-like cells appeared within two weeks, embedded in connective tissue around an implanted silk thread (Silva et al., 1995).

In the second phase (stage 2), a clear bud or "blastema" forms, a process that takes approximately two weeks, and a predictive cellular bulge may be evident already after one week (Somorjai et al., 2012a). Using EM, Silva and colleagues (1998) report that during posterior regeneration in B. platae, the basal lamina under the bud is interrupted, and endothelial-like cells rest directly on a bed of disorganised collagen fibres. In B. lanceolatum, by 14 days post-amputation (dpa), the posterior tip of the nerve cord exhibits a swelling contiguous with the expanded lumen of the regenerating ependymal tube. Transverse muscle fibres, easily identified by F-Actin staining, are clearly dissociated from the damaged myosepta and the notochord begins to lose its characteristic "stack of coins" organisation, leaving a large space filled with amorphous cellular material anterior to the amputation plane. The notochord blastema in contrast is densely filled with cells (Somorjai et al., 2012a; Fig. 2A).

Within 3 weeks, the tail has begun its outgrowth and the nerve cord can clearly be seen to elongate, maintaining its ependymal tube shape and swollen terminal ampulla (stage 3; Fig. 3C). It has the undifferentiated appearance of the caudal nerve cord in unamputated adults, and lacks anatomically definable photoreceptors
we have observed in regenerating notochords of ageing animals (Somorjai et al., 2012a). Detailed histology on sections, and in a larger number of animals at precise regeneration stages will be instrumental in assessing the cellular composition and contributions to cirrus regeneration.

Molecular basis of regeneration

Tail regeneration

Two studies have addressed the molecular basis of posterior regeneration in amphioxus, primarily using immunohistochemistry and candidate gene expression analysis (Somorjai et al., 2012a,b). Members of the developmental signalling pathways Wnt and BMP are expressed in B. lanceolatum in domains consistent with roles in specification and patterning of the blastema. First, Wnt5 is expressed throughout the blastema in cells that are also enriched for β-catenin protein at the membranes (Somorjai et al., 2012b). The absence of β-catenin in the nucleus either suggests that Wnt plays a role in modulating adhesion of blastema cells at this stage, or that the nuclear β-catenin antibody epitope is masked. Second, the stage 2 blastema expresses msox, a downstream target of BMP often expressed in other regenerating systems (Somorjai et al., 2012a). During the elongation phase, the BMP antagonist...
chordin is expressed in new notochord cells, suggesting a role in differentiation. The neural differentiation marker soxB2, the orthologue of vertebrate sox17/21, is also expressed in the regenerating ependymal tube (Somorjai et al., 2012a).

A third study has recently taken a global look at tail regeneration by analysing transcriptomes of the blastema and the unamputated tail (Dailey 2017). As expected, all previously identified players using the candidate approach were recovered (Dailey 2017). A large number of additional genes were also identified from the Wnt, BMP, Notch, FGF and RTK signalling pathways, as well as germine and pluripotency factors (Dailey 2017; see below), many of which have neither been characterised during normal development nor regeneration. However, one of the identified blastema transcripts, Sp5, is expressed in the amphioxus posterior growth zone and is a target of Wnt/β-catenin signalling (Dailey et al., in press). Greater sequencing depth using Illumina in more replicates and at additional regeneration stages are currently underway (Dailey and Somorjai, unpublished). Nevertheless, this initial pilot study has opened up new avenues for understanding the molecular basis of tail regeneration in amphioxus, and provides a comparative framework for identifying processes shared with both ambulacrarians and vertebrates.

**Cirrus regeneration**

Oral cirrus regeneration has been used as a model for understanding the evolution of skeletogenesis (Kaneto and Wada 2011). Markers for a number of genes implicated in vertebrate chordogenesis and osteogenesis including collagens FCol1 and FCol2, SPARC, soxE and runx were expressed specifically in the tips of the regenerating oral cirri. A number of these also mark the tailbud, a possible posterior stem cell (PSC) pool (grey box). Other markers may also play a role in the germline based on expression in B. floridae (Bertrand et al., 2013). Combined with expression data in other chordates, this leads the authors to suggest that co-expression of SPARC-like and Collagen proteins in mesenchymal cells was one of the key steps to skeletal evolution (Bertrand et al., 2013). Indeed, a SPARC gene and runx2 are expressed in prospective sclerotoblast progenitors during scale regeneration in the goldfish, a collagenous dermal skeleton component (limura et al., 2012). It will be interesting to determine whether or not any of these SPARC and runx genes are expressed during tail regeneration, most specifically in notochord precursors.

Axial regeneration likely occurs via an epimorphic process in amphioxus, as blastema cells show considerable proliferation by phospho-histone H3 immunostaining, as does the extending nerve cord (Somorjai et al., 2012a; Fig. 2B). The latter further demonstrates that the nerve cord undergoes active neurogenesis during regeneration. Interestingly the extent of this proliferative activity is significantly lower in older animals, and may account for their reduced capacity to regenerate a complete tail. In contrast to tail regeneration, Kaneto and Wada (2011) report that regeneration of the oral cirri occurs via tissue remodelling, as no increase in proliferation was observed. However, it cannot

![Fig. 3. Evidence for amphioxus stem cell populations. (A-C), nanos transcripts are segregated to the vegetal pole in eggs (A), and inherited by one to a few cells (magenta arrowheads) by PGCs (primitive germ cells) from morula (B) to gastrula (C) stages in cephalochordate embryos (here B. lanceolatum). (D) These cells also express Vasa protein (white arrowheads), as does the posterior neural tube (cyan arrows) at mid-neurula stages [magnification in (E)], as seen by confocal microscopy. (F) Surviving twin of pair of 2-day larvae resulting from dissociation experiment at the 2-cell stage. Note morphological abnormalities in elongation and head/oral structures. (G) Summary of proposed core molecular markers (transcripts and protein) asymetrically labelling PGCs from studies in four cephalochordate species (Blan: B. lanceolatum; Bbel: B. belcheri; Bjap: B. japonicum; Bflo: B. floridae). A number of these also mark the tailbud, a possible posterior stem cell (PSC) pool (grey box). Other markers may also play a role in the germline based on expression in B. floridae and in silico prediction in A. lucayanum (magenta box). The animal-vegetal (an-ve), Anterior-Posterior (A-P) and Dorso-Ventral (D-V) axes are indicated in each panel where appropriate. Cyan: Vasa; yellow: Factin; magenta: DAPI labelled nuclei. ec: ectoderm; me: mesendoderm; *: early blastopore; nt: neural tube; no: notochord; ps: pigment spot. Scale, 50 μm.
be excluded that progenitors contributing to the regenerate may have migrated from elsewhere, or that there may exist different populations of slow or fast cycling stem cells. BrdU pulse-chase labelling may give additional insight into proliferation kinetics. Altogether, these studies support data in other systems suggesting that proliferation-dependent and -independent mechanisms of regeneration may occur in different tissues in the same organism.

Evidence for amphioxus stem cells

Primordial germ cells

Germ cells, by virtue of being able to generate a whole new organism in the next generation, and their close affinity with embryonic stem cells, may be considered stem cells par excellence. In metazoans, two different mechanisms of germine specification have been described: inductive and determined (or "preformation"). In the inductive mechanism, germ cells are specified by signalling, usually relatively late during embryogenesis, while in the determined mode, segregation of germ plasm already in the oocyte determines germ cell fate (Whittle and Extavour 2017).

Although cephalochordates are generally reported as using an inductive mode, recent data in four amphioxus species (all Branchiostoma) suggest rather an inherited mechanism for primordial germ cell (PGC) formation (Wu et al., 2011; Zhang et al., 2013; Dailey et al., 2016). At the two-cell stage, a number of highly conserved germline transcripts including nanos (Fig. 3A-C) and vasa- as well as Vasa protein- are asymmetrically distributed into a single cell, and inherited by few progeny. At the late gastrula stage, up to 8 Vasa+ cells can be identified in B. belcheri (Wu et al., 2011). In neurula stages, these PGCs are nestled posteriorly in endoderm of the tailbud (Fig. 3D, E), and remain as a cluster near the anus in premouth larval stages in all species examined (Wu et al., 2011; Zhang et al., 2013; Dailey et al., 2016).

Given the remarkable conservation of developmental gene expression in Branchiostoma (Somorjai et al., 2008; Yong et al., 2017), comparisons across species, including in silico predictions in Asymmetron lucayanum (Yue et al., 2015) allow a tentative reconstruction of a core set of germine-associated genes in cephalochordates (Fig. 3G). This likely includes nanos, piwi1, vasa, bruno2 and pi10 and a number of Tudor-related genes, which are asymmetrically localised to the vegetal cortex. However, it may also include PUF-domain containing genes, mago nashi or maelstrom, which are more broadly expressed (Yue et al., 2015); these may play roles in germine maintenance or differentiation.

As marker expression is independent of the germine specification mechanism utilised (i.e. nanos, see Fresques et al., 2016), we cannot currently exclude the possibility that other cephalochordate genera utilise non-determinative mechanisms (eg Asymmetron), or that inductive mechanisms may not operate under particular conditions. For instance, removal of PGCs at late stages may permit inductive mechanisms to re-specify germ cells, as seen in the solitary ascidian Ciona intestinalis when the larval tail is amputated (reviewed in Kawamura et al., 2011). However, at least in the earliest cleavage stages, inductive mechanisms do not appear to be operating. Recent blastomere dissociation experiments at the two-cell stage, similar to those performed by Tung (reviewed in Yan 1999), suggest that while morphologically broadly normal twins can be recovered (Wu et al., 2011 and see Fig. 3F), one likely lacks germline as assessed by germ marker expression, and the posterior is malformed (Wu et al., 2011). Taken together, studies of PGC determination in amphioxus such as these will begin to shed light on the nature of stem cell regulation in this taxon.

Somatic progenitors

Reports in a number of animals suggest that the germ-soma divide is not strict, and that mechanisms used to maintain the PGCs/germline may also be utilised in somatic stem cells, supporting the existence of an ancestral multipotency programme already in the last common ancestor of metazoans (Juliano et al., 2010; Fierro-Constain et al., 2017). Thus, many "germline"-associated genes are also expressed in pluripotent somatic progenitors, and can be re-activated during regenerative processes.

In amphioxus, comparative studies suggest the existence of a posterior progenitor/stem cell pool located in the larval tailbud, the growth zone responsible for posterior elongation. Both transcripts and protein of one of the key germine determinants in the PGCs, vasa, localise to this domain, as do nanos and piwi1 and piwi2 (Wu et al., 2011; Zhang et al., 2013; Dailey et al., 2016). Vasa plays a general role in regulating translation in the soma (Poone et al., 2016) and nanos2 has recently been shown to transiently repress translation in PGCs via inhibition of eIF1a in sea urchins (Ouhlen et al., 2017). Thus, we might expect expression of such genes in the adult regenerating tail. Indeed, transcriptomic data from the blastema suggest that "germline" genes are in fact acting as general "stemness factors" in amphioxus (Dailey 2017).

While some data exist in adult amphioxus concerning signalling pathways expressed during regeneration (outlined above), the contribution of stem cells to the process is poorly understood due to current technical limitations. A single study in B. lanceolatum has identified a pool of dividing progenitors positive for Pax3/7 in and around the tail blastema, which appear to decline during differentiation and ageing (Somorjai et al., 2012; Fig. 2B). Pax3/7 expressing muscle satellite-like cells with a role in regeneration have also been identified in Parhyale hawaiensis, a crustacean, suggesting that shared mechanisms for muscle regeneration existed in the common ancestor of bilaterians (Konstainides and Averof 2014). However, the contribution of such Pax3/7+ cells to the amphioxus regenerate -and whether or not the mechanisms employed in amphioxus and vertebrates are indeed homologous-requires further study. Recent research in two salamanders, the axolotl and the newt, suggests that muscle dedifferentiation and satellite cell contributions may differ across species (Sandoval-Guzman et al., 2014). However, the differences observed between the two could also reflect changes in cellular mechanisms and resident stem cell activation during ontogeny (Tanaka et al., 2016). Either way, these have important implications: the first suggests the evolution of an alternate strategy to achieve the same regenerative outcome within urodèles, while the latter has a clear impact on our approach to regenerative medicine. Understanding how amphioxus regenerates muscle may help polarise our views on ancestral mechanisms in chordates.

Outlook

Regeneration and stem cell research in cephalochordates are still at an embryonic stage. We know next to nothing about how different resident progenitor populations contribute to the new regenerate or the cellular mechanisms involved. However, an
understanding of how amphioxus fits into the broader spectrum of animal regeneration generally, and chordate and vertebrate regeneration more specifically, contributes to our understanding of the diversity of mechanisms utilised by animals to repair injuries and replace lost tissues. This in turn has potential biomedical implications: the blastema is effectively a tumour, but undergoing controlled growth, differentiation and patterning. Insight gained into how complex tissue regeneration is regulated in adult amphioxus, and how this potential declines with age, may help inform us about degenerative disease and ageing-associated stem cell misregulation in humans.

Based on studies in other chordates, one can make a number of predictions about amphioxus tail regeneration. As demonstrated in amphibians (Gargioli and Slack 2004; Kragl et al., 2009), notochord, muscle and nerve cord lineages are likely defined early in amphioxus, with little to no lineage switching. However, additional distant sources of progenitors may also contribute to the tail blastema in cephalochordates. For instance, branchial sac stem cells in the solitary ascidian Ciona intestinalis contribute to distal regeneration (Jeffery 2015). Moreover, in some colonial ascidians, small pieces of vessel containing blood cells are sufficient to regenerate an entire organism (Rinkevich et al., 1995), and the endostyle, homologue of the vertebrate thyroid gland, acts as a stem cell niche (Voskoboynik et al., 2008). Although circulating cells are lacking in cephalochordates, amphioxus blood vessels do contain coelomocytes or amoebocytes with some migratory and endocytic capabilities (Rhodes et al., 1982; Monahan-Earley et al., 2013). Further, amphioxus have a well-developed endostyle within the floor of the branchial basket, which could act as a niche due to its close apposition to the circulatory system there, in addition to more local signals originating from the wound epithelium to the underlying tail blastemal cells. The embryonic tailbud is a source of numerous signals in amphioxus (i.e. Wnt, BMP etc.; Bertrand et al., in press) that might regulate proliferation/differentiation of the posterior stem cell pool, similarly to what occurs during posterior regenerative outgrowth. Regeneration and developmental research in amphioxus will therefore have to progress side-by-side within a comparative framework; highlighting differences is as important as identifying commonalities among systems. Ultimately, research in amphioxus, an invertebrate with conserved chordate anatomy and simpler genome, provides an attractive foil and complement to regeneration and stem cell studies in vertebrates.

Acknowledgements
I would like to thank Nicholas Holland for 10 o’clock espresso breaks at the kiosk, Russian translations, and fine wine; Linda Holland for tips on amphioxus care and numerous suppers; and reviewers for constructive criticism that improved the manuscript. Experimental work in my lab is currently funded by the Wellcome Trust ISSF grant 204821/Z/16/Z, the European Union Horizon 2020 research and innovation programme under grant agreement numbers 654428 (“CORBEL”) and 730984 (“ASSEMBLE+”) and the RS MacDonald Charitable Trust. I apologise to the many authors whose primary literature could not be cited due to space restrictions.

References
DAILEY SC, KOZMIKOVA I, SOMORJAI I (2017). Amphioxus Sp5 is a conserved Specificity Protein complement and is modulated by Wnt/b-catenin signalling (DOI: 10.1387/ijdb.1702056b).
Further Related Reading, published previously in the *Int. J. Dev. Biol.*

**Planaria: an animal model that integrates development, regeneration and pharmacology**
Oné R. Pagán
*Int. J. Dev. Biol.* (2017) 61: 519-529

**Reptile genomes open the frontier for comparative analysis of amniote development and regeneration**
Marc Tollis, Elizabeth D. Hutchins and Kenro Kusumi
*Int. J. Dev. Biol.* (2014) 58: 863-871

**Regeneration in spiralians: evolutionary patterns and developmental processes**
Alexandra E. Bely, Eduardo E. Zattara and James M. Sikes
*Int. J. Dev. Biol.* (2014) 58: 623-634

**From the American to the European amphioxus: towards experimental Evo-Devo at the origin of chordates**
Jordi Garcia-Fernández, Senda Jiménez-Delgado, Juan Pascual-Anaya, Ignacio Maeso, Manuel Irinia, Carolina Minguillón, Elia Benito-Gutiérrez, Josep Gardenyes, Stéphanie Bertrand and Salvatore D’Aniello
*Int. J. Dev. Biol.* (2009) 53: 1359-1366
https://doi.org/10.1387/ijdb.072436jg

**The germ line and somatic stem cell gene Cniwi in the jellyfish Podocoryne carnea.**
Katja Seipel, Nathalie Yanze and Volker Schmid

**FGF signalling and blastema growth during amphibian tail regeneration**
P Ferretti, F Zhang, L Santos-Ruíz, JDW Clarke
*Int. J. Dev. Biol.* (2001) 45: S127-S128

5 yr ISI Impact Factor (2013) = 2.879