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SPOTLIGHT

Unravelling spiral cleavage

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ABSTRACT

Snails, earthworms and flatworms are remarkably different animals, but they all exhibit a very similar mode of early embryogenesis: spiral cleavage. This is one of the most widespread developmental programs in animals, probably ancestral to almost half of the animal phyla, and therefore its study is essential for understanding animal development and evolution. However, our knowledge of spiral cleavage is still in its infancy. Recent technical and conceptual advances, such as the establishment of genome editing and improved phylogenetic resolution, are paving the way for a fresher and deeper look into this fascinating early cleavage mode.

KEY WORDS: Cell fates, Cell lineages, Developmental plasticity, Emerging model systems, Phylogeny, Spiral cleavage

Introduction

Spiral cleavage is a distinctive early developmental program displayed by at least eight major animal groups, including annelids (i.e. segmented worms), molluscs (e.g. snails), nemerteans (i.e. ribbon worms) and platyhelminths (i.e. flatworms) (Hejnol, 2010; Henry, 2014; Lambert, 2010) (Fig. 1A). Often mistakenly regarded as the typical cleavage pattern of protostomian lineages, spiral cleavage is instead unique to and probably a synapomorphy (ancestral characteristic) of Spiralia (i.e. Lophotrochozoa *sensu lato*, see below) (Halanych et al., 1995; Giribet, 2008). Spiralia are a morphologically and ecologically diverse group comprising around 10% of the known animal species (Brusca et al., 2016). From a developmental perspective, spiral cleavage is characterised by a 45° shift in the mitotic spindle with respect to the animal-vegetal axis in the transition from the four- to the eight-cell stage (Fig. 1B,C), yet the chirality of this shift might be determined already in the zygote (Meshcheryakov and Beloussov, 1975; Abe and Kuroda, 2019). This shift persists in subsequent divisions, each time alternating directions, either dextrally or sinistrally. Eventually, this results in the cells located at the animal pole of the embryo displaying a compact, spiral-like arrangement, hence the name of the cleavage program (Fig. 1D). In the 19th century, the study of spiral cleavage boosted the study of embryonic cell lineages and supported the use of embryonic data to reconstruct animal relationships (Wilson, 1898; Guralnick, 2002; Maienschein, 1990). The emergence of prominent invertebrate model systems that are not spiral cleavers (such as the fruit fly *Drosophila melanogaster* or the nematode *Caenorhabditis elegans*, both belonging to Ecdysozoa: the moulting animals) meant that the study of spiral cleavage fell behind, and ultimately become one of the most under-investigated, yet widespread, developmental strategies in animals. However, we know today that Ecdysozoa has undergone extensive loss of characteristics (e.g. ciliated epithelia, many gene families, introns) that are preserved between Spiralia and Deuterostomia (Luo et al., 2018; Wang et al., 2017; Roy and Irimia, 2008) – the third major clade of bilaterally symmetrical animals to which vertebrates and humans belong. Therefore, spiralians are important organisms, not only because their unique spiral cleavage enables them to be used to tackle fundamental questions in developmental biology, but also because their phylogenetic position provides a unique window on the bilaterian ancestry.

This Spotlight aims to briefly capture the resurgence that the study of spiral cleavage is experiencing in recent years. Plummeting sequencing prices together with the establishment of molecular and functional experimental approaches in a growing number of species (Neal et al., 2019; Perry and Henry, 2015; Abe and Kuroda, 2019; Zantke et al., 2014) are taking the study of this early developmental program out of its ostracism. We begin with a general overview of the phylogeny of Spiralia and of the species emerging as laboratory research systems, followed by a discussion of some of the features of spiral cleavage that make it uniquely suited to study fundamental questions in developmental biology. We end with a personal perspective on where the study of spiral cleavage and spiralians generally should move to, and what we believe is needed to keep bringing spiralians to the forefront of embryological and evolutionary research.

Spiralian phylogeny: new certainties and lingering doubts

Thirty years ago, the advent of molecular phylogenetics progressively established the subdivision of bilaterally symmetrical animals (Bilateria) into three main superclades: Deuterostomia, Ecdysozoa and Lophotrochozoa (Field et al., 1988; Halanych et al., 1995). By bringing together disparate animal groups such as flatworms, annelids and molluscs, molecular data confirmed what embryologists had suspected for a long time based on the shared presence of spiral cleavage (Schleip, 1929). However, Lophotrochozoa was originally defined as the clade containing all descendants of the last common ancestor of animals with a lophophore (horseshoe-shaped band of ciliated tentacles around the mouth) and/or trochophore (ciliated planktonic larva), without specifying the lineages of these descendants or their relationships in detail (Halanych et al., 1995). The possibility that groups such as Platyhelminthes and Rotifera diverged prior to the ancestor of lophophorates and trochozoans prompted some authors to refer instead to the third domain of Bilateria as Spiralia, and to consider Lophotrochozoa only as its subclade (Giribet, 2008).

At the origin of this naming debate lies the difficulty in accurately reconstructing the internal relationships of Spiralia and the lack of embryological knowledge for many of the more obscure spiralian lineages. Groups such as Platyhelminthes, Gastrotricha and Gnathostomulida have some of the fastest rates of molecular evolution among animals, which causes phylogenetic reconstruction artefacts, such as long branch attraction, that impede the inference of a proper spiralian tree. Recently, several studies have attempted to tackle these issues by expanding the taxon and character repertoire, and more

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Development (2020) 147, dev181081. doi:10.1242/dev.181081

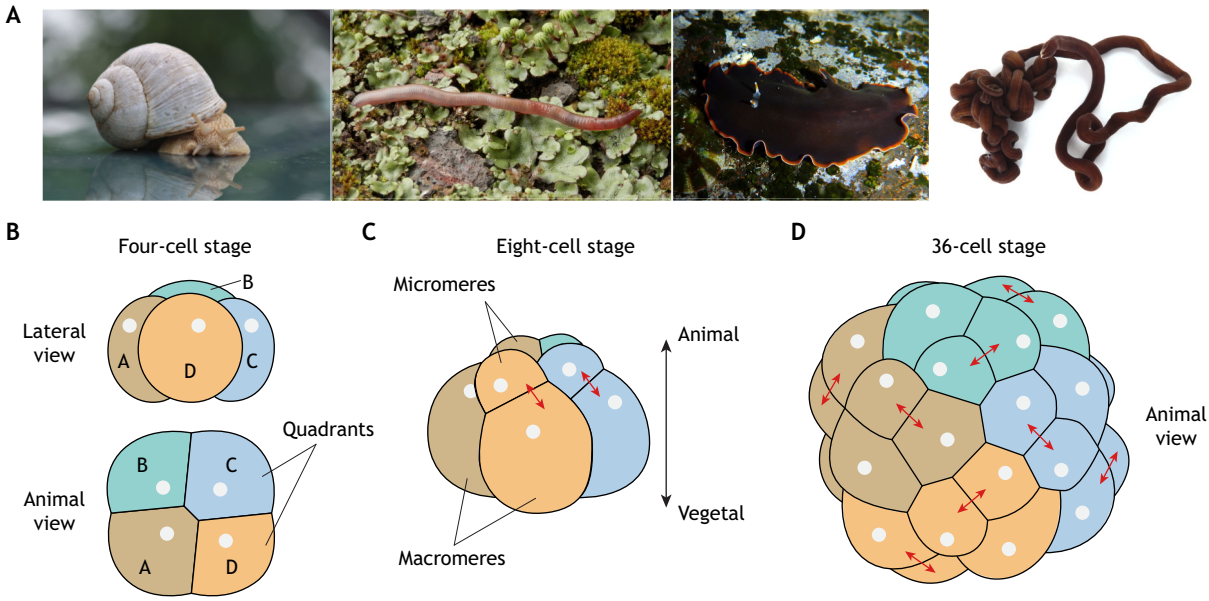

Fig. 1. Spiralians and spiral cleavage. (A) Representatives of the major clades exhibiting spiral cleavage. From left to right, snail (Mollusca; picture by Beocheck), earthworm (Annelida; picture by Ryan Hodnett), flatworm (Platyhelminthes; picture by Sébastien Vasquez) and ribbon worm (Nemertea; picture by Bruno C. Vellutini). (B) Schematic representation of a four-cell stage spiral cleaving embryo, depicting the four embryonic quadrants indicated by the letters A to D. (C) Schematic representation of an eight-cell stage spiral cleaving embryo, showing the small animal micromeres, the larger vegetal macromeres and the direction perpendicular to cleavage (red arrows) shifted ∼45° with respect to the animal-vegetal axis. (D) Schematic drawing of a 36-cell stage spiral cleaving nemertean embryo from an animal view, illustrating the spiral-like arrangement of the micromeres and their cleavage planes. Drawing adapted from Maslakova et al. (2004). In B-D, drawings are not to scale; cell colours in C and D corresponds to the quadrants in B.

importantly by using better inference methods (Marlétaz et al., 2019; Laumer et al., 2019; Kocot et al., 2017), such as the CAT model, in particular, which defines evolutionary profiles and captures the diversity of composition and substitution processes among codon sites (Rodrigue and Lartillot, 2014). These studies have uncovered a new animal clade (‘Gnathifera’) within Spiralia, uniting the enigmatic chaetognaths, rotifers and other neglected lineages, such as gnathostomulids and micrognathozoa (Fig. 2A). Sister-group to the other spiralians, the clade Gnathifera (jaw-bearers) refers to the complex jaw apparatus present in these groups. Strikingly, only Gnathostomulida (i.e. jaw worms) exhibit spiral cleavage within this group (Riedl, 1969), and this needs to be taken with caution, as the single original description of spiral cleavage in these organisms has not been reassessed using more modern methods. If confirmed, this will be of uttermost importance, as it will support the consideration of spiral cleavage as a synapomorphy to the entire clade, and thus the use of the name Spiralia.

Other areas of the Spiralia phylogenetic tree, however, remain strongly disputed depending on methods used in distinct studies. First, the association of Mollusca, the most diverse spiralian group, with Entoprocta (a small clade of mostly sessile and colonial marine animals) in ‘Tetraneuralia’ is only recovered in studies without the fastest evolving taxa (Marlétaz et al., 2019) (Fig. 2A). Similarly, an unexpected association of Platyhelminthes, Nemertea and Annelida (Fig. 2A) contrasts with the association of Platyhelminthes with Gastrotricha (‘Rouphozoa’, Fig. 2B) when more inclusive datasets are used (Laumer et al., 2019). Attempts to resolve these disputes using dataset recoding, whereby individual amino acids are fused into broad biochemical categories, have proven controversial (Hernandez and Ryan, 2019 preprint), but nevertheless have not significantly changed the results reported in the most recent studies (Marlétaz et al., 2019; Laumer et al., 2019). Despite all these uncertainties, all current phylogenies support the belief that spiral cleavage is at least ancestral to the sister clade to Gnathifera, and tell an intricate story of repeated losses of spiral cleavage (Fig. 2A,B; discussed below). This broad phylogenetic framework offers a unique opportunity for exploring the extent to which a cleavage program present in disparate animals that have diverged over millions of years has remained conserved at different levels of biological complexity. Moreover, it also reveals several exciting cases of transition from spiral to radial cleavage, ideal cases with which to explore the relationship between early division patterns, cell lineages and fate specification gene networks.

Spiralian research systems

Spiral cleavage has been studied in a myriad of species, yet in most of the cases the studies are limited to a basic description of the cell lineage. Compared with other areas of biosciences and developmental biology, where a handful of species have become pillars for experimental research (e.g. in vertebrates, arthropods and nematodes), this can be unsettling. This diversity has come with advantages and disadvantages, and it is probably related to the large number of major animal groups exhibiting spiral cleavage, each with distinctive body plans and evolutionary histories that make them fascinating on their own. At the methodological level, the annelid worms *Platynereis dumerilii*, *Capitella teleta* and *Helobdella robusta*, as well as the gastropod mollusc *Crepidula fornicata* are arguably the most settled spiralian research systems (Henry, 2014), with established modern functional (e.g. CRISPR and transgenesis) and imaging approaches (Neal et al., 2019; Perry and Henry, 2015; Zantke et al., 2014; Gline et al., 2011; Song et al., 2002; Weisblat and Kuo, 2014) (Table 1). However, a broad range of other spiralian species have been or are being used to study spiral cleavage employing molecular approaches, including – but not

Development (2020) 147, dev181081. doi:10.1242/dev.181081

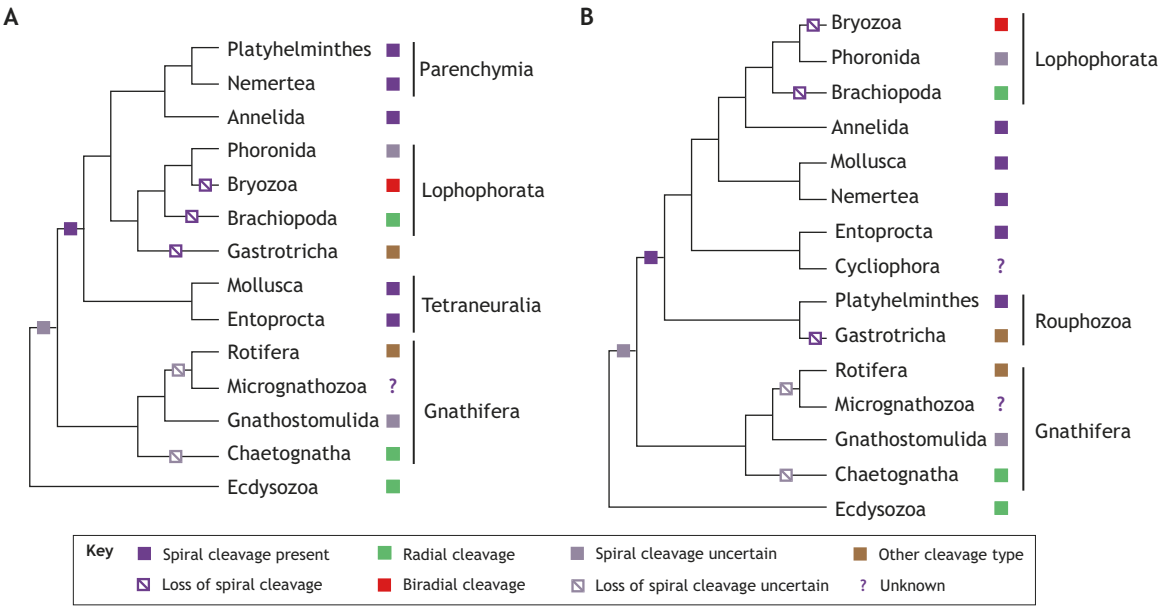

Fig. 2. Spiralian phylogeny. (A) Spiralian topology (based on Marletaz et al., 2019), with a Gnathifera clade including Chaetognatha as a sister to the remaining spiralians, which itself comprises three major clades: Tetraneuralia, Lophophorata and Parenchymia. (B) Spiralian topology (based on Laumer et al., 2019), with Gnathifera also comprising Chaetognatha, but Platyhelminthes branching off together with Gastrotricha in the clade Rouphozoa, intermediate to Gnathifera and the remaining spiralians.

limited to – the annelids *Owenia fusiformis* and *Streblospio benedicti* (Zakas et al., 2018; Martín-Durán et al., 2018); the molluscs *Tritia* (also known as *Ilyanassa*) *obsoleta*, *Biomphalaria glabrata*, *Patella vulgata*, *Lymnaea stagnalis*, *Antalis entalis* and *Acanthochitona crinita* (Wanninger and Wollesen, 2018; Abe and Kuroda, 2019; Lambert and Nagy, 2001; Grande and Patel, 2009; Damen and Dictus, 1994); the nemerteans *Cerebratulus lacteus*, *Lineus ruber* and *Micrura alaskensis* (Martín-Durán et al., 2018; Hiebert and Maslakova, 2015; Henry et al., 2008); the flatworm *Prostheceraeus crozieri* (Girstmair and Telford, 2019); and other spiralian species that have secondarily lost spiral cleavage, such as cephalopod molluscs (Tarazona et al., 2019), the bryozoan *Membranipora membranacea* (Vellutini et al., 2017), and the brachiopods *Terebratalia transversa* and *Novocrania anomala* (Martín-Durán et al., 2016). This combination of established and emerging research systems covering most major lineages of Spiralia is bringing a more comprehensive understanding of spiral cleavage, the plasticity and regularities of this mode of development, and the mechanisms that generate a vast diversity of morphological outcomes from a widely shared embryonic program. However, it also implies that research communities working on a given species are generally small. Therefore, raising some of these organisms to an experimental level comparable with other established research systems outside Spiralia is taking time.

How can spiral cleavage contribute to modern developmental biology?

Its broad phylogenetic distribution among vastly diverse animal lineages (Fig. 2) together with its likely common origin and overall conservation make spiral cleavage a unique developmental program in animals. Several studies have already demonstrated the importance of studying spiral cleavage to infer ancestral developmental characters to bilaterally symmetrical animals (Martín-Durán et al., 2018, 2016; Grande and Patel, 2009; Henry et al., 2008). Probably, the best example is that of the transforming growth factor β (TGFβ) ligand *Nodal*, which controls left-right (LR) axis specification and mesodermal patterning in echinoderms and chordates (i.e. Deuterostomia), and was long considered to be a deuterostomian innovation due to its absence in arthropods and nematodes (Chea et al., 2005). The identification of *Nodal* in molluscs and other spiralians (Grande and Patel, 2009; Grande et al., 2014), and the characterisation of its role in the development of the LR axis in these organisms instead demonstrated that the LR patterning role of the *Nodal* signalling pathway likely dates back to the last common bilaterian ancestor and was secondarily lost in the lineage leading to flies and roundworms. However, the impact of spiral cleavage goes beyond providing an evolutionary perspective to developmental biology. As we illustrate below, spiral cleavage is also a powerful research system for exploring fundamental questions in developmental biology.

Stasis and change in early embryonic cell lineages

Key ontogenetic aspects are broadly conserved in spiral cleaving embryos. Probably the most obvious ones are the subdivision of the embryo in four quadrants, named from A to D (Fig. 1B), and the distinctive twist of the asymmetric mitotic spindle from the eight-cell stage onwards. In addition, cells are usually smaller on the animal pole (the micromeres, named with lowercase letters, a to d) and larger on the vegetal pole of the embryo (the macromeres, named with upper case letters, A to D) (Fig. 1C). How these attributes have remained static over the course of ∼500 million years across animal lineages with markedly different evolutionary trajectories is still a mystery, but some studies indicate that despite the overall conservation at the cellular level, the underpinning molecular mechanisms controlling these basic features of spiral cleavage might vary. For example, the first asymmetric zygotic division in clitellate annelids is controlled by either inherited monastral spindles or the transient downregulation of one of the centrosomes (Ren and Weisblat, 2006). Likewise, the chirality of the shift in the mitotic spindle between the four- and

Development (2020) 147, dev181081. doi:10.1242/dev.181081

Table 1. Exemplary spiral cleaving research systems

Clade	Example species	Public genome	Functional approaches	Imaging approaches
Annelida	*Capitella teleta*	Yes (Simakov et al., 2013)	Yes (e.g. CRISPR) (Neal et al., 2019)	Yes (Meyer et al., 2010)
	Helobdella robusta	Yes (Simakov et al., 2013)	Yes (e.g. morpholino) (Song et al., 2002)	Yes (Gline et al., 2011)
	Platynereis dumerilii	No	Yes (e.g. CRISPR) (Bezares-Calderón et al., 2018)	Yes (Özpolat et al., 2017; Verasztó et al., 2017)
Mollusca	*Crepidula fornicata*	No	Yes (e.g. CRISPR) (Perry and Henry, 2015)	Yes (Lyons et al., 2015)
	Lymnaea stagnalis	Yes (Davison et al., 2016)	Yes (e.g. CRISPR) (Abe and Kuroda, 2019)	Yes (Abe et al., 2009)
Platyhelminthes	*Prostheceraeus crozieri*	No	No	Yes (Girstmair and Telford, 2019)
Nemertea	*Cerebratulus lacteus*	No	Yes (e.g. morpholino) (Henry et al., 2008)	Yes (Henry et al., 2008)

eight-cell stages is controlled by a tandemly duplicated *diaphanous*-related *formin* gene in the pond snail *L. stagnalis* (Davison et al., 2016; Abe and Kuroda, 2019; Kuroda et al., 2016). However, this duplication event is not ancestral to molluscs or even gastropods, and while one of the copies carries a frame-shift mutation in *Lymnaea*, both appear to be functional in the terrestrial pulmonate snail *Bradybaena similaris* (Noda et al., 2019).

The conservation of the spiral cleavage pattern is also related to an overall similarity in the fates of major embryonic regions. Quadrants A to D tend to generate left, ventral, right and dorsal embryonic areas respectively, and the animal-vegetal embryonic axis roughly correlates with the anteroposterior axis. However, the detailed embryonic cell lineages and precise cell fate specification strategies may differ among spiral cleaving embryos (Nielsen, 2005, 2004). For example, a population of cells referred to as 'ectomesoderm', which often contributes to anterior mesodermal structures, is derived from the third tier of micromeres of the quadrants A and B (the 3a and 3b micromeres) in nemertean worms and in the mollusc *Patella*; however, the ectomesoderm comes from the second micromere tier of the quadrant B (2b micromere) in flatworms, micromeres 3a and 3b in the mollusc *C. fornicata*, and micromeres 3a, 3c, 3d, 4d and possibly 2a, 2c and 3b in the annelid *C. teleta* (Meyer et al., 2010; Nielsen, 2005, 2004; Hejnol et al., 2007). Similarly, the overall specification of these cell fates can be strongly controlled by maternal determinants in the so-called unequal or autonomous spiral cleaving species, or rely more on inductive cell-cell interactions in the so-called equal or determinative spiral cleaving species (Henry, 2014). Although classic analyses do not rely on intracellular lineage tracing and need to be viewed with caution, cell lineages in spiral cleavage appear to be far more labile overall than is often depicted, which might form the basis for the morphological diversity of spiralians; however, the mechanisms accounting for this diversity are still poorly understood.

Spiral cleavage has also been lost numerous times over the course of evolution, sometimes to diverge into bizarre cleavage modes, as in many flatworms (Martín-Durán and Egger, 2012), sometimes to reverse to either holoblastic (e.g. in bryozoans and brachiopods) or superficial radially symmetrical patterns (e.g. in cephalopods) (Hejnol, 2010) (Fig. 2). In the bryozoan *Membranipora membranacea*, the loss of the spiral-like arrangement of cells during early development did not affect the overall embryonic cell lineage (Vellutini et al., 2017), which remained similar to that of other spiral cleaving relatives, further supporting that cleavage and cell fates are, or can be to a certain extent, decoupled in some members of Spiralia. This condition significantly differs from other known cases in animal development with highly stereotypical cell division patterns, such as ascidian (Guignard et al., 2018 preprint) and ctenophore embryogenesis (Martindale and Henry, 1999), where cellular arrangements and cell fates are tightly linked. Therefore, spiralians and spiral cleavage can provide a window on the cellular and molecular mechanisms controlling and generating plasticity in embryonic cell fates.

The cellular and molecular control of embryonic patterning

The extensive knowledge of the spiralian cell lineages contrasts with the relatively poor understanding of the gene regulatory networks governing embryonic patterning generally. As mentioned above, LR chirality in gastropod molluscs is under control of early maternally supplied cytoskeleton components that ultimately determine blastomere chirality at the eight-cell stage and the site of expression of the *Nodal-Pitx* signalling pathway (Abe and Kuroda, 2019; Kuroda et al., 2009). However, the extent to which these mechanisms are conserved among spiralians is unclear, as some lineages have lost the *Nodal* ligand (Grande et al., 2014), and the upstream cytoskeleton components appear to vary even among gastropods (Davison et al., 2016; Noda et al., 2019). Moreover, the cytoskeleton and mitotic spindle appear to underpin the differential segregation of mRNAs during spiral cleavage, ultimately controlling micromere quartet identity (Kingsley et al., 2007; Lambert and Nagy, 2002; Rabinowitz and Lambert, 2010).

As in other animal embryos, anteroposterior (AP) and dorsoventral (DV) patterning are intimately linked in spiral cleavage. Descendants of the D quadrant (3D in some molluscs, 2d and 4d micromeres in the annelid *Tubifex*, but a 2D macromere in the annelid *C. teleta*) act as posterodorsal embryonic organiser, controlling the development of the other embryonic fates and bilateral axial identities (Henry, 2014; Hejnol, 2010; Lambert, 2010; Amiel et al., 2013; Nakamoto et al., 2011). In mollusc and some annelid embryos (e.g. *Hydroides hexagonus*), the MAPK signalling pathway is active in the D lineage and is often involved in the specification activity of the posterodorsal embryonic organiser (Lambert and Nagy, 2001, 2003; Koop et al., 2007; Henry and Perry, 2008). In most animal embryos, the canonical Wnt signalling pathway and the bone morphogenic protein (BMP) signalling pathway are often involved in AP and DV specification, respectively, but they appear to exert lineage specific roles in spiralians. For example, canonical Wnt signalling controls binary cell decisions during cleavage in the annelid *P. dumerilii* (Schneider and Bowerman, 2007), but it primarily regulates endomesoderm specification (i.e. gastrulation) in *C. fornicata*, in the nemertean *C. lacteus* and in brachiopod embryos (Martín-Durán et al., 2016; Henry et al., 2008, 2010). Similarly, the BMP pathway controls dorsoventral patterning in the mollusc *Tritia* (also known as *Ilyanassa*) *obsoleta* and brachiopod embryos (Martín-Durán et al., 2016; Lambert et al., 2016), but it does not in the annelid *C. teleta*, where it is instead the activin/Nodal signalling pathway that plays that function (Lanza and Seaver, 2018). As with embryonic cell fates, these data indicate that there is variation in the way spiralian embryos are patterned beneath the highly conserved program of cell

divisions, yet the exact extent of these differences and how they relate to changes in embryonic cell fates is unclear.

The evolution of cell types and morphological novelties

Comprising 15 out of the 32 recognised major animal groups (or Phyla under Linnaean taxonomy), Spiralia is a morphologically and ecologically diverse animal group. Each defined by a relatively distinct body plan, some of these groups are among the most diversified animal clades, such as Platyhelminthes, Mollusca and Annelida. Not surprisingly, there are countless examples of morphological innovations in Spiralia, some of them among the most iconic in the animal tree of life, such as molluscan shells (Wanninger and Wollesen, 2018), and others less known but equally exciting, such as annelid and brachiopod chaetae (Schiemann et al., 2017), and molluscan and brachiopod cartilage (Tarazona et al., 2016). What distinguishes spiralians from other vastly diverse animal groups, such as arthropods and vertebrates, is that, to a large extent, this morphological diversity emerges through the same early spiral cleavage program. For developmental biology this is of great importance, because embryos of very distantly related and morphologically different species can be perfectly matched at the single-cell resolution, allowing the precise identification of the cellular and molecular mechanisms that drive morphological change. For example, molluscan shells emerge from derivatives of the 2a-2d micromere quartet (Mohri et al., 2016; Chan and Lambert, 2014; Lyons et al., 2015), which form an initial cluster of ectodermal cells, the 'shell field', that will differentiate into a novel cell type with biomineralising potential (Wanninger and Wollesen, 2018). However, the 2d micromere and its progeny generate the majority of the segmented trunk ectoderm and the ventral nerve cords in annelids, where they do not differentiate into biomineralising cell types (Meyer et al., 2010). The expression of the transcription factor *engrailed* appears to be an early signal that demarcates the shell field from the rest of the dorsal ectoderm in molluscs (Jacobs et al., 2000), but the upstream mechanisms that generate this divergence in spiral development between molluscs and other spiral cleaving groups are unknown.

Spiralia is also important for exploring the developmental principles governing convergent evolution and gain/loss of morphological traits, even at late ontogenetic stages when differences among embryos are more pronounced. For example, heavily centralised brain centres and/or medially condensed nerve cords evolved secondarily in spiralian groups such as annelids, molluscs and flatworms (Martín-Durán et al., 2018), and so did the complex eyes and the body appendages of cephalopods (Tarazona et al., 2019). Although in some cases, divergence in the molecular repertoire underpin the development of similar structures, such as neuronal cell types and nerve cords (Martín-Durán et al., 2018), the recruitment of relatively well conserved ancestral gene networks govern others, as in the parallel evolution of cephalopod arms (Tarazona et al., 2019). Altogether, these few examples illustrate how spiralians and spiral cleavage may contribute to our understanding of how very similar developmental strategies generate phenotypic diversity, as well as of the mechanisms governing the repeated emergence of similar phenotypic outcomes.

Perspectives

Despite recent advances, major questions remain unanswered in spiralian embryology: when did spiral cleavage evolve? What are the molecular and cellular mechanisms governing spiral cleavage? Are these mechanisms as conserved as the stereotypical cleavage program suggests, or is there widespread developmental variation hidden under a stable cell division program? If the latter, how do developmental programs diversify as the overall cell division patterns remain? And how does morphological diversity emerge from such *a priori* stable early embryonic program? The breadth of stimulating developmental questions that the study of spiral cleavage poses is almost unlimited, as is its capacity to enlighten fundamental biological concepts. However, in order to answer these questions, we need a more solid phylogenetic framework for the inter-relationships between spiralian groups, and to reassess the embryonic development of certain enigmatic groups, in particular gnathostomulids. One can hope that improved genomic resources and, in particular, full genome sequences in all spiralian lineages would help to resolve some of the issues plaguing phylogenomic studies, such as contamination and missing data. This represents a challenge, as many microscopic lineages (e.g. micrognathozoa and gastrotrichs) will prove difficult to sequence, but this endeavour likely represents the next milestone for spiralian phylogeny.

In parallel with phylogenetic efforts, the continued pursuit of more research systems with better genomes and -omics datasets, as well as more functional (e.g. CRISPR and transgenesis) and imaging methodologies, will allow us to dig deeper into the nuts and bolts of spiral cleavage. However, we need to keep promoting and taking advantage of the thriving diversity of organisms employed by the spiralian research community, as it is also the key to attaining a comprehensive perspective on the mechanisms and evolution of this mode of development. Ultimately, this will require a multidisciplinary and coordinated community effort, but the possibility to unwind the mysteries of spiral cleavage is definitely worth the effort.

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