Spiralian model systems

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ABSTRACT The “Spiralia” represent one of the three major clades of bilaterian metazoans. Though members of this clade exhibit tremendous diversity in terms of their larval and adult body plans, many share a highly conserved early pattern of development involving a stereotypic cleavage program referred to as spiral cleavage. This group therefore represents an excellent one in which to undertake comparative studies to understand the origins of such diversity from a seemingly common ground plan. These organisms also present varied and diverse modes in terms of their ecology, development and life history strategies. A number of well established and emerging model systems have been developed to undertake studies at the molecular, genetic, cell and organismal levels. The Special Issue of the Int. J. Dev. Biol. entitled “Spiralian Model Systems” focuses on these organisms and here, I introduce this clade, pointing out different types of studies being undertaken with representative spiralian model systems.

KEY WORDS: bilaterian metazoan, spiral cleavage, life history strategy

The Spiralia (Lophotrochozoa)

Of the three major clades of bilaterians, the Spiralia (Lophotrochozoa) comprise nearly half of the extant metazoan phyla (see Fig. 1). Despite this fact, the group has received relatively little attention compared to the other two clades, the deuterostomes and ecdysozoans, notably in the areas of genetics, as well as molecular, cellular and developmental biology. This is due in part to the long standing predominance of key experimental models positioned within the Ecdysozoa, (e.g., the fruit fly Drosophila and the nematode, C. elegans), and the Deuterostomia, (e.g., chordates such as the Zebrafish and mouse, as well as a few invertebrate representatives from the Echinodermata).

The Spiralia include 14 of roughly 36 metazoan phyla (Fig. 1). The Spiralia include the Lophotrochozoa, and sometimes these terms have been used synonymously. The clade “Lophotrochozoa” was first recognized by Halanych et al., (1995, see also Giribet et al., 2000; Passamanack and Halanych 2006; Helmkampf et al., 2008a,b; Edgecombe et al., 2011) consisting of the Bryozoa, Enteroprot, and Cyclophora. The other group includes the Platyaooza (Cavalier-Smith, 1998; Giribet et al., 2000), which include Gastrotricha, Platylmrminthes, and the groups comprising the “Gnathozoa” or “Gnatiihera” (Gnathostomula, Micronathozoa and Rotifera (Syndermata)). More recently, however, an analysis by Struck et al., (2014), which included additional species, suggests that the Platyaooza are paraphyletic. Their data suggest that, with the exclusion of the Gnatiihera, the Gastrotricha and Platylmrminthes comprise a monophylum, which they term the “Rouphozoa.” They argue that the Rouphozoa together with the other spiralian comprise a monophyletic group called the “Platytrochozoa.” They argue that the Rouphozoa should not be included in the Lophotrochozoa, and that the terms Lophotrochozoa should not be used synonymously with the larger encompassing clade, the Spiralia. Additional lophotrochozoan taxa, with more uncertain affiliations, include the parasitic Acanthocephala (closely related to rotifers), Myzostomida (likely highly derived annelids), and a unique group referred to as the Mesozoa, which includes the Orthinectida and Rhombozoa (see Giribet, 2002, 2008, Hejnol et al., 2009; Edgecombe, 2011). These phyla are listed in Table 1 and a recent view of their phylogenetic relationships is depicted in Fig. 1.

The term “Lophotrochozoa” was derived from two of the prin-
The Spiralia exhibit diverse body plans and life history strategies

Remarkably, the Spiralia have exploited most habitats on earth and exhibit the greatest diversity of body plans compared to any other clade of multicellular organisms (see Fig. 2 and Table 1). In fact, all fundamental grades of organization can be found (Brusca and Brusca, 2003; Ruppert et al., 2003). For instance, groups such as the annelids, and molluscs, exhibit mesodermally-lined true coelomic cavities, while others such as the Platyhelminthes, and entoprocts lack these cavities and possess acoelomate or pseudocoelomate body plans. Members of one phyla, the Annelida exhibit overtly segmented bodies along their anterior-posterior axes (Balavoine, 2014; Weisblat and Kuo, 2014, in this issue). Some groups posses skeletal elements such as the external or internal mineralized shells of molluscs and brachiopods or the hardened exoskeletons found amongst the bryozoans. Others posses specialized external or internal cuticular structures, such as those found in entoprocts, annelids, and gnathostomulids, while many representatives have no skeletal elements at all (e.g., nemerteans, phoronids, Platyhelminthes).

Likewise, different groups exhibit varied modes of development, including many with diverse larval body plans (Fig. 3, see papers by Rockman and Zakas, 2014, Arenas-Mena and Li, 2014, Helm et al., 2014; Boyle and Rice, 2014, Lesoway et al., 2014; Maslakova and Hiebert, 2014, Rockman and Zakas, 2014, all in this issue). As mentioned briefly above, one striking characteristic shared by some members of the annelids and molluscs and possibly also certain nemerteans, bryozoans and cycliophorans is the formation of a trochophore or trochophore-like larvae that possesses a distinct circumferential ciliated band, the prototroch (Fig. 3A,D-E, G). In contrasts, some members of the Nemertea display maximal indirect development via the formation of a feeding larva that contains internal sets of imaginal disks, from which the adult emerges through a radical process of metamorphosis (e.g., heteronemerteans, such as Cerebratulus lacteus, or C. montgomeryi, Fig. 3F, see review by Maslakova and Hiebert, 2014, in this issue).

Other representatives exhibit direct development without the
formation of an intervening larval stage (Fig. 3I). Even within the same genus one can find species with dramatically different modes of development. For instance, the genus of calyptraeid snails, Crepidula contains at least 60 recognized species (Collin, 2003a,b). Some species, such as C. fomicata, C. lingulata and C. plana exhibit indirect development with a planktotrophic feeding veliger larvae (Fig. 3D, Conklin, 1897; Werner, 1955; Fretter, 1972; Collin, 2000). On the other hand, species such as C. adunca, and C. convexa exhibit direct development leading to the formation of crawl-away juvenile snails (Conklin, 1897; Moritz, 1939). Yet others such as Crepipetella dilatata (formerly Crepidula dilatata) and Crepidula cf. onyx form adelphophagic embryos that ingest aborted sibling nurse eggs contained within the same egg capsules (Gallardo, 1977; Chaparro et al., 2002; see paper by Lesoway et al., 2014 in this issue).

Clearly the spiralian “developmental program” represents a highly flexible platform that supported the explosive radiation of these metazoan phyla. As such, the Spiralia provide an excellent group for studies aimed at understanding the developmental mechanisms that underlie the genesis of such diversity. Obviously, they represent a pivotal group in terms of the emergence of the Bilateria. Though currently lacking, a better understanding of the precise phylogenetic relationships amongst these groups will be critical for deciphering the evolutionary trajectory of those fundamental developmental processes that generated such diverse metazoan adult and larval body plans (see Figs. 2-3). The truly remarkable point is that such vastly different body plans originated from an ancestral pattern of early development that involved spiral cleavage.

**Spiral cleavage**

The highly stereotyped spiral cleavage pattern exhibited by many members of the Spiralia is characterized by alternating sets of oblique cell divisions that generate staggered quartets of micromeres located towards the animal pole. The basic pattern is illustrated in Fig. 4. Beginning with the fertilized egg, the first two cell divisions occur along the animal-vegetal axis and are nearly orthogonal to one another. These divisions generate four cells (“blastomeres”) that establish the four basic embryonic quadrants, which are termed A, B, C, and D following the conventional nomenclature refined by Edwin Grant Conklin (1897, see Figs. 4). In many species symmetric divisions generate these four cells, which are all of roughly the same size (Fig. 4A-D, I). In other species asymmetric divisions generate these cells and typically one cell ends up being larger than the others, the so-called D blastomere (Fig. 4A-D’). In either case, each of these four cells subsequently generates a series of animal daughter cells (called “micromeres”), which are formed in alternating clockwise and counterclockwise orientations around the animal-vegetal axis (Fig. 4E-H, E’-H’, J-M). These animal cells are typically smaller and therefore are termed “micromeres,” whereas the four vegetal-most cells are larger and termed “macromeres.” In some cases, such as in nemerteans,
the animal micromeres of the first quartet may actually be larger than the macromeres. Animal micromeres are designated with lower case letters, while the vegetal macromeres are designated with uppercase letters. Hence, the first quartet of micromeres is named 1a, 1b, 1c, and 1d, while the corresponding macromeres are named 1A, 1B, 1C, and 1D. (see Fig. 4E-F, E’F’, J). While the third cleavage division appears to occur at right angles to those of the first and second divisions the cleavage spindles are actually canted such that the micromeres are usually born with a slight clockwise (dextral) twist relative the macromeres when one views the embryos from the animal pole (see Fig. 4E-F, E’F’, J). Subsequently, a second quartet of animal micromeres (2a, 2b, 2c, 2d) is formed by the vegetal macromeres. During this division the spindles become shifted in the opposite direction, such that the second quartet micromeres are usually born with a slight clockwise (dextral) twist relative to the macromeres when one views the embryos from the animal pole (see Fig. 4E-F, E’F’, J). Subsequently, a second quartet of animal micromeres (2a, 2b, 2c, 2d) is formed by the vegetal macromeres. During this division the spindles become shifted in the opposite direction, such that the second quartet micromeres become situated with a slight counterclockwise twist relative to the four macromeres (2A, 2B, 2C, 2D, Fig. 4G-H, G’H’, K). Typically a total of four micromere quartets (collectively referred to as 1q, 2q, 3q, and 4q) are formed and each set is formed with opposing chirality (Fig. 4L-M), though in some species an additional fifth quartet of micromeres may be generated. Of course the individual micromeres belonging to each quartet also undergo further divisions as successive quartets are born and early on these divisions also follow the same alternating oblique orientations. These daughter cells are distinguished from one another by a system of successive superscript numbers (see Conklin, 1897). Typically those daughters born towards the animal pole receive a superscript of 1 while those towards the vegetal pole receive a 2 (e.g., 1b1 and 1b2, Fig. 34-M), and with successive divisions additional superscripts are added (e.g., 1b11 and 1b12).

At some point, the spiral cleavage pattern is interrupted by the occurrence of bilateral sets of cell divisions. Those events represent a key transition in terms of establishing the bilaterian body plan, which is characteristic of both larvae and adults. In most cases the first sign of bilaterality is apparent in the symmetric divisions of cells located in the dorsal D quadrant. For instance, a daughter cell of 1d, 1d12, which is located at the base of the dorsal arm of the “molluscan cross” divides bilaterally to form cells 1d1212 (to the right of the midline) and 1d1211 (to the left of the midline) in the pulmonate...
snail Lymnaea stagnalis. In many cases 2d also exhibits and early bilaterally symmetric pattern of cell divisions (Dohle, 1999).

In the snail Crepidula 4d is the first cell to divide bilaterally to form the ML (left side) and MR (right side) mesendodermal teloblasts well before any of the other fourth quartet micromeres are even born (i.e., 4a, 4b and 4c, see Lyons et al., 2012). These teloblasts form bilaterally symmetrical bands of mesendodermal cells (see Lyons et al., 2012). These cells also appear to generate the primordial germ cells in all cases in which this has been carefully examined (see reviewed by Rebscher, 2014 in this issue). As development continues, the individual germ layers arise from specific cells and the tissues become organized via the processes of gastrulation (see review by Lyons and Henry, 2014, in this issue), organogenesis and morphogenesis to ultimately generate the larval and/or adult body plans.

It should be noted that there are some species in which alternating micromere quartets are formed with the opposite handedness (i.e., the first quartet micromeres are formed in the counter-clockwise direction, etc.), such as in the snail Biomphalaria or even amongst different populations of the same species (e.g., the pond snail Lymnaea peregra, Boycott et al., 1923, 1930; Sturtevant, 1923; Freeman and Lundelius, 1982; Abe et al., 2014, in this issue). Such differences have a profound effect on development, as the early cleavage patterns set up the adult body plan. For instance, in the case of gastropod molluscs such as Lymnaea), the chirality of the adult shell (i.e., right- vs. left-handed coiling) is directly related to the chirality of the early cleavage pattern (i.e., whether the first quartet formed via dextral vs. sinistral cleavages, respectively). The mechanisms that underlay the establishment of left-right asymmetry and changes in shell coiling are described further by other authors contributing to this issue (Abe et al., 2014; Grande et al., 2014, in this issue).

A recent study in annelids (using the leech, Helobdella austenensis) suggests that the key transition to bilateral cleavage may be controlled by zygotic gene expression regulated by members of the Pax family of transcription factors, either PaxB1 and/or Pax2/5/8 (Schmerer, et al., 2013). This transcription factor appears to be necessary for the DNOPQ’” ectodermal proteloblast (equivalent to 2d””) and DM” mesodermal proteloblast (equivalent to 4d) to undergo their transitions to bilateral cleavage. The fascinating development of the Cilatella, or Oligochaeta, including leeches and the sludge worm, Tubifex are described further by Shimizu and Nakamoto (2014, this issue) and Weisblat and Kuo, (2014, this issue). Continued studies of the Spiralia will inform us greatly as to key developmental-evolutionary transitions that have occurred to generate bilaterally symmetrical body plans.

Establishment of the D quadrant

As described above, and depending on the species under consideration, one of two main variations of the spiral cleavage pattern may be observed. In some cases the first two cell divisions are unequal, while in others they are equal. The identity of the D quadrant can be ascertained as soon as the four-cell stage is reached in the former, while the D blastomere is typically much larger than the other cells (Fig. 4A’-D’). On the other hand, the four quadrants cannot be distinguished in the case of the latter (Fig. 4A-D). These differences are closely tied to fundamental differences in the timing and mechanism by which the cell quadrants actually become specified. Multistep models have emerged from

![Fig. 3. Representatives lophotrochozoans, illustrating some of the tremendous diversity of larval body plans. All larvae are shown as dorsal views with the anterior ends toward the top of the figure, except that A and F are left-lateral views and D and J are right-lateral views. (A) trophophore larva of the polychaete annelid, Hydroides hexagonus, (B) Setiger larva of the annelid, Platynereis dumerilii. (C) A sipunculid, “Yellow papillated,” pelagosphere larva collected from the Gulf Stream. (D) Veliger larva of the gastropod mollusc Crepidula fornicata. (E) Trophophore larvae of the chiton, Chaetopleura apiculata. (F) Typical, advanced plakobranch larva with adult worm seen developing internally from imanial rudiments (possibly related to Lineus flavescens). (G) “Trochonemertes” larva of an unidentified nemertean, described as pilidium nielseni, and belonging to an undescribed lineform pilidiophoran species from southern Oregon, otherwise referred to as Micrura sp. (see Maslakova and von Dassow, 2012). (H) Phoronid larval actinotroch collected from Hawaiian waters. (I) ciliated planula larva of an unidentified nemertean, pilidiophoran species collected near Coos Bay, Or. (J) Müllers larva of the polyclad trubellarian flatworm, Hoploplana inquillina. Some figures were kindly provided by M. Martindale (A, H), N. Rebscher (B), M. Boyle (C), S. Maslakova (F, G, I). ad, adult worm; at, apical tuft; ft, foot; lb, ciliated lobes; lo, lophophore; lp, ciliated lappet; pt, prototroch; sg, shell gland; sh, shell; se, setae; vl, velum.]

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experimental data examining these systems that leads to the establishment of the D quadrant and its subsequent activity as an organizer of development (Fig. 5). In the case of species with asymmetric (unequal) cell divisions the larger D quadrant blastomere becomes specified autonomously by virtue of its inheriting specific vegetal determinants (Figs. 4, 5; van den Biggelaar and Guerrier, 1983; Verdonk and Cather, 1983). A specific cell or cells derived from the D quadrant subsequently serve as a key organizer of development to establish the dorso-ventral axis and the fates of adjacent cells (Fig. 5A'-D'; see below). These asymmetric cleavages may take place as a consequence of the asymmetric shifting of the cleavage spindle that dictates where cytokinesis occurs or via the production of vegetal cytoplasmic lobes (so called “polar lobes”) that ultimately become shunted into the D quadrant blastomere during each of these divisions (Guerrier et al., 1978; Verdonk and Cather, 1983; Henry and Martindale, 1999). These determinants for the D quadrant are located in the vegetal region, which are also packaged within polar lobes. On the other hand, in those cases that exhibit symmetric (equal) cell divisions, the D quadrant is not specified until later during development and this occurs conditionally by virtue of cell-cell inductive interactions.

These inductive interaction take place between daughters of the animal first quartet micromeres (the 1q’s) and one of the vegetal macromeres (e.g., the future 3D), typically early during the interval between fifth and sixth cleavage (Fig. 5A-D). Some data suggests that the distinction between these two forms of spiral cleavage may be closer than had been previously appreciated, as there is evidence that animal-vegetal interactions may also be important for the specification of the D quadrant even in the case of unequal cleavers (e.g., in Ilyanassa, Wandelt and Nagy, unpublished data; see Lambert, 2009a,b; Fig. 5B). The nature of these inductive signals is not understood.

The D quadrant organizer

In spiralians, one cell, or in some cases two cells derived from the D quadrant, serve as key embryonic organizers that set up the dorso-ventral axis and direct the development of adjacent cells via inductive interactions (Fig. 5). These cells are set aside relatively early during development. Although these organizer cells reside within the D quadrant, there is a fair degree of heterotopic and heterochronic variation in terms of which particular cell(s) serves

![Fig. 4. Diagrams showing equal vs. unequal forms of spiral cleavage.](image)

(A-H) Early cleavage in equal-cleaving embryos illustrating the formation of the first two quartets of micromeres. Note that all blastomeres are of roughly the same size at the two- (A-B) and four-cell stages (C-D), respectively. A'-H'). Early cleavage in unequal-cleaving embryos showing the formation of the first two quartets of micromeres. Note the larger CD and D blastomeres at the two- (A'-B') and four-cell stages (C'-D'), respectively. The embryos in A-H' are shown as lateral views with the animal pole oriented towards the top of the figure and the vegetal pole towards the bottom. (I-M) Animal pole views of equal cleavage in the mollusc Crepidula fornicta (After Henry et al., 2006, and Conklin, 1897). Cells are labeled following the nomenclature refined by Conklin (1897). In E-H and E'-H' note the alternating oblique orientations of the cleavage spindles that set up the oblique plans of cytokinesis and shunted positions of the micromeres. In most species the first quartet micromeres are generated in a clockwise direction relative to the vegetal macromeres as shown in E-F and E'-F' (i.e., “dextral cleavage” see also I-J). The second quartet is generated in a counterclockwise direction (see G-H and G'-H', see also K). Typically, two additional third and fourth quartets of micromeres are formed (see L-M). Note that in some species, such as Lymnaea peregra, the first quartet is formed with the opposite handedness, being generated in a counterclockwise direction (“sinistral cleavage”) with each successive quartet also alternating their directions, accordingly (not shown here). See text for further details.
Fig. 5. Models summarizing basic mechanisms involved in specifying the dorsal D quadrant and subsequent D quadrant organizer activity during early development in spiralians. A-D highlights these processes in equal-cleaving spiralians. A-D’ illustrates these processes in unequal-cleaving spiralians. (A-B) In the case of equal-cleaving embryos, the D quadrant is established conditionally, as a result of animal-vegetal inductive interactions that involve the animal-most progeny of the first quartet micromeres (1a’ cells). Typically this occurs during the interval between fifth and sixth cleavage when animal cells come into contact with one of the four vegetal macromeres and transmit an unknown signal that triggers this cell to become 3D. This series of events appears to trigger MAPK activation within the 3D macromere (Henry and Perry, 2008). On the basis of observations made in one species, Crepiduala, there may also be an earlier signal that primes the animal micromeres that also involves the activation of MAPK in those cells (Henry and Perry, 2008). (B) Though all four macromeres are capable of becoming 3D, only one emerges, and this could potentially involve some form of lateral inhibition. (C) Once the 3D macromere is specified, in some species it becomes a key organizer of developing that sets up the dorso-ventral axis and directs the development of adjacent cells in the other quadrants. The nature of those signals is not clear, though it appears to trigger the activation of MAPK in a subset of animal micromeres. (D) In some cases 4d may serve as the key organizer (i.e., Crepiduala, Henry et al, 2006). (A’-B’) In the case of unequal-cleaving embryos, the D quadrant is established autonomously as a result of the initial asymmetric cell divisions. The first two cell divisions ultimately segregate vegetal determinants (of unknown nature illustrated here as purple dots) into the D blastomere. (B’) Some evidence suggests that the ultimate fate of the 3D macromere may also require inductive interactions from the animal micromeres, similar to the situation encountered in equal-cleaving embryos (Waldelt and Nagy, unpublished data). (C’) Subsequently, the 3D macromere serves as the key organizer of development, in the same fashion described above for equal cleaving embryos. (D’) In the case of Ilyanassa, the activity of the organizer appears to be prolonged by 4d. (Lambert, 2009). The fate of the D quadrant, and possibly also its organizer activity also involves the activation of MAPK in these unequal-cleaving species (Lambert and Nagy, 2001, 2003; Koop et al, 2007).

Cell lineage fate maps

Not only is the cleavage pattern highly conserved, but so to are the general fates of the individual blastomeres. These observations first became apparent from comparative analyses of cell lineages compiled by investigators working at the Marine Biological Laboratory in Woods Hole, MA. The very first of these was carried out by Charles Otis Whitman, who examined development of the leech Clepsine (Whitman, 1878, 1887). Leeches, like other oligochaetes, exhibit a modified form of spiral cleavage involving the formation of germinal bandlets that generate most of the adult ectoderm, endoderm and mesoderm (see review by Weisblat and Shankland, 1985; Weisblat and Kuo, 2014 in this issue). In fact, Whitman may be regarded as the “father” of cell lineage analysis. His student Frank Rattray Lillie and other individuals including Edmund Beecher Wilson and Edwin Grant Conklin, subsequently assembled cell lineage fate maps for a number of different spiralian systems including various annelids, Nereis, Anica foetida, Spio fuliginosus, the polyclad Leptoplana (Wilson, 1892; Mead, 1897), molluscs, such as the slipper snail Crepidula fornicata (Conklin, 1897) the
freshwater bivalve *Unio* (Lillie, 1895). Additional work was carried out by their counterparts in Europe (e.g., Heymons, 1893; Wierzejski, 1905). That early work has been extended in recent decades using modern cell-autonomous lineage tracers for a number of species (i.e., the gastropod mollusc *Crepidula fornicta* and *C. convexa*). Hejnol et al., 2007; Lyons et al., 2012, and Ilyanassa obsoleta, Render 1991, 1997; Chan and Lambert 2014; the polyclad turbellarian *Hoploplana inquilina*, Boyer et al., 1996, 1998; and the annelids *Capitella teleta*, Meyer et al., 2010, Meyer and Seaver, 2009, 2010, and *Platyneris dumerilii*, Ackerman et al., 2005; Fischer and Arendt, 2013). Together, this body of work has revealed that the ultimate fates of these quadrants are, to a large extent, homologous across the embryos of different spiralian phyla. Generally speaking, the first three quartets of macromeres give rise to ectodermal tissues, and components of the nervous system, including the photoreceptors (typically derived from 1a and 1c). Specific combinations of cells derived from the second and/or third quartets also generate mesodermal tissues that contribute to the larval and adult body plans (the so-called "ectomesoderm", see review by Lyons and Henry in this issue). The cells of the fourth quadrant typically generate endodermal tissues of the digestive tract, though one cell, the mesentoblast 4d, also serves as a mesodermal progenitor (the so-called "endomesoderm"). In many, but not all cases this cell contributes to the formation of the hindgut intestine. The fourth quadrant macromeres may or may not form endodermal tissues, depending on the species being examined. As mentioned previously, the D quadrant is the first one to be specified in the embryo and its organizing activity subsequently directs the development of the other cell quadrants.

The positions of the four embryonic quadrants bear a specific relationship to the future dorso-ventral and left-right axes. Some authors have stated that the A, B, C, and D quadrants generally correspond to the right, ventral, left and dorsal sides of the embryo,
respectively, but those relationships are oversimplified. Because individual micromeres within each quadrant are generated with an alternating clockwise/counter-clockwise direction, they occupy slightly different positions relative the dorsoventral and left right axes at the completion of these cleavage divisions. Thus, in many cases, the progeny of the first quadrant, 1a, 1b, 1c, 1d, occupy left-ventral, right-ventral, right-dorsal, and left-dorsal positions, respectively (Henry and Martindale, 1999). The second quadrant micromeres occupy left (2a), ventral (2b), right (2c), and dorsal (2d) positions. The third quadrant micromeres exhibit axial relationships similar to those of the first. Finally, the fourth quadrant micromeres exhibit axial relationships similar to those of the second. Of course, these are generalities and, in fact, there has been some significant modification of these cleavage patterns and cell fates over the course of metazoan evolutionary history, as described below (see Henry and Martindale, 1999, and the review by Seaver, 2014, in this issue).

Differential localization of mRNAs: specification of the micromere quartets

Elegant work by Lambert and Nagy (2002, see also Kingsley et al., 2007) showed that specific mRNAs are localized to particular cells during early cleavage in the snail *Ilyanassa obsoleta,* and subsequently that some of these mRNAs actually play a role in specifying the fates of the various micromeres (see Swartz et al., 2008; Rabinowitz, et al., 2008; Rabinowitz and Lambert, 2010; Chan and Lambert, 2011). These mRNAs become scattered throughout the cytoplasm, centrosomes, and the cell cortex to ultimately become differentially localized to specific daughter cells during cleavage. These localized mRNAs are thought to play key roles in establishing an animal-vegetal pre-pattern that distinguishes the different tiers of micromeres within these embryos (see Lambert, 2009a,b, 2010). Subsequent inductive interactions of the dorsal D quadrant organizer then refine this pattern to impart further complexity within each micromere quadrant. Similar patterns of localized mRNAs also appear in the gastropod *Crepidula fornicata* (Henry et al., 2010c), and this could be a universal mechanism to distinguish cell fates in the Spiralia.

Evolution of the spiral cleavage program

Although spiral cleavage appears to represent a key aspect of the ancestral mode of development in this group of organisms, it has clearly undergone tremendous modifications, being completely lost in several groups such as the bryozoans, brachiopods, and some polychaetes, which means there are many gaps in our understanding of the other phyla. More widely used systems for developmental biology include the molluscs, *Lymnaea, Ilyanassa* (Gharibian et al., 2009), *Crepidula* (Henry et al., 2010a,b), *Patella, Dentalium,* and the annelids, *Platyneris* (Fischer et al., 2010), *Capitella, Hydrodies* and leeches such as *Helobelia* and *Hirudo* (Weisblat and Kuo, 2009). The nemertean *Cerebratulus lacteus* has also been used in some developmental studies (Henry and Martindale 1998; Henry, 2002). More recently there has been considerable interest in the evolution of mechanisms that control asymmetry and establishment of the left-right axis. Gastropods snails have been the subjects of these studies for several years (including, *Lymnaea, Lottia,* and *Biomphalaria,* see papers by Grande et al., 2014; Abe et al., 2014 and Liu et al., 2014, in this issue). Annelids have figured prominently in comparative

**Spiralian model systems**

Over the years, a number of different spiralian taxa have served as models for a variety of studies, and some of these are listed here in Table 2 along with certain features that make these systems so useful. In general, they have been chosen for distinctive advantages that each has to offer, which includes ease of collection/culture, generation time, experimental accessibility (absence of egg investments, ease of microinjection, dissection) ability to examine gene expression/function, access to other resources such as genomic information or EST collections, etc.). Most of these representatives reside within the molluscs, Annelida, and Platyhelminthes, which means there are many gaps in our understanding of the other phyla. More widely used systems for developmental biology include the molluscs, *Lymnaea, Ilyanassa* (Gharibian et al., 2009), *Crepidula* (Henry et al., 2010a,b), *Patella, Dentalium,* and the annelids, *Platyneris* (Fischer et al., 2010), *Capitella, Hydrodies* and leeches such as *Helobelia* and *Hirudo* (Weisblat and Kuo, 2009). The nemertean *Cerebratulus lacteus* has also been used in some developmental studies (Henry and Martindale 1998; Henry, 2002). More recently there has been considerable interest in the evolution of mechanisms that control asymmetry and establishment of the left-right axis. Gastropods snails have been the subjects of these studies for several years (including, *Lymnaea, Lottia,* and *Biomphalaria,* see papers by Grande et al., 2014; Abe et al., 2014 and Liu et al., 2014, in this issue). Annelids have figured prominently in comparative
studies examining the origins and mechanisms of segmentation (see review by Balavoine, 2014; and Weisblat and Kuo, 2014, in this issue). In fact spiralian have contributed greatly to the recent resurgence of the field of development and evolution and to our understanding of metazoan phylogeny.

The Spiralia contain many systems that are excellent for understanding life history strategies related to transitions between different developmental modes, as well as the process of metamorphosis. As mentioned above, members of the calyptopsidean snails (e.g., species in the genus Crepidula) exhibit a tremendous array of developmental modes including forms with direct development and others with planktotrophic feeding larval development or yet others with intermediate forms of development (Henry et al., 2010a, b; Lesoway, et al., 2014 in this issue). Several species exhibit protandric hermaphroditism, like various Ophryotrocha and Crepidula species. For instance different members of the genus Ophryotrocha exhibit different modes including those with separate sexes (gonochoristic) while others exhibit different forms of hermaphroditism (Åkesson, 1973, 1975, 1994; Paxton and Åkesson, 2010), making them excellent systems for understanding factors that influence sexual development.

Various spiralian exhibit remarkable abilities to undergo asexual reproduction and many can regenerate missing body parts. Numerous studies focusing on regeneration have been carried out using the flatworms Schmidtea mediterranensis and Dugesia japonica (covered extensively in an earlier issue of this journal (UDB, volume 56, 2012). Annelids such as Ophryotrocha and Pristina can regenerate missing posterior segments and represent excellent systems to study these phenomena (Pfannenstiel, 1974; see articles by Bely, 2014, and Szabó and Ferrier, 2014, in this issue).

Certain systems have been used extensively for studies of neurobiology, such as those with large, easily accessible neurons and relatively simple nervous systems that support complex behaviors, like the squid Loligo and Aplysia (Abbott et al., 1995) and leeches such as Hirudo (Muller et al., 1981). Several have been used in behavioral studies of learning, memory and behavior, such as Lymnaea, (Benjamin and Kemenes, 2009, Feng et al., 2009), the limpet, Lottia gigantea, (Stimson, 1970, 1973) and the leech (Stent, et al., 1984; Muller et al., 1981). Annelids such as the leech and the polychaetes Capitella and Platynereis have also been used to study the development of the nervous system (Stent, 1984; Meyer and Seaver, 2009; see paper by Helm et al., 2014 in this issue). Due to the ease with which one can obtain large quantities of gametes, many cell biological, molecular and biochemical studies have been carried out using species such as the surf clam Spisula. The oligochaete Tubifex (the “slug worm”) and the soil oligochaete Enchytraeus coronatus have served as models for studies of toxicity, as well as in developmental biology (see paper by Shimizu and Nakamoto, 2014 in this issue), and serve as important environmental water quality indicator species or in soil toxicity tests, respectively. Studies examining the biology of bio-fouling organisms have examined different organisms such as the calcareous tube dwelling annelid Hydroides (Nedved and Hadfield, 2009) and the encrusting bryozoan Bugula neritina (Callow and Callow, 2002; Mukaki et al., 1997). The Bobtail Squid (Euprymna scolopes) has served as a model for understanding the nature of eukaryote-prokaryote mutualism (Lee et al., 2009). The freshwater snail Biomphalaria, which represents the aquatic host for a key human parasite Schistosoma has been studied in order to understand these host-parasite interactions, as a potential means to control this debilitating disease (Morgan et al., 2001).

Though the Spiralia currently lack a well established genetic model system, their tremendous strength lies in the rather broad understanding we have of their biology and, as mentioned above, their diverse body plans provide us with excellent subjects in which to undertake comparative studies aimed at understanding the evolution of triploblast bilaterian metazoans. It is only a matter of time before we develop tractable systems in which to undertake genetic analyses and, in fact, some labs are already working towards this end using species, such as the annelids Streblospio (see Rockman and Zakas, 2014, in this issue) and Platynereis (e.g., lab of Dr. Detlev Arendt, EMBL, Heidelberg, Germany).

The papers featured in this issue highlight many of these tremendous systems and provide examples of the remarkable work that is being carried out by investigators from around the globe. This body of work concentrates mainly on those groups that exhibit the ancestral mode of development that involves spiral cleavage.

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