Brefeldin A and monensin inhibit the D quadrant organizer in the polychaete annelids Arctonoe vittata and Serpula columbiana

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**SUMMARY** The D quadrant organizer is a developmental signaling center that is localized to the vegetal D quadrant in different spiral-cleaving lophotrochozoan embryos and may be homologous to axial organizing regions in other metazoans. Patterning by this organizing center creates a secondary developmental axis and is required for the transition from spiral to bilateral cleavage and later establishment of the adult body plan. Organizer specification in equal-cleaving embryos is thought to involve inductive interactions between opposing animal and vegetal blastomeres. To date, experimental demonstration of this interaction has been limited to molluscs and nemerteans. Here, we examine three families of equal-cleaving polychaete annelids for evidence of animal—vegetal contact. We find that contact is present in the polynoid, Arctonoe vittata, but is absent in the serpulid, Serpula columbiana, and in the oweniid, Owenia fusiformis. To interfere with cell signaling during the period predicted for organizer specification and patterning in A. vittata and S. columbiana, we use two general inhibitors of protein processing and secretion: Brefeldin A (BFA) and monensin. In A. vittata, we detail subsequent embryonic and larval adult development and show that treatment with either chemical results in radialization of the embryo and subsequent body plan. Radialized larvae differentiate many larval and adult structures despite the loss of bilateral symmetry but do so in either a radially symmetric or four-fold radially symmetric fashion. Our results suggest that the D quadrant organizer is functionally conserved in equal-cleaving polychaetes, but that details of its specification, induction, and patterning have diverged relative to other spiral-cleaving phyla.

**INTRODUCTION**

Developmental organizers are signaling centers that pattern the surrounding cells or region. A developmental organizer commonly patterns the early embryo through gastrulation for proper establishment of the adult body plan in diverse and distantly related animal phyla (Martindale 2005). These organizers were first identified through classical experiments and have since been shown to express similar sets of transcription factors and signaling pathway components (reviewed in Martindale 2005; Lebreton and Jones 2006). Within taxa as divergent as cnidarians, molluscs, arthropods, and chordates, the organizing regions are often localized, at least initially, to the vegetal pole. Although clear differences exist between clades, organizer loss or duplication results in a similar loss or duplication of the primary body axis in each (Crampton 1896; Wilson 1904; Browne 1909; Penners 1924; Spemann and Mangold 1924; Tyler 1930; Holm 1952). Morphologically, these organizing regions include the dorsal blastopore lip, or Spemann's organizer, in the frog, the blastopore rim and adult hypostome in anemones and polyps, the cumulus, a germ disk thickening, in spiders, and the vegetal D quadrant in molluscs and other spiral-cleaving lophotrochozoans. Here, we refer to these and other similar taxon-specific organizers collectively as axial organizers, i.e., developmental organizers required for proper establishment of the primary body axis. Although the homology of axial organizers and the body plans they give rise to remain to be determined, functional and molecular genetic similarities in development suggest that the now distinct axial organizers of extant metazoans may share a potentially ancient origin and that the ancestral organizer may have played a fundamental role in early body plan evolution. Thus, the comparative characterization of axial organizers in a variety of metazoans offers potential new insight as to the enigmatic origin and diversification of animal body plans, particularly with respect to poorly understood, but phylogenetically important, clades, including spiral-cleaving lophotrochozoans, such as annelids.
Spiral-cleaving lophotrochozoans include annelids, molluscs, nemerteans, kamptozoans, and polyclad flatworms (Halanych 2004). Early development is characterized by spiral cleavage, which, through its stereotypic patterns of division and determinant segregation, creates a 4-fold radially symmetric developmental architecture that is polarized along the animal–vegetal axis, allowing individual blastomeres to be identified in a nomenclature of quadrants and quartets (Wilson 1892; Sweet 1998; Lambert and Nagy 2002). Before gastrulation, a signaling center localized to the vegetal D quadrant, commonly known as the D quadrant organizer, patterns the early embryo, thereby establishing a secondary developmental axis (Crampton 1896; Clement 1962). As shown by patterns of division (van den Biggelaar 1977), cell lineage contribution (Dictus and Damen 1997), and gene expression (Lartillot et al. 2002b) in gastropod molluscs, organizer patterning transforms the developmental architecture, such that it becomes bilaterally symmetric and is polarized along two perpendicular axes: the primary animal–vegetal axis and the secondary organizer-dependent axis. The nature of this secondary axis is poorly understood. It is commonly referred to as either the anteroposterior or dorso-ventral axis, with the D quadrant or D quadrant midline as posterior or dorsal, respectively. However, cell lineage studies indicate that blastomere contributions at the time of patterning are neither axis nor structure specific and that the adult axes arise later in development after gastrulation (Dictus and Damen 1997). In molluscs, annelids, and nemerteans, experimental loss or duplication of the vegetal D quadrant results in the subsequent loss or duplication of bilateral symmetry in the embryo and later body plan, i.e., radialization and twinning (Crampton 1896; Wilson 1904; Tyler 1930; Clement 1952; Render 1983; Henry and Martindale 1987; Henry 2002). Thus, the D quadrant organizer is an axial organizer. In molluscs and nemerteans, the blastomere 3D functions as the organizer (Crampton 1896; Clement 1962; Wilson 1904; Henry 2002). In contrast to axial organizers in other metazoans, the D quadrant organizer acts only as a transient signaling center and is not maintained during gastrulation (Clement 1962). Still, in various molluscs and annelids, a number of genes characteristic of, although not specific to, axial organizers in other phyla are expressed during gastrulation within midline lineages whose origin is directly dependent upon patterning by the D quadrant organizer (Arendt et al. 2001; Nederbragt et al. 2002; Lartillot et al. 2002a, b). Overall, classical experimental and recent molecular studies collectively suggest that the D quadrant organizer in spiral-cleaving lophotrochozoans is potentially homologous at some level to axial-organizing regions in other metazoans.

Specification of the D quadrant organizer is often distinct in even closely related species, particularly in equal- versus unequal-cleaving embryos (Freeman and Lundelius 1992; van den Biggelaar and Haszprunar 1996). In general, organizer-specific determinants are localized to the vegetal cortex (Clement 1968; Guerrier 1968). In many unequal-cleaving embryos, such as the gastropod mudsnail *Ilyanassa obsoleta*, the vegetal region is then repeatedly segregated to a single-daughter blastomere through either unequal cleavage or polar-lobe formation during early divisions, such that, with establishment of the embryonic four quadrants at the four-cell stage, a single lineage is already destined to give rise to the D quadrant organizer. How 3D’s organizing function is later activated remains unclear in many unequal-cleaving embryos but must occur before the transition from spiral to bilateral cleavage, as this transition is dependent upon patterning by the organizing center (Clement 1962; Lambert and Nagy 2001). In equal-cleaving embryos, such as the gastropod limpet *Patella vulgata* and the pond snail *Lymnaea stagnalis*, the vegetal region is equally partitioned during cleavage and the vegetal lineages of all four quadrants remain equivalent, exhibiting the same developmental potential to act as the D quadrant organizer until midway through the sixth cleavage (van den Biggelaar and Guerrier 1979; Arnold et al. 1983; Freeman and Lundelius 1992). In this case, organizer specification involves selecting one of four equivalent 3Q vegetal blastomeres to take on the organizing role of 3D (van den Biggelaar 1977). This is achieved through contact-dependent, inductive interactions between cells in the animal and vegetal poles (van den Biggelaar and Guerrier 1979). Specifically, the animal 1q11 and vegetal 3Q blastomere quartets extend into the cleavage cavity at the 32-cell stage and establish contact with one another (van den Biggelaar 1977). Following contact, one of four vegetal blastomeres becomes highly central-ized within the cleavage cavity, occluding its counterparts from their animal contacts, and establishes contact with nearly all other blastomeres (van den Biggelaar 1977). Experimental inhibition of animal–vegetal extension and contact, including the use of monensin, a potent Na (+) ionophore, and Brefeldin A (BFA), an inhibitor of protein translocation in the Golgi, results in the radialization of subsequent development (Martindale et al. 1985; Kuhtreiber et al. 1988; Gonzales et al. 2007). In particular, a single 3Q blastomere is not centralized, the transition from spiral to bilateral cleavage does not occur, and the subsequent body plan is radially or 4-fold radially symmetric, similar to lobeless development in unequal-cleaving embryos (Martindale et al. 1985; Kuhtreiber et al. 1988; Gonzales et al. 2007). Despite noted differences in organizer specification, both equal- and unequal-cleaving embryos activate Mitogen-Activated Protein Kinase (MAPK) in 3D just before organizer patterning, and both are similarly radialized by treatment with an inhibitor of MAPK activation, U0126 (Lambert and Nagy 2001; 2003; Lartillot et al. 2002b), suggesting that the divergent specification systems converge onto a pathway or network before patterning. In addition, variations and intermediate forms of the two systems clearly exist, for instance, the slipper snail *Crepidula*
fornicata, where cleavage is unequal with a polar-lobe but animal–vegetal contact is required for organizer specification and where 4d, rather than 3D, may function as the organizing center (Henry 2006).

To determine whether 3D’s role as the D quadrant organizer in gastropods is representative of spiral-cleaving lophotrochozoan development in general, it is necessary to test for the organizer in other phyla, including annelids. Annelids are a paraphyletic clade of segmented, or secondarily non-segmented, worms (Halanych 2004). The group is composed of some 80 families split into three large groups: the polychaetes, oligochaetes, and clitellates. Monophyly and phylectic relations within and between the different groups are unresolved (Halanych 2004). However, equal-cleaving polychaetes may retain many ancestral features (Nielsen 2004). Development in polychaetes is described in classical studies (Wilson 1892; Mead 1897; Treadwell 1901) and is reviewed for the Nereid, Platynereis dumerilii (Fischer and Dorresteijn 2004). The presence of the D quadrant organizer in polychaetes is suggested by a number of different studies. The organizer-dependent transition to bilateral cleavage was first recognized in spiral development in the nereid, Neanthes (formerly Nereis), and is classically detailed for a number of equal-cleaving families, including the polynoid, Lepidonotus, the hesionid, Podarke, and the echiuran, Thalassema (Wilson 1892; Mead 1897; Treadwell 1901; Torrey 1903). In contrast to molluscs, this transition in polychaetes occurs after the onset of the sixth cleavage in the animal 1q and vegetal 3Q quartets, rather than before it (Freeman and Lundelius 1992).

Despite this difference, experimental work in polar lobe-forming species indicates that the vegetal D quadrant functions as an organizing center. In the polar lobe-forming sabellarian, Sabellaria cementarium, removal and suppression of the polar lobe at the first cleavage, which normally localizes to the vegetal region of the D blastomere, results in the respective loss and twinning of bilateral symmetry in the embryo and body plan (Hatt 1932; Novikoff 1938; Render 1983). In the equal-cleaving polynoid, Lepidonotus sp., animal–vegetal contact occurs before the transition to bilateral cleavage (Mead 1897) and is suggestive of organizer specification. However, in contrast to equal-cleaving molluscs and nemerteans, contact in Lepidonotus is between the animal 1q and 4Q sixth-cleavage quartets, rather than the 1q and 3Q fifth-cleavage quartets (Freeman and Lundelius 1992). In the equal-cleaving serpulid Hydroides, MAPK is activated in the sixth cleavage 4d mesentoblast, a pattern that may be homologous to its organizer-related activation in the fifth-cleavage 3D blastomere in molluscs (Lambert and Nagy 2003). Overall, characteristic elements of D quadrant organizer specification and patterning in molluscs are present in annelids (Freeman and Lundelius 1992; Lambert and Nagy 2003) but the presence and function of the organizing center remain untested in equal-cleaving polychaetes.

To test for the D quadrant organizer in equal-cleaving polychaetes, we used the chemicals BFA and monensin, two small organic molecules that rapidly and reversibly inhibit protein processing and secretion in eukaryotes through distinct mechanisms (Mollenhauer et al. 1990; Dinter and Berger 1998; Mayer 2003). As demonstrated in P. vulgata and other molluscs (Kuhntreiber et al. 1988; Gonzales et al. 2007), both chemicals show great promise as simple yet effective experimental tools with which to inhibit the inductive interactions underlying organizer specification in species where more specific molecule genetic tools have not yet been developed. We initially looked for evidence of animal–vegetal contact in three families of equal-cleaving polychaetes: the polynoid, Arctonoe vittata, the oweniid, Onnini fusiformis and the serpulid, Serpula columbiana. We then tested for the presence and function of the organizing center in A. vittata and S. columbiana by using BFA or monensin to interfere with cell signaling during the interval predicted for organizer specification and patterning, i.e., after fifth cleavage but before the onset of bilateral cleavage. We examined the effect of treatment on subsequent development, focusing on A. vittata in particular. Our results demonstrate that a functional homolog of the D quadrant organizer is present in equal-cleaving polychaetes, although its blastomere identity remains unknown.

METHODS

Collection and culturing
A. vittata, O. fusiformis, and S. columbiana adults were collected from intertidal localities on San Juan Island, Washington between 1996 and 2004. A. vittata spawned after removal from its host, the keyhole limpet Diadema aspera, whereas O. fusiformis and S. columbiana spawned upon removal from their tubes. Successful A. vittata fertilizations exhibited >90% synchrony within 5 min of first and second cleavage. Developmental synchrony of O. fusiformis and S. columbiana embryos was uncharacterized, or was variable and <90% within 5 min. Embryos and larvae were cultured in 0.45 μm filtered seawater (FSW) at ambient sea table temperatures (10–12°C).

Treatment with BFA and monensin
One millimolar BFA (Sigma, St. Louis, MO, USA) and monensin (Sigma) stocks were prepared in 100% ethanol and stored for up to 6 months at −20°C. Based on previous BFA and monensin dose–response experiments in the gastropod mollusc P. vulgata (Kuhntreiber et al. 1988) (per obs), A. vittatae and S. columbiana embryos were incubated for 1–5 h in freshly prepared 1 μM BFA or monensin FSW solutions. Embryos were then washed five times with FSW after treatment. To test for the stage-specific specificity of treatment relative to organizer specification versus patterning in A. vittata, BFA and monensin treatments were initiated either before or else after the onset of bilateral cleavage. Treatments in S. columbiana were initiated only before the onset of bilateral cleavage. To show that observed experimental phenotypes are
specific to either BFA or monensin and not to the solute EtOH, control experiments of 0.001% EtOH treatment were made in *A. vittata*. To score treatments in all species, experiments of tens of thousands of embryos were initially surveyed on a dissecting scope. Subsamples of 50–100 larvae were then examined at higher magnifications on dissecting or compound microscopes and scored as normal, radialized, abnormal, or other.

**Embryonic and larval analysis**

To assess animal–vegetal contact in *A. vittata*, *O. fusiformis*, and *S. columbiana*, and cleavage patterns in *A. vittata*, embryos were fixed every 20–30 min through early gastrulation and prepared for whole-mount or semithin sectioning according to Kuhtreiber et al. (1988). For each stage, 20–30, or more, embryos were examined and sketched, and of these, a selected few were either photographed or else drawn using camera lucida and the drawings were then scanned and traced using Adobe Illustrator and Photoshop. In *A. vittata*, all cells and patterns of cleavage (spiral vs. bilateral) were identified for each set of divisions through the 64-cell stage; however, for later stages, only landmark cells and those preparing to divide were identified. In *O. fusiformis* and *S. columbiana*, only blastomeres of the more animal and vegetal quartets were fully identified and the transition to bilateral cleavage was not characterized. Overall, developmental rates (timing of cell divisions after fertilization and the onset of ciliation and swimming) in all three species were nearly identical, i.e., within 20–30 min or less of one another. To assess larval morphology in *A. vittata*, larvae were photographed live, or fixed and analyzed by scanning electron microscopy according to Kuhtreiber et al. (1988). To assess muscle differentiation in *A. vittata*, larvae were photographed live, or fixed and stained with Phalloidin (Molecular Probes, Carlsbad, CA, USA) for confocal analysis according to Wanninger and Haszprunar (2002). A minimum of 20 larvae were examined per stage.

**RESULTS**

**Animal–vegetal contact in equal-cleaving polychaetes**

To survey initially for animal–vegetal contact in different families of equal-cleaving polychaetes, we fixed a developmental series for light microscopy, the series extending from fertilization through the onset of gastrulation, in *A. vittata*, *O. fusiformis*, and *S. columbiana*. In *A. vittata*, animal 1q

111

and 1q

112

and vegetal 4q and 4Q quartets extended into the cleavage cavity following their formation at the 44-cell stage and established contact by the 56-cell stage, some 2 h after the 32-cell stage onset and before the transition to bilateral cleavage (Figs. 1A, 2, and 4, A–E). In contrast, the same blastomeres in *O. fusiformis* and *S. columbiana* exhibited only a slight extension into the cleavage cavity. In these embryos, the cleavage cavity was still present around the 128-cell stage, some 4–5 h after onset of the 32-cell stage (Fig. 1, B and C). In *O. fusiformis*, a more general animal–vegetal contact was observed several hours later but appeared to be a feature of gastrulation. *S. columbiana* was not examined at these later stages.

**Development in *A. vittata***

To design experiments to test for the D quadrant organizer in *A. vittata*, we first made a detailed description of early development. Proceeding from fertilization, the primary features of early development were spiral cleavage, a transition to bilateral cleavage, gastrulation in the larval trochophore, and initial establishment of the adult body plan in the larval presetiger, i.e., establishment of adult axes and analagen but limited adult structures.

In the embryo, we focused on the presence and timing of developmental features characteristic of organizer specification and patterning in equal-cleaving molluscs and nemerteans. These features included animal–vegetal interactions (specification) and the transition from spiral to bilateral cleavage (patterning). The division chronology of early cleavage in *A. vittata* is previously undescribed and is summarized in Fig. 2. Divisions were synchronous within, but not necessarily between, blastomere quartets along the animal–vegetal axis (Fig. 2). Sets of divisions occurring in rapid succession exhibited a slight variability with regard to the sequence of division between embryos. The fifth cell-cycle was the last cycle to be fully completed before the onset of the next.

![Fig. 1. Animal–vegetal contact is present in *Arctonoe vittata* but absent in *Owenia fusiformis* and *Serpula columbiana*. (A) shows contact between the 1q

111

and 4Q quartets at 9 h in *A. vittata* (lateral). (B) and (C) show the absence of contact at 11 h in *O. fusiformis* and in *S. columbiana*, respectively (lateral and lateral). Scale bar = 20 μm. c, contact; cc, cleavage cavity.](image-url)
concluded with the division of the 2q quartet at the 32-cell stage (Fig. 2). A subsequent resting stage, which occurs in equal-cleaving gastropods, was absent and the sixth cell cycle was initiated in the animal 1q11 blastomeres and vegetal 3Q blastomeres (Fig. 2). Cell divisions remained spiral until midway through the sixth cell cycle, when a localized transition to bilateral cleavage occurred in blastomeres along the D quadrant midline (Figs. 2 and 4, F and G). A cleavage cavity was present in the embryo beginning at the 32-cell stage (Fig. 4A).

Newly formed animal 1q111 and 1q112 and vegetal 4q and 4Q blastomere quartets extended into the cleavage cavity at the 44-cell stage and established contact with one another around the 56-cell stage (Figs. 2 and 4, B–E). Contact occurred specifically between the animal-pole 1q111 and vegetal-pole 4Q quartets and between the more peripheral animal 1q112 and vegetal 4q quartets (Fig. 4, B–E). Both sets of quartets maintained contact through the onset of bilateral cleavage and into later development (Fig. 4, B–E). Centralization of a single

Fig. 2. Arctonoe vittata undergoes a developmental transition from spiral to bilateral cleavage. The division chronology of early development in A. vittata shows the developmental timing of animal–vegetal contact and the subsequent transition to bilateral cleavage.
Fig. 3. Brefeldin A (BFA) and monensin treatment before bilateral cleavage results in radial development in Arctonoe vittata and Serpula columbiana. (A) shows a schematic of the developmental timing of BFA and monensin treatments and shows that treatments initiated after onset of the 32-cell stage result in radial development in A. vittata and S. columbiana, whereas treatments initiated after the onset of bilateral symmetry result in normal development in A. vittata (S. columbiana was not tested). (B) shows a bar chart showing the number of normal versus radialized embryos resulting from 32-cell onset BFA treatments made at different concentrations and shows that concentrations of 1 μM radIALIZED development, whereas concentration of 0.1 μM had no discernable effect. (C) and (D) show normal and monensin-radialized 8-day A. vittata presetigers. (C) shows normal development with the body plan symmetrically organized about the adult anteroposterior and dorsoventral axes (posterior). (D) shows monensin-radialized development with the body plan radially organized about a novel aboral–oral axis (lateral). (E–H) show normal and monensin-radialized gallocyanin-stained 7-day S. columbiana presetigers. (E and F) show normal development with the body plan symmetrically organized about the adult anteroposterior and dorsoventral axes (lateral and posterior). (G and H) show monensin-radialized development with the body plan radially organized about a novel aboral–oral axis (lateral and oral). Scale bar = 10 μm. a, anus; ab, aboral; an, anterior; d, dorsal; e, extruded material; es, eyespot; fg, foregut; g, uncharacterized gut; hg, hindgut; o, oral; po, posterior; pr, prototroch; s, stomodeum; sl, stomodeum-like opening; st, stomach; v, ventral.
blastomere did not occur before the onset of bilateral cleavage, or in later stages (Fig. 4E). After contact, divisions became increasingly asynchronous both between and within quartets (Fig. 4, F and G). Patterns of division asynchrony within quartets were bilaterally symmetric about and polarized along the B–D quadrant midline (Fig. 4, F and G).

The transition to bilateral cleavage was initiated at the 56-cell stage, shortly after animal–vegetal contact (Figs. 2 and 4). Although not all divisions contributing to this transition were true bilateral cleavages, i.e., divisions in which the cleavage spindle is oriented with respect to a plane of bilateral symmetry, division asynchronies and asymmetries within quartets were bilaterally symmetric with respect to the B–D quadrant midline and created a more global pattern of bilateral symmetry in the embryo. The transition to bilateral cleavage was first evident during the sixth division of the 2q^ quartet (Fig. 4, F and G). Blastomere 2d^ was bisected by the D quadrant midline and future plane of bilateral symmetry, but divided spirally, producing a large 2d^ blastomere and a small 2d^ blastomere (Fig. 4G). Equivalent 2q^ blastomeres outside the D quadrant midline also divided spirally, but with a reversal in the size of daughter blastomeres, producing small 2a^ blastomeres and large 2a^ blastomeres (Fig. 4, F and G). At the seventh division, patterns of asynchrony within the 2q^ daughter blastomeres were bilaterally symmetric with respect to the B–D quadrant midline, with 2d^ dividing before 2a^ and 2d^ dividing after 2a^ (Fig. 4, F and G). In the third quartet, the sixth division of 3q occurred before animal–vegetal contact and was spiral with radial symmetry (Fig. 2). The seventh division of 3q^ occurred after contact and was a mixture of bilateral and spiral cleavages with bilateral symmetry. Blastomeres 3c^ and 3d^ lay to either side of the D quadrant midline and divided in mirror image, whereas 3a^ and 3b^ lay to either side of the opposing B quadrant midline and divided slightly later with spiral cleavage (Fig. 4G). Presumably, the 4d mesentoblast underwent its characteristic bilateral division across the plane of symmetry, but this division was not observed through the 93-cell stage (Fig. 2). The 4D blastomere was equally likely to be a cross-furrow or noncross-furrow blastomere in different embryos. Overall, novel asymmetries and asynchronies were observed within quartets relative to the D quadrant midline following animal–vegetal contact. These asymmetries and asynchronies suggested the underlying formation of a secondary developmental axis and transformed the developmental architecture from 4-fold radial to bilateral symmetry (Fig. 2). In the prototrochal region, divisions remained synchronous and spiral through the 93-cell stage; however, in contrast to the orientation predicted by spiral cleavage, seventh division of the 1q^ quartet, i.e., the quartet that previously established contact with the 4q blastomeres, was not dextrorotary but either laeotropic or, perhaps, radial (Fig. 4K). Ciliation of the primary trophoblasts, which were arranged in a 4-fold radially symmetric pattern of four clusters of four cells around the middle of the embryo, and of the nonmotile apical tuft, which was located at the animal pole, began at around the 68-cell stage (Fig. 4, B–D and K).

We next examined larval development, focusing on morphological features that characterized the developing body plan of the gastrulating trochophore and subsequent presetiger. A distinct blastopore was not observed in the embryo through the 111-cell stage (data not shown); however, a general ingression of cells at the vegetal pole was later observed in the 18-h trochophore (Fig. 5D). The blastopore became slit-like and appeared to give rise to both the stomodeum and anus through convergence of the lateral lips by 27 h (Fig. 5, A–C). However, it is possible that either the stomodeum, anus, or both formed as secondary invaginations unrelated to the blastopore during the interval between 18 and 27 h. The stomodeum and anus formed opposing invaginations at either end of what had been the vegetal pole and were bisected by the B–D quadrant, or now bilateral, midline (Fig. 5, C and E). The apical tuft was present at the anterior tip of the pre trochal episphere in 12- to 27-h trochophores, whereas paired meniscotrochal cilia lay to either side of the anterior ventral midline (Fig. 5D). The four sets of primary trophoblasts shifted from their initially opposing positions at the midpoint of the embryo to form a ring of beating cilia, the prototroch. The prototroch gradually became the base of the larva over the course of vegetal ingestion and gastrulation (Fig. 5D).

Overt morphogenetic movements appeared to be localized to the posterior hyposphere, i.e., posttrochal or future trunk region. Here, the initial stomodeal invagination migrated, perhaps through ingestion of B quadrant midline lineages, along the B quadrant midline to a more anteroventral position just beneath the prototroch and gave rise to the stomodeum proper and foregut in the pre tiger. A distinct blastopore was not observed in the embryo through the 111-cell stage (data not shown); however, a general ingression of cells at the vegetal pole was later observed in the 18-h trochophore (Fig. 5D). The blastopore became slit-like and appeared to give rise to both the stomodeum and anus through convergence of the lateral lips by 27 h (Fig. 5, A–C). However, it is possible that either the stomodeum, anus, or both formed as secondary invaginations unrelated to the blastopore during the interval between 18 and 27 h. The stomodeum and anus formed opposing invaginations at either end of what had been the vegetal pole and were bisected by the B–D quadrant, or now bilateral, midline (Fig. 5, C and E). The apical tuft was present at the anterior tip of the pre trochal episphere in 12- to 27-h trochophores, whereas paired meniscotrochal cilia lay to either side of the anterior ventral midline (Fig. 5D). The four sets of primary trophoblasts shifted from their initially opposing positions at the midpoint of the embryo to form a ring of beating cilia, the prototroch. The prototroch gradually became the base of the larva over the course of vegetal ingestion and gastrulation (Fig. 5D).
Fig. 4. Monensin-radialized Arctonoe vittata embryos establish animal–vegetal contact but do not make the transition to bilateral cleavage patterns. (A–G) show normal development. A shows initiation of animal–vegetal extension into the cleavage cavity (lateral). (B–E) show contact between the central 1q^111 and 4Q quartets and between the peripheral 1q^112 and 4q quartets (animal midsction +3, 0, −3 μm, and lateral). (F) and (G) show bilateral cleavage in sixth division asymmetry of 2q^2 and seventh division asynchrony of 2q^1 and 3q, respectively (animal and vegetal). (H–J) show monensin-radialized development. (H) shows contact between the central 1q^111 and 4Q quartets (lateral). (I and J) show spiral cleavage with 4-fold radial symmetry in the 2q^2 and 3q quartets, respectively (animal and vegetal). Brefeldin A-radialized embryos exhibited identical patterns of contact and division. (K) and (L) show the laeotropic, rather than dexiotropic, seventh division of the 1q^112 and its similar orientation to the sixth division in the 1q^12 quartet. (K) shows a 68-cell control (animal). (L) shows a 68-cell monensin-radialized embryo (animal). Semithin sections, (A–F), (H), (I), (K), (L); Whole-mounts, (G), (J); *dividing cells.
Fig. 5. Brefeldin A (BFA)-radialized larvae undergo radial patterns of gastrulation to form a radially symmetric mouth at the oral-pole. (A–K) show scanning electron micrographs of normal and BFA-radialized development. (A–G) show the normal transformation of the blastopore into mouth and anus in the trochophore and presetiger. (A) shows the blastopore as a slightly oblong invagination at the vegetal pole in an 18-h trochophore (vegetal). (B) and (C) show the apparent convergence of the lateral lips to form the stomodeum and anus in 27- and 40-h trochophores (ventral). (D) and (E) show 2-day trochophores with fully formed mouth and anus at opposing ends of the posterior pole and bisected by the plane of bilateral symmetry (lateral and ventral). (F) and (G) show bending of the mouth by 90° and ciliary differentiation of the mouth and neurotroch in 4-day presetigers (lateral and posterior). (H–K) show the radial transformation of the blastopore into a radial mouth at the oral pole in BFA-radialized trochophores and presetigers. (H) and (I) show stomodeal-like invaginations formed at 90° to one another around a central disk, or, perhaps, invagination, in 2-day trochophores. (J) and (K) show a single circular ciliated oral field with a single stomodeal-like opening in 4-day presetiger. Scale bar = 10μm; a, anus; at, apical tuft; bl, blastopore; d, central disk; ll, blastopore lateral lips; in, stomodeal-like invagination; lg, lateral glands; m, meniscotrochal cilia; n, neurotroch; pr, prototroch; s, stomodeum; sl, stomodeal-like opening.

BFA and monensin treatment in A. vittata
In a preliminary investigation of the effect of BFA and monensin treatments on development in A. vittata, embryos were incubated in either drug beginning at the 32-cell stage and through the onset of bilateral cleavage (Fig. 3A). This interval includes the period during which organizer specification and patterning is predicted to occur. Following highly synchronous fertilizations, a 1.5–3.5-h treatment with a minimum concentration of 1μM BFA initiated at timepoints within 90 min after the onset of fifth cleavage resulted in nearly 100% radialized development in thousands of embryos (Fig. 3, A and B). Consequently, there was a loss of bilateral

band beneath the prototroch (Fig. 6E). Paired eyespots were present at 8 days (Fig. 3C).
Radialized development in A. vittata

To further explore the apparent loss of patterning by the organizing center in A. vittata, we characterized the development of monensin- and BFA-radialized embryos and larvae. In radialized embryos, animal–vegetal extension of the 1q^111, 1q^112, 4q, and 4Q quartets still occurred during the period of treatment (Fig. 4H). However, in contrast to normal development, subsequent patterns of division within quartets remained synchronous and spiral, with a 4-fold radial symmetry throughout the embryo. This radiaUzation of the embryo represented a loss of divisions specific to the D quadrant midline (Fig. 4, I and J). Division of the 2q^2 quartet resulted in small 2q^21 blastomeres and large 2q^22 blastomeres (Fig. 4I), identical to 2a^2–2c^2 divisions in controls. Similarly, the seventh division of the 2q^22 and 2q^21 quartets remained spiral and synchronous within each quartet (data not shown). The seventh division of the 3q^2 quartet was also spiral and synchronous, identical to 3a^2–3b^2 divisions in controls (Fig. 4J). The seventh division of the 4q quartet did not occur before the 92-cell stage. In the pretrochal region, seventh division of the 1q^112 quartet was either laeotropic or possibly radial (i.e., spindles aligned parallel to the animal–vegetal axis), a pattern identical to controls (Fig. 4L). Formation of the多语句...
internalization of the vegetal region during gastrulation. Thus, the vegetal region failed to occur, as a single stomodeal-invasion. In forming the stomodeum, 48-h radialized trochophores exhibited four sites of invagination. These sites were perpendicular to one another and formed a 4-fold radially symmetric ring around a disk region that centered on the vegetal pole (Fig. 5, H and I). Owing to larval retention of the embryonic vitelline membrane and the radial symmetry of development, it was not possible to clearly identify either the invaginations or disk region as stomodeal or anal; however, the entire region gave rise to a large circular stomodeal-like structure at the oral pole, suggesting that the four invaginations and disk were stomodeal in nature (Fig. 5, J and K). The stomodeum and foregut were ciliated similar to controls but, in contrast to controls, the stomodeum now formed a flat radial circle that was bent at a right angle (Figs. 5, J and K, and 6, F and G). Similarly, the gut, which normally runs anterior, turns 180 degrees, and runs posterior, was a simple in-pocketing that ran parallel to the aboral–oral axis. The gut appeared to be everted in the later stages of development (data not shown).

In the pretrochal region, anterior tufts of meniscotrochal cilia and larval lateral glands differentiated in a 4-fold radial pattern oriented at right angles from one another (Fig. 6, C and D). Larvae exhibited muscular contractions and Phalloidin staining of preeversion presetigers revealed radial rings of muscle bands encircling the stomodeum and foregut, stacked along the aboral–oral axis and extending up into the aboral region that centered on the vegetal pole (Fig. 5, H and I). Owing to larval retention of the embryonic vitelline membrane and the radial symmetry of development, it was not possible to clearly identify either the invaginations or disk region as stomodeal or anal; however, the entire region gave rise to a large circular stomodeal-like structure at the oral pole, suggesting that the four invaginations and disk were stomodeal in nature (Fig. 5, J and K). The stomodeum and foregut were ciliated similar to controls but, in contrast to controls, the stomodeum now formed a flat radial circle that was bent at a right angle (Figs. 5, J and K, and 6, F and G). Similarly, the gut, which normally runs anterior, turns 180 degrees, and runs posterior, was a simple in-pocketing that ran parallel to the aboral–oral axis. The gut appeared to be everted in the later stages of development (data not shown).

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### Table 1. Scoring normal and radialized development in *A. vittata*, *S. colombiana*, and *O. fusiformis*

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Character change(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sarcopterygi</strong></td>
<td>200.* Metapterygial segmentation: absent (0) → present (1). This change is optimized to the sarcopterygian stem group below the divergence of the extended ‘onychodont’ clade comprising <em>Onychodus, Strunius, Achoania,</em> and <em>Psarolepis.</em></td>
</tr>
<tr>
<td><strong>Latimeria</strong></td>
<td>196. Pectoral fin (A: 6): unrotated (0) → rotated (1). Although the state for this character cannot be assessed for <em>Shoshonia,</em> the pectoral fin appears unrotated in <em>Miguashaia.</em> If <em>Shoshonia</em> is placed below or as sister to <em>Miguashaia,</em> then pectoral fin rotation would have evolved on the branch leading to <em>Latimeria</em> in Fig. 3. Regardless of the condition in <em>Shoshonia,</em> the cladistic solution presented here indicates that fin rotation is homoplastic between lungfishes and coelacanths.</td>
</tr>
<tr>
<td><strong>Tetrapodmorp</strong></td>
<td>198. Pectoral fin web: asymmetrical (0) → symmetrical (1).</td>
</tr>
<tr>
<td><strong>Triktaadik</strong></td>
<td>201. Pectoral radials: all bear fin rays or lepidotrichia (0) → some ‘naked’ (1).</td>
</tr>
<tr>
<td><strong>Sauripterus</strong></td>
<td>204.* Ball-shaped caput humeri (JA: 15): absent (0) → present (1).</td>
</tr>
<tr>
<td><strong>Tiktaadik and</strong></td>
<td>212. Distal fin or limb domain expanded across A-P axis (C: 1): no (0) → yes (1).</td>
</tr>
<tr>
<td><strong>higher</strong></td>
<td>214. Pectoral fin radials (F: 116): unjointed (0) → jointed (1).</td>
</tr>
<tr>
<td><strong>Triktaadik</strong></td>
<td>215. ‘Preaxial’ radials only (0) → ‘preaxial’ plus ‘postaxial’ radials (1).</td>
</tr>
<tr>
<td><strong>tetrapodomorph</strong></td>
<td>201. Pectoral radials: do not bifurcate (0) → bifurcate (1).</td>
</tr>
<tr>
<td><strong>Dipnomor</strong></td>
<td>205.* Body of humerus (AJ: 89): cylindrical (0) → flattened with rectangular cross-section (1).</td>
</tr>
<tr>
<td><strong>Glyptolepis</strong></td>
<td>209. Radius of equal length or shorter than humerus (C: 17): no (0) → yes (1).</td>
</tr>
<tr>
<td><strong>Neoceratodus</strong></td>
<td>114. Pectoral fin radials (F: 117): ‘preaxial’ radials only (0) → ‘preaxial’ plus ‘postaxial’ radials (1).</td>
</tr>
<tr>
<td><strong>Neoselachii</strong></td>
<td>215. ‘Preaxial’ radials only (0) → ‘preaxial’ plus ‘postaxial’ radials (1).</td>
</tr>
<tr>
<td><strong>Taxon</strong></td>
<td>200.* Metapterygial ‘axis’ of pectoral fin skeleton (jointed ‘axes’ only) (F: 115): short (0) → long (1).</td>
</tr>
<tr>
<td><strong>Neoceratodus</strong></td>
<td>208.* Humeral radials (A: 8): present (0) → absent (1).</td>
</tr>
<tr>
<td><strong>Pecora</strong></td>
<td>202. Pectoral radials: all bear fin rays or lepidotrichia (0) → some ‘naked’ (1).</td>
</tr>
<tr>
<td><strong>Neoceratodus</strong></td>
<td>204.* Ball-shaped caput humeri (JA: 15): absent (0) → present (1).</td>
</tr>
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<td><strong>Pectoral</strong></td>
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<td>209. Radius of equal length or shorter than humerus (C: 17): no (0) → yes (1).</td>
</tr>
</tbody>
</table>

blastopore and subsequent morphogenetic movements were not observed directly; however, anterior migration of the vegetal region failed to occur, as a single stomodeal-like structure differentiated with radial, or perhaps 4-fold radial, symmetry at what had been the vegetal pole (Fig. 5, I and K). In general, the differentiation of larval adult structures occurred in a radially or 4-fold radially symmetric pattern about a novel aboral–oral axis. The aboral pole of the aboral–oral axis corresponded to the animal pole; however, despite the general similarity of spatial position, the lineage composition of the oral pole did not correspond to that of the vegetal pole. This difference was due to the radially symmetric internalization of the vegetal region during gastrulation. Thus, the aboral–oral axis of radialized larvae was parallel to but distinct from the animal–vegetal axis of the embryo.

The origins of the radialized stomodeum were unclear. However, based on the later relative position of the proto-troctoch, it appeared to be derived from second- or third-quartet lineages from all four quadrants. In forming the stomodeum, 48-h radialized trochophores exhibited four sites of invagination. These sites were perpendicular to one another and formed a 4-fold radially symmetric ring around a disk
A developmental signaling center homologous to D quadrant vegetal contact. Although the early embryo and trochophore segments were made at similar developmental stages in *S. co-*animal-vegetal contact between the fifth cell-cycle Iq” and tive to other spiral-cleaving phyla. Specification with overt polychaetes has diverged, both among polychaetes and related to other spiral-cleaving phyla. Given that radialization in other spiral-cleaving ancestors, or are secondarily equal-cleaving and have conserved noncontact specification from an unequal-cleaving ancestor. Alternatively, in the equal-cleaving hesionid *P. obscura*, overt contact is initially absent. However, fine filamentous protrusions extend between animal and vegetal blastomeres before bilateral cleavage (Treadwell 1901) and it is suggested, although not demonstrated, that filamentous contact may underly organizer specification in *P. obscura* and *Hydroides* sp. (Lambert and Nagy 2003). It is unclear from our results whether filamentous contact occurs in *O. fusiformis* and *S. columbiana*, or whether it might precede overt contact in *A. vittata*. If present in all three, it could provide a unifying mechanism for organizer specification in contact and noncontact equal-cleaving polychaetes, or even different equal- and unequal-cleaving species and phyla. Clear comparative documentation and functional studies of non-, filamentous, and overt animal–vegetal contact will help to resolve the evolution and conservation of organizer specification in equal-cleaving polychaetes and other spiral-cleaving lophotrochozoans.
Developmental timing of organizer patterning

We found that development in *A. vittata* is generally similar to other equal-cleaving polychaetes (Mead 1897; Treadwell 1901; Torrey 1903) and in agreement with previous descriptions (Pernet 2000). One distinction is that in *Podarke* and *Thalassoma*, the 1q112 of the annelid cross are the first quartet to exhibit bilateral symmetry in cleavage, with asymmetric divisions at the B quadrant midline and symmetric divisions at the D quadrant midline and subsequent divisions asynchronous between midlines (Treadwell 1901; Torrey 1903). In the polynoid, *Lepidonotus* sp., 1q112 divisions are all equivalent and spiral, but become bilateral and asynchronous in subsequent patterns of divisions, similar to subsequent divisions in *Podarke* and *Thalassoma* (Mead 1897). In *A. vittata*, 1q112 divisions are identical to *Lepidonotus*, but we did not follow subsequent divisions in sufficient detail to determine whether they take on a bilateral pattern. If bilateral, it is possible that, in the polynoids *A. vittata* and *Lepidonotus* sp., the organizer-dependent secondary axis is established within the 1q112 quartet, as it is in *Podarke* and *Thalassoma*, but is not morphologically evident. More generally, the timing of the developmental versus morphological onset of the organizer-dependent secondary axis is a critical distinction that will be central to understanding the D quadrant organizer in *A. vittata* and other spiral-cleaving lophotrochozoans.

BFA and monensin developmental targets in annelids and molluscs

The direct targets of BFA and monensin are distinct (Mollenhauer et al. 1990; Dinter and Berger 1998; Mayer 2003); however, both chemicals similarly inhibit protein processing and secretion and treatment with either results in an identical radialization of development. This suggests that downstream molecular, subcellular, or physiological effects of either treatment converge to interfere with the same developmental process or processes and that the radialization phenotype is not the result of chemical-specific toxicity. Both the radialization phenotype, with its characteristic loss of bilateral symmetry in the embryo and body plan, and the specificity of treatment, i.e., radialization is limited to treatments made during the period predicted for organizer specification and patterning, suggest that BFA and monensin, either directly or indirectly, inhibit patterning by the D quadrant organizer. It is also possible that additional, unrecognized developmental processes unrelated to the organizer also act within the same developmental window, are sensitive to treatment, and have contributed to the observed phenotypes.

Organizer specification in molluscs versus annelids

BFA- and monensin-radialized development in *A. vittata* is, in a general sense, similar to BFA- and monensin-radialized development in equal-cleaving gastropods and chitons (Kuhntreiber et al. 1988; Gonzales et al. 2007). However, while the direct targets and immediate effects of either chemical are largely conserved across eukaryotes (Mollenhauer et al. 1990; Dinter and Berger 1998; Mayer 2003), and although within a species, the initially distinct effects of each chemical converge to radialize development similarly, details of how radialization is achieved differ between phyla, if not between species, in a development-specific manner. In both annelids and molluscs, development is radialized only if treatment is initiated before the transition to bilateral cleavage. In radialized gastropods and chitons, BFA and monensin inhibit organizer specification by blocking, at the morphological level, 1q11 and 3Q extension and contact (Kuhntreiber et al. 1988; Gonzales et al. 2007). However, similar treatments in *A. vittata* do not block extension and contact still occurs in radialized development, making it unclear whether contact is functionally required. In addition, the absence of clear morphological markers also makes it unclear when it is that BFA and monensin act during organizer specification and patterning, or even if they interfere with the same or different features of either process. Still, the actions of both drugs are clearly downstream of extension and contact and, therefore, distinct from their mode of action in molluscs. The combined absence of centralization and occurrence of an additional division in animal and vegetal quartets make it unclear which blastomeres, or blastomeres, function as the organizing center in equal-cleaving polychaetes. Similar to other spiral-cleaving lophotrochozoans tested, the organizing center might be localized to vegetal lineages of the D quadrant or D quadrant midline, but we have no direct evidence of this in *A. vittata* and, given clear morphological differences with regard to contact and centralization, the organizer might reside in additional, or distinct, lineages. Based on MAPK activation patterns, it is suggested that 4d in polychaetes acts as the functional homolog of 3D in molluscs (Lambert and Nagy 2003). However, MAPK has numerous roles in early development (Schohl and Fagotto 2002) and it is unclear whether its role in *Hydroides* embryos is related to the organizer, or might be directed toward other potentially related or unrelated functions, such as endomesoderm specification. Future blastomere ablation experiments within the animal and vegetal quartets, functional assays of MAPK in polychaetes, and the identification and functional testing of a clear molecular marker for the D quadrant organizer are all important steps toward establishing whether contact is required for organizer specification and which blastomeres take on its organizing function in polychaetes.

Models of organizer specification and patterning

Current mollusc-based models of organizer specification and patterning are problematic when applied to equal-cleaving polychaetes. A stochastic model has been proposed for orga-
nizer specification in equal-cleaving gastropods (Arnolds et al. 
1983). In this model, all four vegetal 3Q blastomeres make 
over a contact and a single blastomere, designated 3D, 
select as the organizer through contact-dependent animal 
interactions (Arnolds et al. 1983). However, despite develop-
mental equivalence within the 3Q quartet, 3D is almost al-
ways a cross-furrow, and only rarely a noncross-furrow,
blastomere (van den Biggelaar 1976, 1977; van den Biggelaar 
and Guerrier 1979). Therefore, it is proposed that organizer 
specification is stochastically determined by differences in the 
relative amount or duration of contact that each 3Q blasto-
more makes with overlying animal quartets and that, due to 
the geometry of spiral cleavage, the more central and elevated 
cross-furrow blastomeres have an inherent advantage. This 
model has two problems in equal-cleaving polychaetes. The 
first is that both S. columbiana and O. fusiformis do not es-
ablish animal-vegetal contact and so organizer specification 
does not involve overt contact-dependent interactions with 
the animal pole. The second is that A. vittata establishes overt 
contact, but animal contacts remain equivalent between veg-
etal blastomeres and the plane of bilateral symmetry, which is 
a proxy for the location of the organizing center, is as likely to 
bisect cross-furrow as noncross-furrow blastomeres. These 
features both suggest that spatially and stochastically deter-
ined relative differences in animal contacts by vegetal blastomeres do not play a role in D quadrant organizer spec-
ification in A. vittata. An understanding of what, if any, el-
ements of organizer specification are conserved between molluscs and annelids will require the characterization of un-
derlying molecular mechanisms in each clade.

Mechanistic models of organizer patterning in molluscs commonly suggest that the D quadrant organizer acts as a signaling point-source within the radially symmetric develop-
mental architecture established by spiral cleavage (Raven 
1976; van den Biggelaar and Guerrier 1979; Sweet 1998; 
Lambert and Nagy 2003). In general, these models argue that 
3D centralization places 3D in contact with nearly all other blastomeres in the embryo but that the onset or duration of contact and the position of blastomeres relative to the nucle-
us, vegetal cortex, or some unknown subcellular feature of 3D 
diffs between blastomeres in a bilaterally symmetric fashion. It is this combination of centralized global contact and spatial 
symmetry that then allows 3D to pattern the embryo, cre-
ating the distinct, symmetrical developmental identities ob-
erved within quartets relative to the D quadrant midline. In 
contrast, our results show that while the functional homolog 
of the D quadrant organizer is present in equal-cleaving polychaetes, global centralized contact by a vegetal blasto-
mere is absent in both S. columbiana (no centralized contact) and 
A. vittata (centralized contact is equivalent and localized to 
intermediate neighbors for each 4q and 4Q blastomere). Thus, novel noncontact noncentralized and contact noncen-
tralized mechanisms of organizer patterning are required for 
equal-cleaving polychaetes, although the underlying molecu-
lar genetic pathways of patterning may still be conserved for spiral-cleaving lophotrochozoans in general.

Developmental function of the D quadrant organizer

The developmental function of the D quadrant organizer in spiral-cleaving lophotrochozoans is enigmatic. Organizer pat-
tern is said to establish anteroposterior, dorsoventral, or 
structure-specific developmental fates (Clement 1962; van den 
Biggelaar and Guerrier 1979; Martindale et al. 1985; Lambert 
and Nagy 2001; Martindale et al. 2002b). However, lineage 
traces indicate that axis- or structure-specific fates are estab-
lished later in development (Serras and van den Biggelaar 
1990; Dictus and Damen 1997). Radialized development pro-
vides a functional test of the D quadrant organizer because 
patterning by the organizer is absent. Previous studies recog-
nized a limited ability of radialized larvae to differentiate dis-
organized masses of adult tissue (Wilson 1904; Clement 1952, 
1962; Atkinson 1971; van Dongen 1976; Kuhtreiber et al. 
1988; Lambert and Nagy 2001, 2003) and it has been recently 
shown in BFA- and monensin-radialized molluscs that these 
structures are organized about a novel aboral-oral axis (Go-
zales et al. 2007). Similarly, we show here that radiaUzed A. 
vittata larvae differentiate various larval and adult structures 
in a radial or 4-fold radial fashion that is organized both 
around, i.e., larval glands, menisotrochal cilia and stomo-
deum, and along, i.e., gut and muscles, a novel aboral-oral 
axis. This radialized body plan is reminiscent of the 4-fold 
radially symmetric developmental architecture that is estab-
lished by spiral cleavage prior patterning by the organizer in 
normal development. However, due to morphogenetic move-
ments in gastrulation, the aboral-oral axis is parallel, but not 
identical, to the animal-vegetal axis. Similar to radialized 
molluscs (Gonzales et al. 2007), the ability of radialized A. 
vittata larvae to gastrulate demonstrates that patterning by 
the D quadrant organizer is not required for gastrulation to 
proceed. It also argues against, although does not disprove, 
the idea that the radialized development is a passive result of 
default developmental fates or autonomous differentiation. 
Instead, we propose that the organizer-dependent develop-
mental architecture at the onset of gastrulation, whether ra-
dial or bilateral, dictates subsequent patterns of gastrulation 
and it is active patterning during gastrulation that regionally 
establishes lineage contributions to and architecture of the 
adult body plan. In this way, organizer-dependent patterning 
of the embryo is transformed into the body plan of the adult 
without directly patterning adult anteroposterior and dorso-
ventral axes, or directly specifying specific larval and adult 
structures. Even if this is true, the nature of the organizer-
dependent secondary axis remains unknown, as is the rela-
tionship of the organizing center in spiral-cleaving
lophotrochozoans with axial organizers in other metazoans. Future research focusing on genome-enabled spiral-cleaving lophotrochozoans, including the polychaete annelid Capitella sp1, the ciliate annelid Helobdella robusta, and the gastropod mollusc Lottia gigantea, will be central to understanding both the developmental function of the D quadrant organizer and the potential role that an ancestral axial organizer may have played in early animal evolution.

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We wish to thank B. Pernet, C. Hermans, and R. Straumann for guidance and advice on local polychaetes, R. Rubicz for field and lab assistance, G. Zwaan for preparing and drawing semithin sections of A. vittata, L. Nederbragt for contributions on the O. fusiformis analysis, J. Priano and M. Rice for their generosity of lab space and assistance with SEM, V. Foe, S. Santagata, G. V. Dassow, and A. Wanninger for advice on confocal microscopy, and the NIH Center for Cell Dynamics for the generous use of their confocal. We also thank E. Begovic, D. Rokhsar, K. O’Day, and two anonymous reviewers for their helpful comments and suggestions regarding the manuscript. We greatly appreciate the generosity, kindness, and support of staff and scientists at the University of Washington Friday Harbor Laboratories, the Smithsonian Marine Station in Fort Pierce, and the former Department of Experimental Embryology at the University of Utrecht. E. Gonzales was supported by NSF and Smithsonian predoctoral fellowships and an NIH Training Grant to the University of California Center for Integrative Genomics.

REFERENCES


