

# Axis specification in animal development

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## Summary

Axis specification is the first step in defining specific regions of the developing embryo. Embryos exploit asymmetries, either pre-existing in the egg or triggered by external cues, to establish embryonic axes. The axial information is then used to generate regional differences within the embryo. In this review, we discuss experiments in animals which address three questions: whether the unfertilized egg is constructed with pre-determined axes, what cues are used to specify the embryonic axes, and how these cues are interpreted to generate the initial regional differences within the embryo. Based on mapping the data onto an animal phylogeny, we then propose a scenario for how this primary developmental decision occurred in ancestral metazoans.

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## Introduction

A central question in developmental biology is how the fertilized egg can produce the many diverse regions of an adult organism. Eggs typically have very few initial asymmetries built into them. To create additional asymmetries, embryos often utilize external cues. Asymmetries commonly take the form of a polarity present throughout the embryo, or cytoplasmic determinants localized to a specific region of the embryo; these provide the starting point for regional diversification within the embryo. Asymmetries in only two planes are sufficient starting information from which to elaborate the full complement of cell types, usually by way of many cell-cell interactions between the unique groups of cells which have inherited these asymmetries.

What sort of cue might be used to specify an embryonic axis? A cue would need to be consistently available to each developing embryo in order for development to proceed successfully. It would need to be present at the appropriate time, and it would need to be capable of representing an asymmetry to the embryo. Embryos experience very few such cues. Most of the good potential cues they do experience, including the position of the oocyte relative to surrounding cells, the site of sperm-egg fusion, and even gravity, are in fact used in some animals; however, there are also examples of early cellular asymmetries which are not utilised for specifying embryonic axes. The presence of an axis must therefore be inferred by experiment; the most direct way to test for an axis is to physically bisect an egg or embryo in order to isolate the poles of a hypothetical axis, and then determine if the halves develop differently and according to their respective fates. This experiment has been carried out in many of the major animal phyla.

The relevant experiments are discussed below for each phylum for which data exists, along with a list of classes in each phylum or subphylum, to aid in indicating for which classes information is currently available. The two major body axes are ordinarily established separately; we treat each separately in this paper. The conclusions are summarized in Table 1. After reviewing the experiments, we use this data to address how axis specification may have occurred in ancestral metazoans.

## Cnidaria

*Anthozoa, Hydrozoa, Scyphozoa*

The majority of these animals are radially symmetrical. Information on the specification of the anterior-posterior (aboral-oral) axis exists only for hydrozoans. Several potential cues exist for anterior-posterior axis specification (Fig. 1A). The site opposite where the developing oocyte contacts the gastrodermis is normally where the polar bodies are formed, where the egg is fertilized and where first cleavage initiates; this site becomes the gastrulation site (in hydrozoans which gastrulate by unipolar ingression) and the posterior end of the larva<sup>(1,2)</sup>.

Centrifuging fertilized eggs can alter the site of first cleavage initiation relative to the site of polar body formation. When this is done, the site of first cleavage initiation becomes the posterior end, suggesting that the site of polar body formation and fertilization do not specify the anterior-posterior axis<sup>(3)</sup>. It is possible to create two simultaneous first cleavage initiation sites, by reversibly inhibiting first cleavage with cytochalasin, and then centrifuging before the second cleavage cycle<sup>(4)</sup>. Both cleavage initiation sites give

**Table 1.** Cues used to specify embryonic axes in animal development

Axial organization	Phylum	Axes specified in oogenesis	Axes specified later, cue:
Radial	Cnidarian	None	<b>1 AP</b> by 1st cleavage initiation site
Biradial	Ctenophore	None	<b>1 AP</b> by 1st cleavage initiation site
Bilateral	Nemertean	<b>1 AP*</b>	
	Nematode	None	<b>1 AP</b> by sperm component <b>2 DV</b> by skewing of mitotic spindle
	Arthropod	<b>1 AP, 2 DV</b>	
	Mollusc		
	Unequal cleavers	<b>1 AP</b>	<b>2 DV</b> unequal cleavages partition factors in new plane
	Equal cleavers	<b>1 AP</b>	<b>2 DV</b> by cell position and induction
	Annelid		
	Unequal cleavers		<b>DV</b> unequal cleavages partition factors in new plane
	Equal cleavers		
	Brachiopod		
	Articulate	<b>1 DV</b>	<b>2 AP</b>
	Inarticulate	<b>1 DV, 2 AP</b>	
	Echinoderm	<b>1 AP</b>	<b>2 DV</b>
	Chordate		
	Urochordate	<b>1 DV*</b>	<b>2 AP</b> by sperm aster in second phase of ooplasmic segregation
Vertebrate			
Amphibians	<b>1 AP</b>	<b>2 DV</b> by sperm aster or gravity, causing rotation of cortex	
Fish		<b>AP</b> by gravity	
Bird		<b>AP</b> by gravity	
Mammal			

See text for details on this summary.

Abbreviations: 1, 2. First, second axes, where order of axis specification is known; AP, antero-posterior axis; DV, dorso-ventral axis.

\*Axis specified but not determined in oogenesis; i.e. the poles when isolated generally still retain the ability to form whole animals.

rise to gastrulation sites, and two posterior ends develop (Fig. 1B). These results suggest that the first cleavage initiation site is the asymmetric cue which specifies the anterior-posterior axis.

How the cleavage initiation site is interpreted to specify a region as the posterior is unclear. One possibility is that the cleavage initiation site establishes an asymmetric distribution of a determinant, which is partitioned by cleavage into cells of only that region, determining the fate of these cells to form the posterior pole. However, when presumptive anterior and posterior halves of the embryo are separated from each other at later stages (between the eight-cell stage and early gastrulation), both halves can still form complete larvae, indicating that each half has the ability to form both anterior and posterior regions. Marking cut halves has shown that each half additionally retains the original anterior-posterior polarity (Fig. 1C). The results indicate that the first cleavage initiation site establishes a polarity throughout the embryo but does not cause immediate regional determination. We refer to this situation, in which axial polarity is present in the parts of an embryo, as a *global polarity*. It will be interesting in the future to explore the molecular basis for this form of polarity. There is also evidence in the ctenophores, nemerteans and ascidians to suggest that an axis may be initially established as a global polarity. These cases differ from what has been found

in other animals, such as nematodes and amphibians, where axis specification involves immediate fate determination in a region of the embryo.

## Ctenophora

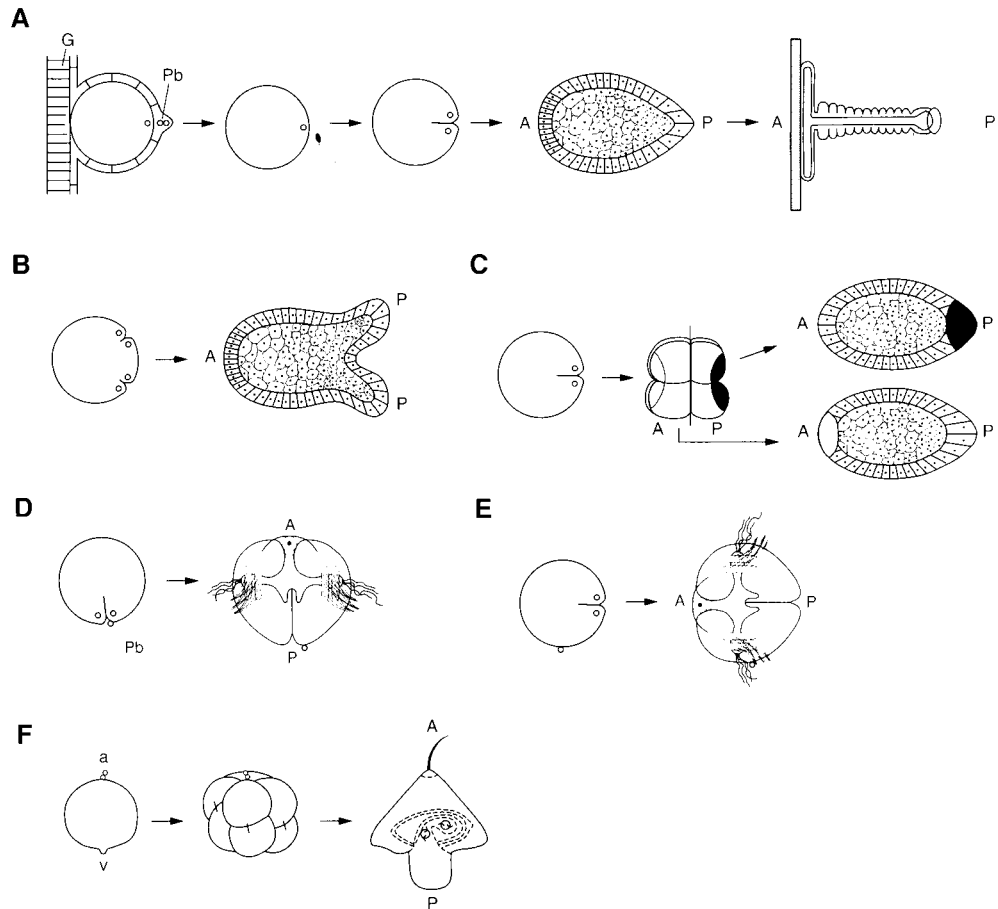
### *Nuda, Tentaculata*

These animals are biradially symmetrical. Information on the establishment of the anterior-posterior axis exists for both classes. The site of first cleavage initiation usually corresponds to the site of polar body formation and becomes the posterior pole of the embryo (Fig. 1D). In some natural cases and also in centrifugation experiments, the first cleavage initiation site can be different from the site of sperm-egg fusion and the site of polar body formation (Fig. 1E). In these cases the first cleavage initiation site becomes the posterior pole, suggesting that, as in the Cnidaria, the primary axis is specified by the site of first cleavage initiation<sup>(5)</sup>.

There is little information on how the cleavage initiation site can establish the anterior-posterior axis. Houliston *et al.*<sup>(6)</sup> have presented evidence for a wave of cytoskeletal reorganization emanating from the region of the zygote nucleus just prior to first cleavage; whether this plays a role in axis specification is unclear.

By cutting blastomeres at various times, it was deter-

**Fig. 1.** Cnidaria (A-C), Ctenophora (D,E) and Nemertea (F). (A) Normal hydrozoan development. Oocyte in ovary after polar body formation, egg at fertilization with sperm entering at animal pole, embryo midway through first cleavage, planula larva and polyp shown. All stages are drawn in the same orientation. The oral end of the polyp corresponds to the posterior end of the planula larva. (B) Embryo with two first cleavage initiation sites gives rise to a two-tailed planula larva (and a bifurcated polyp with two oral ends). (C) Experiment in which the presumptive anterior and posterior poles of an embryo were each marked before bisecting the embryo at the four cell stage. Two half-sized planulae develop, each with the original polarity of the embryo before bisection. (D) Normal ctenophore development. The site of polar body formation usually coincides with the site of first cleavage initiation, and becomes the posterior end of the larva. (E) When the site of polar body formation and the first cleavage site do not coincide, the site of first cleavage initiation becomes the posterior end. (F) Normal anoplan development. The animal pole becomes the anterior end of the pilidium larva. Cleavage is spiral. g, gastrodermis; Pb, polar bodies; A, anterior; P, posterior. These labels are used similarly in other figures.



mined that there is a segregation of developmental potential during the first three cleavages so that the presumptive anterior (aboral) region of the embryo acquires the ability to differentiate comb plates and the presumptive posterior (oral) region acquires the ability to differentiate photocytes<sup>(7)</sup>. These experiments suggest that the anterior-posterior axis is specified as a global polarity, as in the hydrozoans, and that segregation then takes place along this axis during the first three cleavage cycles.

The other axis in ctenophores is determined by the two-cell stage. The first cleavage always occurs along the anterior-posterior axis and the plane of cleavage corresponds with the plane of symmetry, which separates the two tentacles of the adult. If the blastomeres of the two-cell stage are isolated, each blastomere undergoes a partial cleavage program and becomes a tentacular half of the adult<sup>(8)</sup>.

## Nemerteans

### *Anopla, Enopla*

Fate-mapping studies in anoplans indicate that the animal half of the egg forms the anterior ectodermal covering of the larva including the apical tuft, while the vegetal half forms the gut and lappet ectoderm<sup>(9)</sup> (Fig. 1F). The plane of first

cleavage corresponds to the plane of bilateral symmetry in 50% of the cases, and to the frontal plane in the other 50%<sup>(10)</sup>.

When eight-cell stage anoplan embryos are cut to create animal and vegetal halves, the animal half forms a tuft but no gut, while the vegetal half forms a gut but no tuft, indicating that regional specification has occurred along the animal-vegetal axis by this stage<sup>(11)</sup>. Cutting the unfertilized egg similarly and then fertilizing the halves often results in two complete larvae, although vegetal halves of unfertilized eggs can form a tuft more often than animal halves can, suggesting that the ability to form a tuft may actually be localized somewhat to the opposite (vegetal) pole<sup>(12)</sup>. Cuts at various times up to the eight-cell stage have documented the time course through which the potential to form gut and tuft are localized to each pole. Inhibition of meiotic aster formation prevents this localization, and early induction of asters causes precocious localization, suggesting a role for the cytoskeleton in the process<sup>(12)</sup>.

Dorsal-ventral asymmetry is determined in anoplans some time after the four-cell stage: under normal circumstances not all of the cells at the four-cell stage give rise to muscle, but if the four cells are isolated, each can form a small but normal pilidium larva with muscle cells<sup>(13)</sup>.

**Nematoda**

*Adenophorea, Secernentea*

Most work on axis specification has involved the class Secernentea. In *C. elegans*, several potential cues exist for anterior-posterior axis specification (Fig. 2A), including the oocyte nucleus and a cytoplasmic bridge to the other oocytes, at the presumptive anterior pole, and the site of sperm entry at the presumptive posterior pole. When eggs are fertilized at the abnormal end of the egg, the embryo develops with a reversed anterior-posterior axis<sup>(14)</sup>, indicating that the unfertilized egg has no predetermined axes, and that the sperm specifies initial asymmetry in the embryo (Fig. 2B).

How does the sperm specify the anterior-posterior axis? After fertilization, a sperm-associated component directs a reorganization of cytoplasm, which has been implicated in segregating putative determinants. This reorganization is microfilament-dependent, and may involve the contraction of a cortical microfilament network to one pole of the egg. Several gene products which become asymmetrically distributed by first cleavage have been identified in *C. elegans*. An understanding of how these gene products function may reveal how the cue for axis specification is interpreted to generate the difference between the cells of the two-cell stage<sup>(15,16)</sup>.

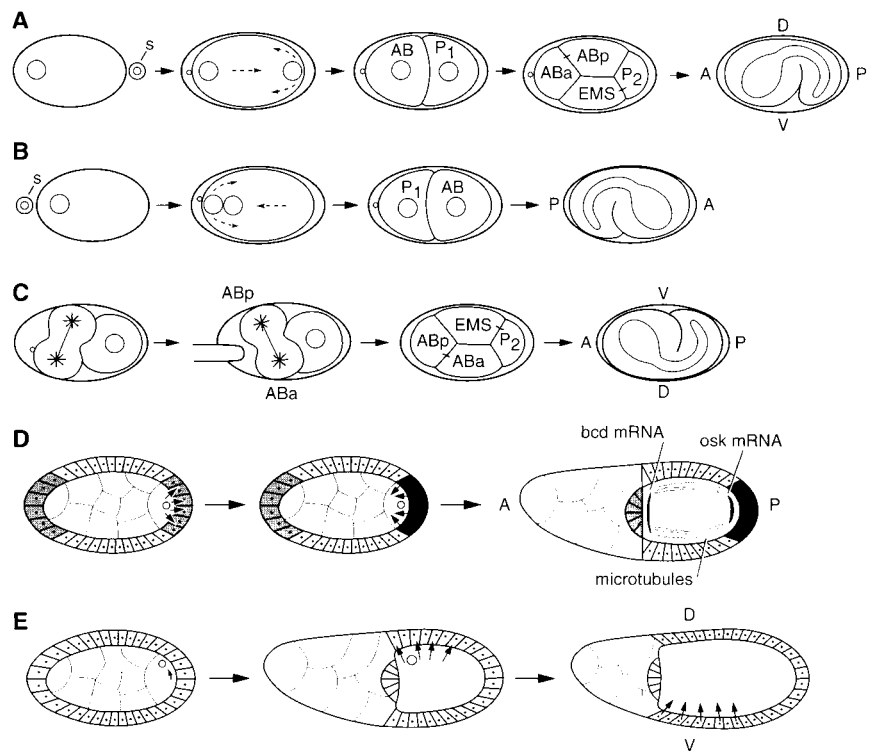
Observations of fertilization, the cytoplasmic reorganization and axis formation in other secernenteans suggest similar conclusions, but not every secernentean develops in this way (B. G., manuscript in preparation). In a species of *Acroboloides* the relationship between sperm entry and the an-

terior-posterior axis is uncoupled, indicating that some other cue must specify the anterior-posterior axis. Other manifestations of asymmetry are also altered: these embryos lack a visible cytoplasmic reorganization during the first cell cycle, and germ-line-specific P granules are segregated at a different time.

Experiments the Adenophorean *Enoplus brevis* indicate that the anterior-posterior axis is not specified by the two-cell stage: marking a cell at the two-cell stage has shown that the contribution of each cell to the developing worm is variable between individuals<sup>(17)</sup>.

Secernentean embryos appear radially symmetrical through the two-cell stage. The first visible dorsal-ventral asymmetry in *C. elegans* is the skewing of the axis of AB cell division at the two-cell stage (Fig. 2C). This skewing can probably occur in any direction, as altering the direction of skewing can lead to normal development<sup>(18)</sup>. The orientation of the cells of the four-cell stage with reference to each other appears to then determine the dorsal-ventral axis<sup>(18,19)</sup>.

Throughout the animal phyla, many cases exist in which a cue has not yet been identified for the specification of a given axis. It is possible in some of these that asymmetry arises in the absence of any cue, by the tipping of an imbalance present in the embryo, in a random direction. The results above suggest that the specification of the dorsal-ventral axis in *C. elegans* represents one such case; the specification of the dorsal-ventral axis in equally cleaving molluscs is another (see below).



**Fig. 2.** Nematoda (A-C) and Arthropoda (D,E). (A) *C. elegans* development. Egg at fertilization, cytoplasmic rearrangement (arrows indicate direction of cortical and cytoplasmic flow), two-cell stage, four-cell stage, elongated embryo. All stages are drawn in the same orientation. (B) Experiment in which the sperm enters in the opposite end of the egg. Development proceeds normally but with a reversed anterior-posterior axis. (C) Experiment altering the direction of skewing of the dividing AB cell. Development proceeds normally but with a reversed dorsal-ventral axis. (D) Development of the AP axis in *Drosophila*. Oocyte to follicle cell signalling, follicle cell to oocyte signalling, arrangement of microtubules and specific mRNAs. (E) Development of the DV axis in *Drosophila*. Migration of the oocyte nucleus, oocyte to follicle cell signalling, ventralizing signal from follicle cells. s, sperm; bcd, bicoid; osk, oskar.

## Arthropoda

*Chelicerata, Crustacea, Insecta, Myriapoda, Pycnogonida*

Relevant information is available only in the Insecta. The elaboration of many different body regions from a few initial asymmetries has been worked out in great detail in *Drosophila*, as a result of thorough genetic and molecular analysis of development. Here we address only the initial sources of asymmetry in development.

Oogenesis in *Drosophila* is meroistic: each oocyte develops from one cell of a clone of 16 cells, the other 15 of which develop as nurse cells. The egg chamber consists of these 16 cells and a surrounding monolayer of follicle cells. Each oocyte develops in the posterior end of its egg chamber (toward the more mature end of the ovariole). The position of the oocyte represents the earliest apparent asymmetry along the anterior-posterior axis; how the oocyte attains this position is unclear. This position allows the oocyte to signal to only the nearer of two sets of polar follicle cells, which lie at the ends of the egg chamber. Evidence for local signaling to follicle cells is provided by mutants in which the oocyte is situated centrally and contacts neither set of polar follicle cells: neither end is specified as posterior and the embryo develops with two anterior ends<sup>(20)</sup>.

Both the signal and receptor for this oocyte-follicle cell interaction have been identified: Gurken, a protein with EGF repeats, activates Torpedo/DER, an EGF receptor homologue, in the polar follicle cells<sup>(20)</sup>. These posterior follicle cells then produce an as yet unidentified signal, which is received by the oocyte and acts to polarize the oocyte cytoskeleton. The polarized cytoskeleton plays a role in localizing bicoid mRNA to the presumptive anterior and oskar mRNA to the presumptive posterior<sup>(21)</sup> (Fig. 2D). How the diverse cell types arranged along the anterior-posterior axis develop from these asymmetric distributions of mRNAs is understood in some detail<sup>(22)</sup>.

The dorsal-ventral axis is specified by a similar set of interactions between the oocyte and follicle cells<sup>(20,23)</sup> (Fig. 2E). In this case, however, the initial asymmetry appears to be the position of the oocyte nucleus. After the oocyte cytoskeleton is polarized by the posterior follicle cell signal, the oocyte nucleus migrates anteriorly along the cortex on the polarized cytoskeleton, on what will become the dorsal side of the embryo. This migration may represent the establishment of dorsal-ventral asymmetry; alternatively, the direction of migration may be specified by an earlier, unidentified cue. Near the oocyte nucleus, the oocyte then signals to follicle cells, using the same signal/receptor interaction as was used in anterior-posterior axis specification. Gurken accumulates near the oocyte nucleus and interacts with nearby follicle cells. Activation of Torpedo in these cells keeps them from producing a ventralizing signal, restricting ventral signaling to the follicle cells on the opposite side of the oocyte. The ultimate result of ventral signaling is the formation of a nuclear gradient of Dorsal protein, a transcrip-

tion factor which acts to specify cell fates along the dorsal-ventral axis<sup>(23)</sup>. The anterior-posterior and dorsal-ventral axes are normally established at 90° from each other, although mutants exist in which this angle varies, and in some mutant embryos the two axes can overlap<sup>(24,25)</sup>.

Little is known about axis specification in other arthropods. The mechanisms used must vary to some extent, as some of the proteins involved in elaborating regions in *Drosophila* must diffuse across the syncytial embryo, and many arthropods do not have syncytial embryos.

## Mollusca

*Aplacophora, Bivalvia, Cephalopoda, Gastropoda, Monoplacophora, Polyplacophora, Scaphopoda*

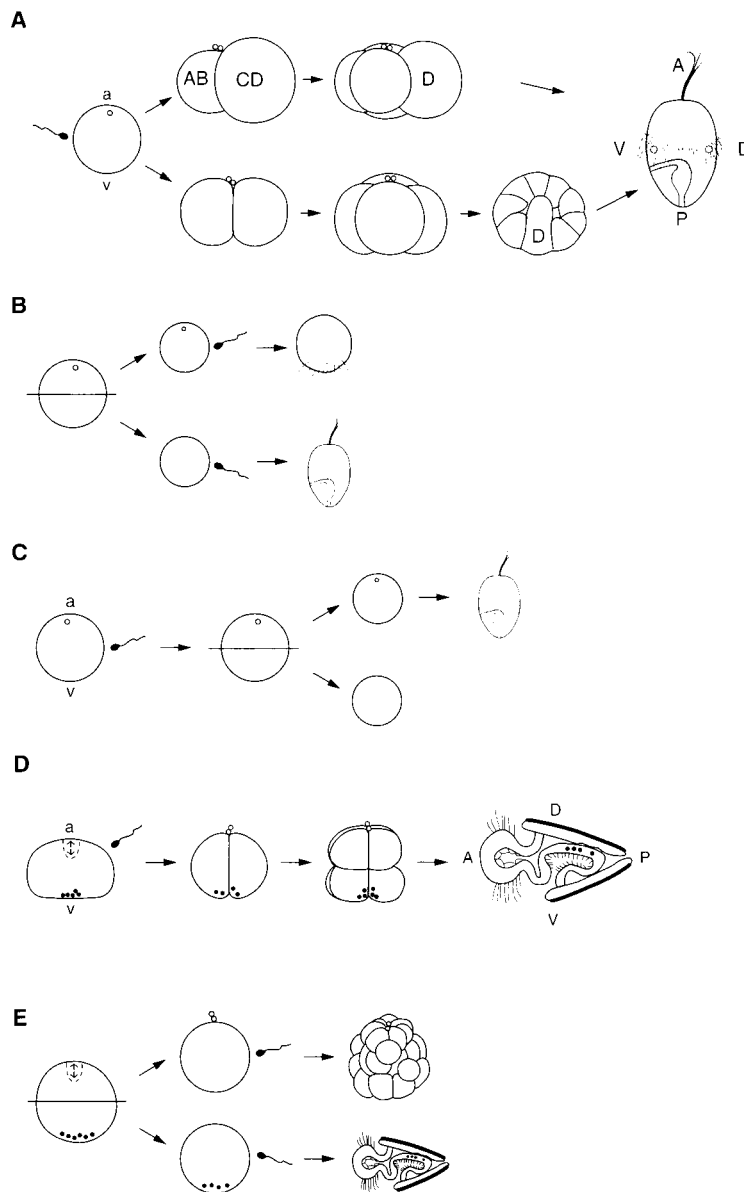
All molluscs, with the exception of cephalopods, exhibit spiral cleavage. In some molluscs the first two cleavages are equal, while in others they are unequal, generally producing a large cell (named D) at the four-cell stage (Fig. 3A). Unequal cleavage is accomplished by either simple unequal cytokinesis or by producing a polar lobe at the vegetal pole which is inherited by one cell.

In all classes of molluscs there is a visible cytoplasmic segregation of yolk-free cytoplasm toward the animal pole. This segregation appears to begin at different times in different species, from the time of polar body formation through the early cleavages. Whether this segregation plays a role in axis specification or cell fate specification is not known.

In eggs of scaphopods and gastropods, which form polar lobes, regional specification along the animal-vegetal axis occurs during oogenesis. In the scaphopod *Dentalium*, experiments have been done where matured oocytes were cut in half and each half was fertilized<sup>(26)</sup>. While the animal halves formed typical animal-half-derived structures such as velar cilia, they never formed a D macromere or vegetal-specific structures (Fig. 3B). The vegetal halves formed normal larvae, suggesting that regional specification occurs along the animal-vegetal axis during oogenesis.

In the equally cleaving gastropod *Limax*, compression experiments have been done to push the meiotic aster to a new position between production of the two polar bodies, causing the second polar body to be given off at roughly 90° from the first polar body<sup>(27)</sup>. Cytoplasmic streaming and cleavages were oriented with respect to the site of second polar body formation, and development proceeded normally and tilted 90° from the site of first polar body formation, indicating that the anterior-posterior axis is labile at this stage.

The establishment of dorsal-ventral asymmetry in molluscs with spiral cleavage occurs as a consequence of D macromere specification. The D macromere has special roles in inducing overlying micromeres to form bilateral ectodermal structures, and in forming the mesentoblast (4d cell), which generates the bilateral bands of mesoderm in the embryo. Specification of one of the four quadrants as D establishes the dorsal-ventral axis.



**Fig. 3.** Mollusca (A,B), Annelida (C) and Brachiopoda (D,E). (A) Normal molluscan development. Molluscs with unequal early cleavage are represented in the upper sequence: egg at fertilization, two-cell stage, four-cell stage with large D cell, trochophore larva. Forms with equal cleavage are represented in the lower sequence: two-cell stage, four-cell stage, one of the macromeres contacting the animal pole cells (viewed in frontal section); this macromere becomes the D macromere. In both forms the animal pole becomes the anterior end and the D quadrant gives rise to the dorsal side of the larva. (B) If the unfertilized egg is bisected meridionally and both halves are fertilized, the animal half develops as an incomplete larva, whereas the vegetal half forms a normal half-sized trochophore larva. (C) Experiment in which a fertilized annelid egg was cut into animal and vegetal halves. Only the animal half divides and develops. It forms a complete trochophore larva. (D) Normal inarticulate brachiopod development. Egg at fertilization, two-cell stage, eight-cell stage, larva. The animal pole becomes the dorsal side of the larva. (E) Experiment in which the unfertilized egg is bisected meridionally and both halves are fertilized. The animal half develops incompletely, whereas the vegetal half forms a normal half-sized larva.

D quadrant specification occurs in two different ways in the molluscs. In cases where unequal cleavage occurs, the inequality of the cleavage causes vegetal pole components to be inherited by the large D macromere at the four-cell stage. The vegetal pole material has been implicated in specifying the D quadrant<sup>(28)</sup>. The position where D will form in the embryos with unequal cytokinesis is affected (though not strictly determined) by the site of fertilization<sup>(29)</sup>.

In the equal cleavers, D quadrant specification occurs at a later stage by induction, and any of the four macromeres can be induced to become D<sup>(30)</sup>. One of the four macromeres moves to the center of the blastula and becomes the only macromere to contact animal pole cells, and as a consequence forms the mesentoblast (Fig. 3A). Removing any one macromere will not prevent D quadrant

specification, indicating that no one macromere is predetermined to become D. Deleting animal pole cells or preventing contact with the animal pole cells can prevent D quadrant specification and lead to radialized development, suggesting that these are the cells which induce the central macromere<sup>(28)</sup>. Freeman and Lundelius<sup>(31)</sup> have presented evidence that dorsal-ventral axis specification by equal cleavage and induction is ancestral for the coelomate spiralian, and unequal cleavage is a mechanism which has been derived in some groups.

In cephalopods, the large egg forms a clear cap of cytoplasm at its animal pole following fertilization. Cleavage is superficial in the cytoplasmic cap. Experiments have been done in which the process of cytoplasmic reorganization was disrupted in just a region of the embryo, or discrete parts of

the embryo surface were UV-irradiated after first cleavage<sup>(32,33)</sup>. These treatments cause local defects in the resulting embryos, suggesting that discrete regions of the embryo are specified at least as early as first cleavage. Transplantation experiments have suggested that the information that specifies patterns of differentiation lies in the yolk syncytial layer, which underlies the cells of the early embryo<sup>(34)</sup>.

## Annelida

### *Polychaeta, Oligochaeta, Hirudinida*

All annelids exhibit spiral cleavage. Experiments on the establishment of initial developmental asymmetry along the animal-vegetal axis have been done only on polychaetes. There is a visible cytoplasmic segregation in these eggs, as there is in the molluscs. *Nereis* and *Sabellaria* eggs have been cut into animal and vegetal halves after fertilization and polar body formation but before first cleavage<sup>(35,36)</sup> (Fig. 3C). Under these conditions only the animal halves develop, and they form normal larvae, suggesting that, unlike molluscs, the vegetal region may not be critical for development. In contrast, removal of vegetal material from *Chaetopterus* embryos at the two-cell stage gave incomplete embryos similar to those obtained from the experiments in molluscs<sup>(37)</sup>.

Dorsal-ventral axis specification appears similar to the mechanisms used by the molluscs. In many polychaetes and all oligochaetes and leeches, the first two cleavages are unequal. One macromere of the four-cell stage is larger than the other three, representing the first visible dorsal-ventral asymmetry. In some polychaetes the first two cleavages are equal. One quadrant develops as D, forming the mesentoblast (4d cell), although it is not known how the mesentoblast is specified in equally cleaving polychaetes.

## Brachiopoda

### *Articulata, Inarticulata*

Fate-mapping studies on the inarticulate brachiopod *Glottidia* indicate that the animal half of the egg forms dorsal ectoderm, while the vegetal half forms ventral ectoderm, endoderm and mesoderm, and is the site of gastrulation (Fig. 3D). When unfertilized eggs are cut into animal and vegetal halves and each half is fertilized, the animal half cleaves but does not form a dorsal valve, while the vegetal half forms a normal, half-sized larva<sup>(38)</sup> (Fig. 3E). Similar experiments have yielded similar results on the articulate brachiopod *Terebratalia*<sup>(39)</sup>. The results suggest that the dorsal-ventral axis is specified during oogenesis.

In the inarticulate brachiopod *Glottidia*, fate-mapping studies also indicate that the plane of first cleavage coincides with the plane of bilateral symmetry of the larva, and the plane of second cleavage separates the presumptive anterior from the presumptive posterior. When an embryo is cut in half at the four-cell stage along the second cleavage plane, one half forms an anterior half-larva, and the other forms a posterior

half-larva, suggesting that the anterior-posterior axis is specified by this stage. When a cut is used to create bilateral halves of unfertilized eggs and each half is then fertilized, in about 50% of the cases only one member of the pair forms the anterior-specific apical lobe. The result suggests that the anterior-posterior axis may also be in place in the unfertilized egg<sup>(38)</sup>. In the articulate brachiopod *Terebratalia*, there is no fixed relationship between the plane of first cleavage and the axis of bilateral symmetry, and the anterior-posterior axis is not specified until just before gastrulation<sup>(39)</sup>.

## Echinodermata

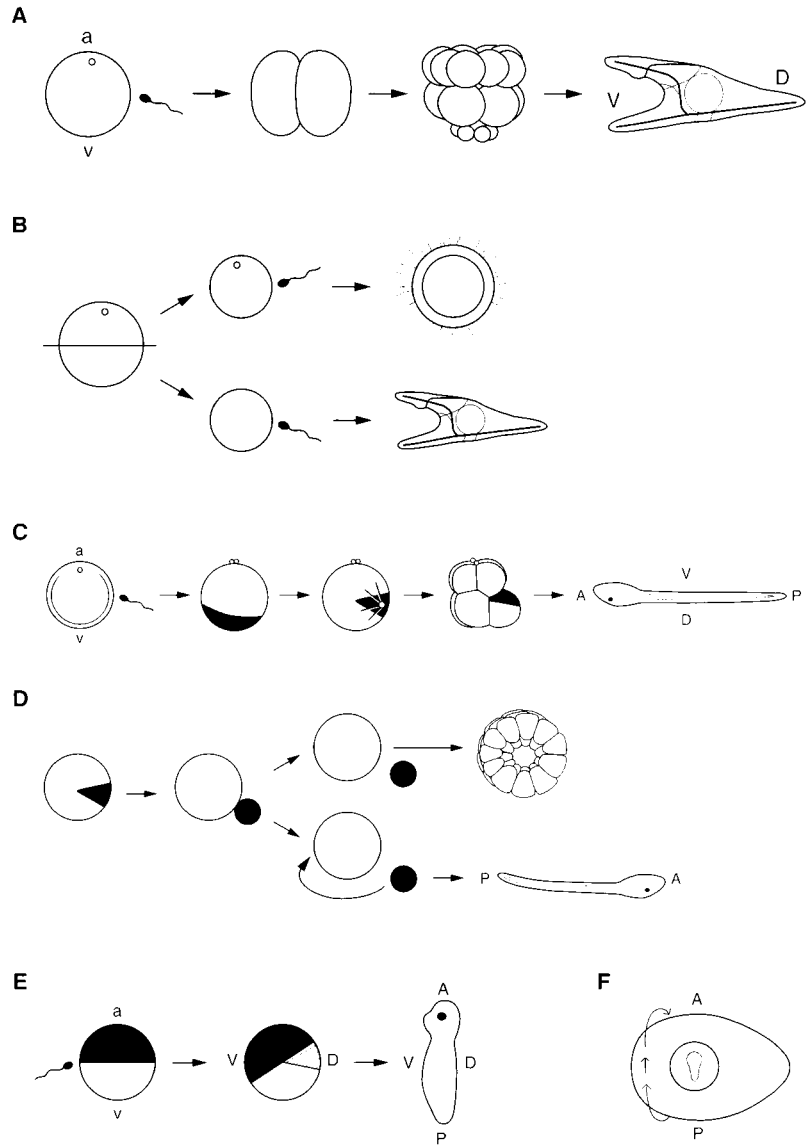
### *Asteroidea, Crinoidea, Echinoidea, Holothuroidea, Ophiuroidea*

The only two echinoderm classes for which we have data on animal-vegetal axis specification are the asteroids and echinoids. Cleavage in echinoderm eggs is radial; the first two cleavage planes occur along the animal-vegetal axis (Fig. 4A). Fate maps have been constructed for both asteroids<sup>(40)</sup> and echinoids<sup>(41-42)</sup>. In both classes, the vegetal region of the egg normally gastrulates and forms ectodermal, mesodermal and endodermal structures, while the animal region forms the ectoderm of the anterior region of the larva, including the oral ectoderm.

In echinoids and asteroids, experiments have been done where eggs were cut into animal and vegetal halves prior to fertilization, and each half was fertilized (Fig. 4B). In most cases the animal halves developed into ciliated blastulae while the vegetal halves developed into normal larvae with endodermal and mesodermal structures<sup>(42-45)</sup>. In an elaboration on this experiment, Kiyomoto and Shirai<sup>(46)</sup> have isolated animal fragments of oocytes, fertilized them, and then fused each with a small vegetal fragment of an oocyte. The resulting embryos developed normally and formed endodermal and mesodermal structures. These experiments suggest that asymmetries exist in the oocyte before fertilization.

A cue for dorsal-ventral (oral-aboral) axis specification in echinoids and asteroids has not been found. The orientation of the first cleavage plane and the plane of bilateral symmetry of the larva can vary in the different species which form larvae, and in cases where a relationship has been claimed it has always been approximate, suggesting it does not play a role in axis specification<sup>(47)</sup>. Other evidence for no fixed oral-aboral axis in the cleavage stage embryo derives from blastomere isolation experiments, in which isolated cells of the two- or four-cell stage can go on to form normal larvae<sup>(42)</sup>.

The situation in the direct developing echinoid *Heliocidaris erythrogramma* differs: fate-mapping studies have shown that the first cleavage plane separates the future dorsal and ventral sides of the adult<sup>(48)</sup>. When blastomeres were separated at the two-cell stage, one half developed as a ventral half-larva while the other developed as a dorsal half-larva<sup>(49)</sup>. Subsequent work by Henry *et al.*<sup>(50)</sup> has demonstrated that this lateral difference exists prior to first cleavage.



**Fig. 4.** Echinodermata (A,B), Urochordata (C,D) and Vertebrata (E,F). (A) Normal echinoid development. Egg at fertilization, two-cell stage, 16-cell stage with micromeres at vegetal pole, pluteus larva. The vegetal pole becomes the site of gastrulation. (B) Experiment in which the unfertilized egg is bisected meridionally and both halves are fertilized. The animal half develops as a ciliated ball of cells, whereas the vegetal half forms a normal half-sized pluteus larva. Experiments have been done to control for the effect of cutting and size: when the egg is bisected laterally (such that each half contains both animal and vegetal poles) and both halves are fertilized, both form normal, half-sized pluteus larvae. (C) Normal ascidian development. Cytoplasm inherited by muscle cells (called myoplasm) is shaded. Egg at fertilization, after capping of myoplasm at vegetal pole, after crescent formation (sperm aster shown), eight cell stage, tadpole larva. The animal pole becomes the ventral side and the site of crescent formation becomes the posterior side. (D) Experiment in which the myoplasm is removed, leading to radialized development (no apparent AP axis), or removed and fused to the presumptive anterior side, leading to a reversed AP axis. (E) Normal *Xenopus* development. Egg at fertilization, after cortical rotation and formation of gray crescent, tadpole larva. The animal pole becomes roughly the anterior end; the side opposite sperm entry is where the grey crescent forms, gastrulation begins, and the dorsal side develops. (F) Chick egg, viewed from above. The embryo develops on the upper surface of the yolk, which remains relatively stationary inside the rotating egg. Rotation results in one end of the embryo being tilted down. The direction of the egg's rotation is shown.

### Chordata

*Subphylum Urochordata: Appendicularia, Ascidiacea, Thaliacea*

The only urochordate class where we have information on the establishment of axes is the ascidians (Fig. 4C). Visible animal-vegetal asymmetries are present in ascidian eggs<sup>(51)</sup>. Actin filaments, endoplasmic reticulum and myoplasm are present throughout the cortex of the egg, except at the animal pole. One of these components, the myoplasm, has been implicated in muscle cell fate specification. Upon fertilization, ascidian eggs undergo a major cytoplasmic reorganization. During the first phase of this process, part of the cell membrane and the underlying cortex are translocated to the vegetal pole. This is accomplished by the contraction of cortical microfilaments to the vegetal pole, triggered by sperm entry, possibly *via* a

release of calcium. Experiments suggest that the dorsal-ventral (animal-vegetal) axis is established in the unfertilized egg as a global polarity: animal and vegetal half-eggs, when fertilized, also undergo a vegetally-oriented contraction<sup>(52)</sup>, and both halves can develop normally<sup>(53)</sup>.

Contraction of the cortex to the vegetal pole is associated with regional specification along the dorsal-ventral axis. Experiments involving deletion of vegetal pole material after contraction, transplantation of cytoplasm, or unilateral UV irradiation have suggested that the vegetal pole at this time contains factors essential for muscle and endoderm development, gastrulation and formation of the second (anterior-posterior) axis<sup>(54)</sup>.

The embryo appears radially symmetrical until the second phase of cytoplasmic reorganization, as the myoplasm moves with the sperm aster to a subequatorial position where

it forms the 'yellow crescent' and marks the future posterior end of the larva<sup>(55,56)</sup>. Based on observations of sperm aster and myoplasm movement, on the association of myoplasm with the aster, and on the dependence of this movement on intact microtubules, it has been hypothesized that the sperm aster's position specifies the AP axis by moving the myoplasm as the aster grows and migrates anamally<sup>(56,57)</sup>.

The role of the yellow crescent of myoplasm in anterior-posterior axis specification has been tested<sup>(58)</sup>. When the yellow crescent region is removed after the second phase of cytoplasmic reorganization, a radialized embryo develops with no anterior-posterior axis (Fig. 4D). When the yellow crescent is removed from the presumptive posterior and then fused with the presumptive anterior region of the same egg, the resulting larva develops fairly normally but with a reversed anterior-posterior axis, suggesting that the yellow crescent region is sufficient to specify one side of the embryo as the posterior.

*Subphylum Vertebrata: Agnatha, Amphibia, Aves, Chondrichthyes, Mammalia, Osteichthyes, Reptilia*

In amphibians with holoblastic cleavage, regional specification occurs along the animal-vegetal axis (which becomes roughly the anterior-posterior axis of the embryo) during oogenesis (Fig. 4E). Visible asymmetries of yolk and pigment exist in *Xenopus* eggs, though a putative developmentally relevant asymmetry has not yet been tested by bisecting unfertilized eggs. The most convincing evidence for a developmental asymmetry in the unfertilized egg is that mRNAs have been identified which are localized to the vegetal pole, and appear to require proper localization in order for development to proceed normally (*Vg1* and *Xwnt11*)<sup>(59-61)</sup>. Translocation of factors to the vegetal pole of the egg occurs by two distinct mechanisms, one of which has been shown to require microtubules<sup>(62,63)</sup>.

Apparent radial symmetry persists in anuran amphibians until, during the first cell cycle, the cortex rotates 30° relative to the central cytoplasmic mass<sup>(64)</sup>. Rotation causes a 'gray crescent' to form at an equatorial position which then predicts the site of gastrulation and the dorsal side. The direction of rotation is specified by the sperm entry point, although the direction of rotation can be altered by tipping the eggs and gravity will determine its direction. Microtubules present in the sperm aster or near the cortex might drive rotation, as microtubule depolymerization prevents rotation<sup>(65)</sup>. Kessler and Melton<sup>(66)</sup> have suggested that rotation results in the localization of active Vg1 protein to the presumptive dorsal side, where it induces dorsal mesoderm.

Within the bony fishes (Osteichthyes), experimental work on axial specification has been done on the Chondrostei and the Teleostei. Sturgeons (Chondrostei) have yolky eggs; most of the yolk is located at the vegetal pole of the egg, while non-yolky cytoplasm is located primarily at the animal pole. The egg is surrounded by a chorion with a micropyle situated above the animal pole, which is the site of

fertilization. Immediately after spawning and fertilization, the egg is fixed to a substrate and a cortical reaction creates a space between the egg and its chorion. Because the yolk is heavier than the non-yolky cytoplasm, the egg then rotates in the chorion and orients with its cytoplasmic disk upward. Just before first cleavage a light crescent forms on one side of the cytoplasmic disk. The position of the crescent is a predictor of the site of gastrulation and the anterior-posterior axis of the embryo. The crescent always forms in the plane of rotation opposite the lowest position of the egg prior to rotation. If eggs are rotated in one direction for 90° and immediately rotated in another direction through more than 90°, the second rotation will override the first and establish the plane of bilateral symmetry<sup>(67)</sup>.

Teleosts have large, yolky eggs where cleavage takes place in a blastodisc on top of a yolk layer. A possible role for gravity in specifying the dorsal-ventral axis in teleosts has not been resolved<sup>(68,69)</sup>. Long<sup>(70)</sup> has suggested that anterior-posterior asymmetry arises in *Salmo* first in the yolk, which then induces antero-posterior asymmetry in the blastodisc. Long removed blastodiscs from embryos which already had shown signs of an antero-posterior axis, and placed on each yolk syncytial layer a blastodisc from a younger animal, which had not yet shown signs of an antero-posterior axis. Each new blastodisc developed in the same orientation as the old one, suggesting that the yolk can impart positional information to the blastodisc. Whether this positional information normally first arises in the yolk has not been further examined.

Birds (Aves) have large, yolky eggs with a blastodisc, like the teleosts. The anterior-posterior axis of the chicken egg is entrained by gravity. As the fertilized egg rotates and travels down the oviduct, cleavage, epiblast formation, and hypoblast formation take place. The epiblast will form the embryo, while the hypoblast forms extraembryonic tissue. It has been known since the last century that the anterior-posterior axis of the chicken embryo is set up prior to laying<sup>(71)</sup>. Von Baer's rule states that when an egg is viewed with its pointed end to the right, the embryo sitting on top of the yolk will be perpendicular to the long axis of the egg, with its posterior end toward the observer (Fig. 4F). Rotation of the egg in the oviduct causes the embryo to lie with one side pointing down, which becomes the anterior. Kochav and Eyal-Giladi<sup>(72)</sup> showed that it is gravity and not rotation per se which entrains the anterior-posterior axis, by removing eggs and hanging the embryos in various orientations. The critical time during which this effect occurs is the 14th to 16th hour of rotation in the uterus<sup>(73)</sup>. It is not clear how a gravitational cue is translated into axial specification in birds. Experiments of Khaner<sup>(74)</sup> show that at the time of laying, axial information resides in the epiblast. Von Baer's rule is also true in reptiles, raising the possibility that they too may specify their anterior-posterior axis by gravity<sup>(75)</sup>.

In the mammals, very little is known about the establishment of the anterior-posterior axis. In armadillos, which are

naturally polyembryonic, the orientation of each embryo relative to the others can be predicted before an axis is apparent, suggesting that an external cue might orient the axes<sup>(76)</sup>. Cells can be removed from cleavage-stage mouse embryos with no effect on development, indicating that the regions of the embryo are not determined until later<sup>(77)</sup>. The data suggest that in the early mammalian embryo either an axis may be specified but not determined, or that different mammalian embryos specify their axes differently.

The dorsal-ventral axis in fishes, birds and mammals is established with respect to the yolk (in fishes or birds) or to the blastocoelic space (mammals). How this axis is specified is unknown.

### The left-right axis

Specification of the left-right axis is a distinct problem from the specification of the first two axes, in that left and right are always specified with reference to the first two axes, rather than by an absolute asymmetry in the egg. Experiments in many animals have shown that reversal of either the anterior-posterior or dorsal-ventral axes affects the position of left-right asymmetries, such that structures typical of the left side of the animal develop on the animal's new left side, and structures typical of the right side of the animal develop on the animal's new right side<sup>(78)</sup>. Several early, asymmetrically expressed genes have been discovered in vertebrates<sup>(79)</sup>; however, the original question remains – how left and right are distinguished based on information from two orthogonal axes.

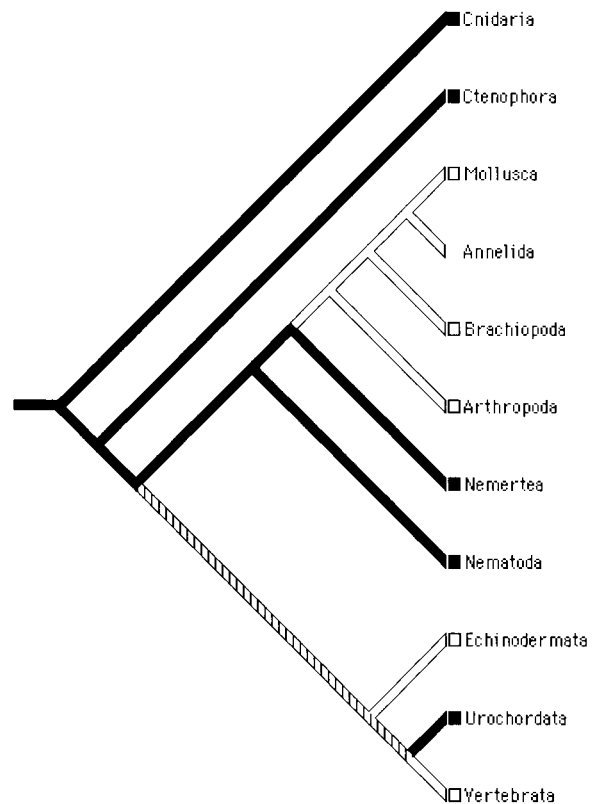
### Conclusions

The time that each axis arises varies between phyla. The primary axis can arise as early as oogenesis (most animals) or as late as first cleavage (cnidarians, ctenophores). This axis can exist in one of two forms: as a global polarity, along which determination of regions occurs through early cleavages (cnidarians, for example), or in the form of immediate determination of fate in regions of the embryo (amphibians). Although both the cues and the time at which axes are specified varies between animals, the cytoskeleton is used in many and perhaps all cases to translate an asymmetric cue into an internal asymmetry. For example, the asymmetric contraction of an actin network in the cortex has been implicated in establishing asymmetry along the primary axis of animals such as nematodes and ascidians, while the movement of determinants along tracks of microtubules has been implicated in others, such as the insects. The various uses of the cytoskeleton by many animals may be derivations of a mechanism used by ancestral metazoans, or may reflect the paucity of other mechanisms available to embryos for generating internal asymmetry (i.e. convergent evolution).

It is interesting to note that the dissociated and reaggre-

gated cells of some embryos will develop an axis<sup>(2)</sup>, suggesting that embryos have the ability to self-organize an axis over time, yet all embryos studied to date appear instead to have a mechanism to specify their primary axis near the beginning of development.

The secondary axis can be specified as early as oogenesis (some arthropods and inarticulate brachiopods) to as late as gastrulation (articulate brachiopods). There is no case known where the secondary axis is specified as a global polarity. The secondary axis arises by a variety of distinct mechanisms in various animals. In some cases, the cytoskeleton has been implicated in moving putative determinants (such as ascidians and amphibians), while in other cases, the secondary axis arises by a pattern of cleavage which partitions putative determinants into a new plane (unequally cleaving molluscs and annelids), the skewing of primary asymmetries into a new plane by cell movement (nematodes), or one of a group of equivalent cells attaining



**Fig. 5.** Evolution of primary axis specification. Black, no regions yet determined in the egg; white, regions already determined in the egg. Reconstruction of the state of this character through the phylogeny is by parsimony<sup>(80)</sup> based on the state of the character in each phylum (squares). Cross-hatching represents a branch for which neither character state can be assigned based on the data. The phylogeny drawn is a rough consensus of molecular phylogenies, mainly from Christen *et al.*<sup>(81)</sup> and Wainright *et al.*<sup>(82)</sup>. Our conclusion is unchanged by some of the common inconsistencies in metazoan phylogenies, including placing the ctenophores as a sister taxa to the cnidarians, or placing the nematodes as arising from before the protostome-deuterostome split.

a unique position in the embryo (equally cleaving molluscs). For both axes, embryonic inductions are then used widely to elaborate many more regions from the initial few.

How were the cues for axis specification chosen as the various developmental programs arose? One possibility is that the cues used by ancestors were re-used; alternatively, each new developmental program could have been opportunistic in choosing from available cues. There are very few cases in which a cue is used by multiple, closely related phyla. One potential case is the use of first cleavage initiation site in cnidarians and ctenophores. More commonly, a particular cue is used either by just one phylum, or is shared by multiple, distantly related phyla. Examples include the use of cell positioning and cell-cell interactions in *Drosophila* and equally cleaving molluscs, and the use of the sperm in nematodes and amphibians. In such cases, the mechanisms used to interpret the cue often vary markedly between the phyla which use them. There are also a few cases where the cue has evolved within a phylum. These data suggest that the selection of cues has been opportunistic, with each new developmental program exploiting the cues available to it.

### Axis specification in ancestral metazoans

Little is known about how development occurred in the ancestral metazoans, although there have been a number of speculations dating from Haeckel. Based on mapping the data collected here onto an animal phylogeny and reconstructing ancestral character states by parsimony (Fig. 5), we present a scenario for axis specification in ancestral metazoans. We propose that unlike model organisms such as *Drosophila* and *Xenopus*, the ancestral animals constructed eggs with no regions pre-determined, and the embryonic axes were specified after fertilization. Additionally, ancestral animals may have established asymmetry in the form of a global polarity. The features which we propose as ancestral are shared by several of the basally branching phyla: the cnidarians, ctenophores, nematodes and nemerteans have no regions determined in the egg before fertilization. Often coupled with this trait is the specification of the primary axis in the form of a global polarity (cnidarians, ctenophores, nemerteans and possibly others, as this has not been directly tested in many phyla). We propose that the mechanisms used by other phyla for specifying axes during oogenesis are evolutionarily derived mechanisms. That is to say, some animals, such as the ascidians, use global polarity but have invented a mechanism for specifying this polarity as early as oogenesis. Others may have lost the use of global polarity and instead specified one or even both axes by regional determination in the egg. While mapping the presence/absence of regional determination in eggs onto an animal phylogeny suggests that eggs without pre-determined regions are ancestral for the metazoa, data are currently available for only nine phyla. The best way to test this hypothesis will be to conduct experiments on axis speci-

fication in more of the thirty or so phyla, and to map the results onto a robust phylogeny of the metazoans.

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