Origin of Bilaterian Body Plans: Evolution of Developmental Regulatory Mechanisms

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An argument is proposed to explain the origin of large metazoans, based on the regulatory processes that underlie the morphogenetic organization of pattern in modern animals. Genetic regulatory systems similar to those used in modern, indirectly developing marine invertebrates are considered to indicate the Precambrian regulatory platform on which were erected innovations that underlie the development of macroscopic body plans. Those systems are genetic regulatory programs that produce groups of unspecified "set-aside cells" and hierarchical regulatory programs that initially define regions of morphogenetic space in terms of domains of transcription factor expression. These ideas affect interpretation of the development of arthropods and chordates as well as interpretation of the role of the genes of the homeotic complex in embryogenesis.

Large animals may first have existed during the latest Precambrian (Vendian) period, and fossils dated to the initial period of the Cambrian (*1*) indicate the de novo appearance at this time of a diverse assemblage of bilaterian forms. By bilaterian we mean all deuterostome and protostome metazoans, including flatworms. An interpretation of the mechanism of the evolutionary process that caused the appearance of large and complex animals continues to be a major challenge. It was already evident a quarter of a century ago that novel morphological forms in animal evolution result from changes in genetically encoded programs of developmental gene regulation (*2*).

In this article, we discuss the higher level regulatory processes that program the morphogenesis of modern bilaterians. The adult body plans of many bilaterian phyla are first represented in fossils dated at or near the Precambrian-Cambrian boundary (*3*, *4*) (Fig. 1). The sudden advent of remains of such great morphological diversity is sufficiently dramatic, possibly occurring within 25 million years (*1*), that the remarkable evolutionary event they reflect has become known as the "Cambrian explosion" (*3*–*6*). Only taxa that have a low fossilization potential, such as nematodes, seem to be lacking from the Cambrian assemblages.

In interpreting the paleontological data we must consider whether the Cambrian explosion of fossils actually represents a sudden explosion of altogether new animal forms (*5*). The absence of Precambrian remains related to most modern taxa could reflect the fact that Precambrian animals lacked the shells and exoskeletons that are so prominent in the Cambrian fossil assemblages; or these animals might have existed only as microscopic organisms; or both. In either case, the Cambrian explosion would have to be regarded at least partly as an explosion not of novel metazoan phyla but of fossils. The consequence would be that at least some metazoan phyla may have originated before the Cambrian boundary. The only direct evidence for this derives from the relatively slim library of macroscopic Precambrian fossils. Among these are probable cnidarians, such as *Charniodiscus* (*7*–*9*); probable bilaterians, such as *Dickinsonia* and *Spriggina* (*4*, *7*, *9*); and unequivocally bilaterian trace fossils (*10*). If just one adult bilaterian body plan existed, then the type of genetic mechanism responsible for its development must have evolved before the first appearance of that organism in the Vendian.

We propose a period when Metazoa were represented only by microscopic forms similar to modern marine larvae. This would constitute a cryptic, pre-Ediacaran evolutionary phase that left no fossil record (or at least none so far recovered). These putative micrometazoan ancestors would not have left a fossil record because of their small size and probable lack of skeletonization (*4*). Even the very recent fossil record includes no remains of marine larvae that lack exo-

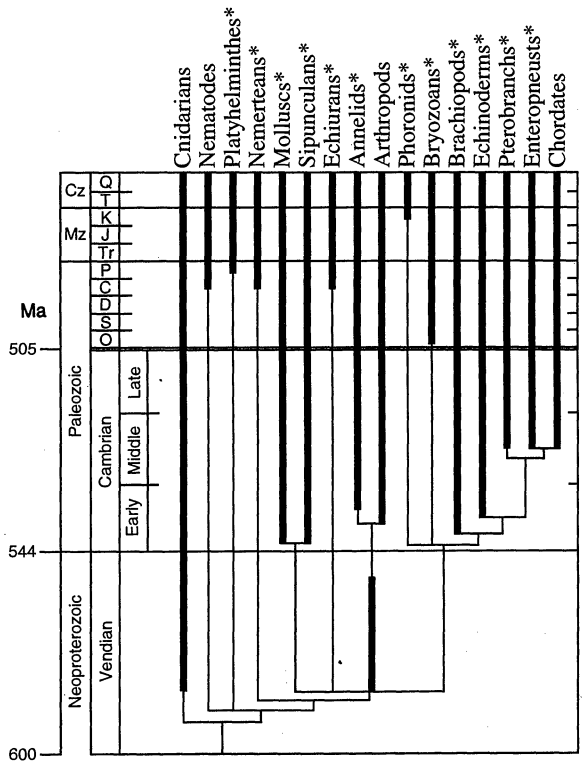

Fig. 1. The origin and diversification of metazoan phyla. The time scale at the left side of the figure (*1*) ends at the Cambrian-Ordovician boundary. Ma, million years ago. Above this boundary, geologic periods are indicated symbolically, without reference to a time scale, by the single-letter code given below. A tentative phylogenetic scheme of most major phyla is presented, with the known stratigraphic range (*4*, *39*, *50*) based on fossil recovery (solid bars). The range includes all fossils that are more closely related to one of the taxonomic groups shown at the top of the chart than to any other of these taxa; for example, hyoliths are considered to be allied to the sipunculans (*4*). The inferred depth of divergences among the phyla is also shown (thin lines). The solid bar leading up to annelids and arthropods represents the Ediacaran taxa mentioned in the text. The depths of the divergences shown are minimal; the split between cnidarians and bilaterians, for example, could have occurred well before 600 Ma. The phylogenetic scheme is derived in part from (*40*, *44*, *51*). Except for the annelid-arthropod and enteropneust hemichordate-chordate sister groupings, the evolutionary mechanisms discussed in the text are independent of the topology. Also indicated is the widespread distribution of maximal indirect development among the phyla, as indicated by an asterisk after the phylum name. Note that some cnidarians also develop indirectly, but their larvae are structured differently from those of bilaterians. Abbreviations are as follows: Mz, Mesozoic; Cz, Cenozoic; O, Ordovician; S, Silurian; D, Devonian; C, Carboniferous; P, Permian; Tr, Triassic; J, Jurassic; K, Cretaceous; T, Tertiary; and Q, Quaternary.

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skeletons (*11*). Such larvae were undoubtedly present in recent deposits from which larger fossils are abundantly recovered, but their minute and delicate structures were not preserved.

Indirect Development

In indirect development, the processes of embryogenesis differ from those later mobilized to generate the adult body plan. Our thesis is that the embryos and larvae of modern, indirectly developing marine taxa provide specific insights into the evolutionary mechanism by which bilaterians might have arisen. In organisms that display a completely indirect process of development, which we refer to below as "maximal indirect development," the embryo produces a larva that bears no morphological resemblance to the adult, which arises through a separate postembryonic developmental process occurring during the larval period. The primary larvae of marine invertebrates that develop by maximally indirect processes are usually of minute dimensions, typically ≤1 mm, and are composed of only a few thousand cells or less. Such larvae consist of an epithelial body wall one cell thick that surrounds the original embryonic blastocoel, now expanded. The blastocoel is traversed by a regionally differentiated gut (Fig. 2A). In maximal indirect development, the adult form arises from undifferentiated cells, often referred to as the "imaginal rudiment," that are set aside from participation in embryogenesis itself.

Maximal indirect development, as found in many marine invertebrates, must be distinguished from indirect development as this term is commonly applied to holometabolous insects or to typical frogs. These insects and frogs generate "secondary larvae" (*12*), that display many fundamental aspects of the adult body plan, such as metamerism in holometabolous insects or the dorsal central nervous system in amphibian tadpoles. Although further aspects of the adult body plans of these animals, such as appendages, are formed by postembryonic processes, by this measure insect and frog embryos develop directly, as do all terrestrial metazoans. Because most of the embryos that have been intensively studied are terrestrial (for example, *Drosophila*, amphibians, or *Caenorhabditis elegans*), it is easy to forget or ignore the fact that maximal indirect development in its true form is a widespread, general, and basic mode of bilaterian development (Fig. 1). Maximal indirect development is the rule rather than the exception in many taxa (*13*), such as echinoids, in which more than 80% of species begin life as feeding larvae and produce their adult forms indirectly (*14*). Maximal indirect development is a property that is shared among organisms on almost all branches of the bilaterian tree.

Direct development is a frequently observed evolutionary derivative of maximal indirect development. Loss of feeding capability, shortening of the larval phase, and precocious development of the juvenile form are features of direct development. Both direct and indirect development are often found even in marine species belonging to the same genus, which have diverged only recently. We are convinced that the direction of the transition is from indirect to direct development (*15*) and not the reverse (*16*). Genetic changes toward direct modes of development may happen rapidly on an evolutionary scale (*14, 15*). To approach the problem of the evolutionary origin of adult bilaterian forms, we focus on maximal indirect development, on the premise that this represents the ancestral mode by which adult body plans are ontogenically produced.

The sea urchin *Strongylocentrotus purpuratus* undergoes a drastic metamorphic transition. The process by which the adult body plan is formed is shown in Fig. 2, B and C. The mesodermal set-aside cells from which the juvenile body plan arises are the coelomic pouches, which derive from the archenteron (*17*). At the end of the larval stage, there are about 150,000 cells, or about 100 times more than at the end of embryogenesis (*18*), but more than 90% of these are included in the adult rudiment. After embryogenesis, the cells of the larva itself replicate only two to three more times, on average, during the whole of postembryonic larval life. At metamorphosis, the remaining larval structures are jettisoned and autolyzed. This example illustrates the almost brutal independence of the developmental process by which the adult body plan is generated.

Indirect development in the polychaete *Polygordis neapolitanis* produces the trochophore (Fig. 3), a spheroid larva characterized by a thick equatorial belt of ciliated cells (the prototroch) (*19*). Within the larval blastocoel are also the neuroectodermal and mesodermal germ-band precursor cells that generate the segmented body plan of the adult. These set-aside cell populations arise from fifth- and sixth-cleavage progeny of one of the blastomeres of the four-cell embryo (the D blastomere). In this example, one of the embryonic axes is retained as the anterior-posterior (A-P) axis of the juvenile, and there is a gradual rather than an abrupt metamorphosis from larval to adult form. Nonetheless, the development of *P. neapolitanis* clearly represents a case of maximal indirect development.

Type 1 Embryogenesis: Characteristics and Evolutionary Significance

In early development, the key mechanism is the process by which the fates of cells in different regions of the embryo are assigned.

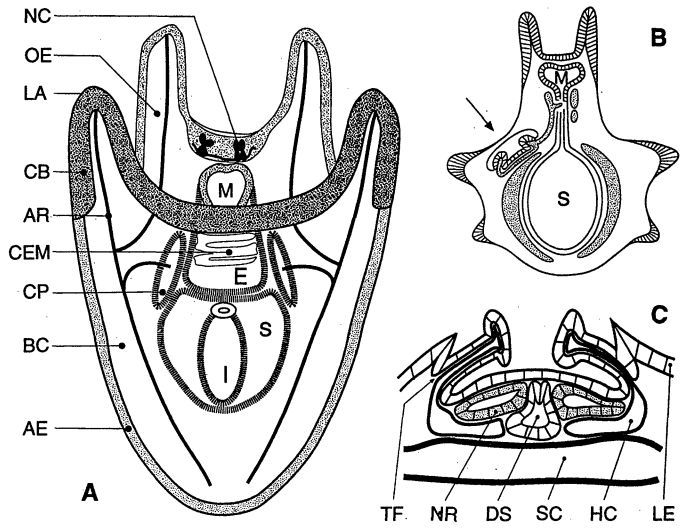

Fig. 2. Diagrammatic representations of indirect development in the sea urchin *S. purpuratus*. (**A**) Larva at completion of embryogenesis. One-cell-thick ciliated epithelia form the larval skin surrounding the embryonic blastocoel and the gut derived from the original embryonic archenteron. (**B**) Schematic diagram of the partially developed larva. After the onset of larval feeding, the trimeric coeloms arise. These are among the set-aside cells that give rise to most of the adult form. The ventral surface of the adult body develops from the apposition of the oral epithelium and the left coelom (arrow) (*52*). (**C**) Diagram of a portion of the mature imaginal rudiment. The pentameral symmetry of the adult body plan is first evident as the coelom forms a torus and five outgrowths push into the vestibule from the wall of the torus, thus forming the five primary tube feet. The characteristic sea urchin endoskeleton forms from test plates that organize around the periphery of the adult primordium within the larval blastocoel. Abbreviations are as follows: AE, aboral ectoderm; AR, skeletal rods; BC, embryonic blastocoel; CB, ciliated band; CEM, esophageal muscles; CP, coelomic pouches; DS, tooth sacs; E, esophagus, HC, hydrocoel, or left middle coelom; I, intestine; LA, larval arms; LE, larval epithelium; M, mouth; NC, serotinergic neurons; NR, circumoral nerve ring; OE, oral ectoderm; S, stomach; TF, tube feet; and SC, somatocoel, or left posterior coelom.

This process is termed specification, and its immediate consequence is the installation of differential patterns of gene expression in the cells whose progeny give rise to the diverse structures of the embryo. Comparative analysis of modes of embryonic specification across Metazoa (*20*) reveals that almost all major taxa, with the exceptions of insects and vertebrates, share essentially common mechanisms of embryonic specification. This general form of early development is termed Type 1 embryogenesis; it occurs primitively in all the taxa included in Fig. 1 and underlies both direct and indirect developmental processes. Therefore, the emergence of Type 1 embryogenesis must have preceded the divergence between cnidarians and bilaterians and hence must long antedate the Cambrian radiation. This implies the prior existence of animals that used Type 1 specification processes for their development but were not macroscopic bilaterians. We now propose that these metazoans provided the genetic platform on which the bilaterian diversification ultimately took place, and that their grade of organization is that of the primary larvae derived from Type 1 embryogenesis.

In Type 1 embryogenesis, cleavage begins immediately after fertilization and proceeds for a species-specific set number of divisions, often in the range 10 ± 2. By the end of cleavage, all the blastomeres have been specified. The embryo is now divided into a set of polyclonal lineages, each element of which gives rise to a certain differentiated cell type or types. The cell lineage of each morphological structure in the complete embryo is invariant within species. The invariance of the lineage follows from the canonical positions of the successive cleavage planes with reference to each other. These planes separate the lineage founder cells, which thus always appear in certain mutual positional relations with respect to the axes of the egg (*20*). Specification of the founder cells involves both conditional and autonomous mechanisms (*21*). These mechanisms define the identity, positions, and differentiated fates of the progeny of all the founder cells in situ, before the embryonic cells acquire any capability for cell motility.

This seems an efficient way to organize spatially the differential cellular functions of an embryo de novo, but it seems to work only for building the morphological structures of small larvae, consisting of at most a few thousand, sometimes just a few hundred, cells. The restriction may follow from the fact that both the number of cleavage divisions and the relative orientations and positions of the cleavage planes are important in that signal-mediated specifications continue to occur in place throughout cleavage. In Type 1 embryos, the individual structures of the larva consist only of the relatively small number of cells that can be generated during the remainder of cleavage according to their founder cell specifications, plus those cells that arise in the few subsequent divisions that some of these lineages undergo later in embryogenesis. For example, in completed embryos of *S. purpuratus* (*22*), the skeletogenic system consists of 64 cells, the gut of ~120 cells, and the muscle of 20 cells, and the embryo has only eight neurons; in *C. elegans* (*23*) there are 81 body muscle cells, 37 pharyngeal muscle cells, and 69 gut cells; and in the gastropod *Patella* (*24*), the swimming organ of the completed embryo contains 32 cells.

Type 1 larvae consist of many of the same basic differentiated cell types of which modern bilateral metazoans are composed. Typical Type 1 embryos generate the specialized differentiated cell types that form, for example, the larval gut, muscle, ganglia, eyes, ectoderm, and skeleton. Thus, they must use the large batteries of differentiation structural genes that enable each of these cell types to execute its particular functions, including particular biochemical functions and motility and structural functions that involve specialized cytoskeletal organization and intercellular interfaces. This in itself probably requires several thousand diverse genes (*25*). Furthermore, the differentiation gene batteries must be controlled, and Type 1 embryos also use regulatory genes that encode the transcription factors that organize the coordinate expression of differentiation genes. A key distinguishing feature of Type 1 embryos is that cohorts of downstream genes encoding specified differentiation products begin to be expressed during cleavage (*20, 26*). Thus the Type 1 specification process leads at once to the lineage-specific activation of differentiation gene batteries.

The case of maximal indirect development shows that by itself the Type 1 repertoire of developmental mechanisms does not suffice to build a macroscopic adult body plan. The adult organism is generated from the larval imaginal rudiment by a process that is distinct from that by which the larva is generated from the early embryo. Thus, in indirectly developing marine larvae, the processes leading from embryo to larva and from the imaginal rudiment to adult are entirely separable. An illustration of this is shown in Fig. 4. The photograph in Fig. 4A shows a sea urchin larva of a strain that was recovered in an inbreeding experiment (*27*). The larva displays a normal advanced morphology (compare Fig. 4B), but it has entirely failed to embark on rudiment development. In no apparent way has this compromised its own embryonic or larval developmental process.

Pattern Formation

In order to produce the body plans of macroscopic animals, a qualitatively different developmental regulatory mechanism than that used in Type 1 embryogenesis is needed. We refer to a fundamental initial aspect of this mechanism as "pattern formation"; we use this term in a precise sense (*28*) to denote regulatory mechanisms that are required to partition undifferentiated regions of an embryo into areas of specific morphogenetic fate. For example, a sheet of undifferentiated embryonic cells may be programmed in a given species to give rise during development to some structure, such as an appendage. The pattern formation process to which we refer is that which initially defines the area of cells whose progeny produces the appendage (the "progenitor field") (*29*), and then within this field defines the subregions that give rise to each part of the structure. This usage focuses on the upstream regulatory processes of morphogenesis rather than on the subsequent morphogenetic mechanisms by which the three-dimensional

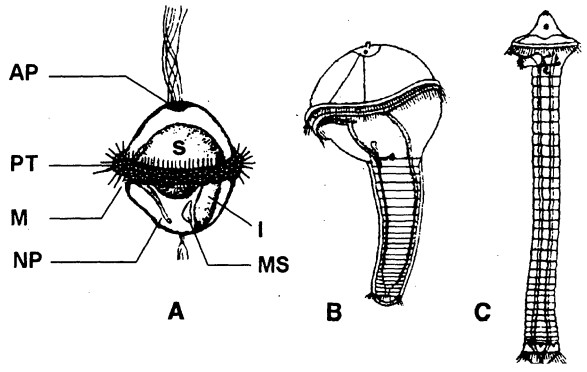

Fig. 3. Indirect development of the polychaete worm *P. neapolitanus*. (**A**) Trochophore larva, the product of embryogenesis, with a thick band of ciliated cells at the equator. Within the blastocoel reside the mesodermal set-aside cells that give rise to the germ band rudiment. AP, apical plate; I, intestine; M, mouth; MS, mesodermal strand; NP, nephridium; PT, prototroch; S, stomach. (**B**) The larva beginning to form the metameric segments of the adult body plan. The portion of the trochophore larva above the prototroch remains essentially unchanged, enabling the larva to continue its free-swimming habit. When about 30 segments have formed, the structures of the adult head appear. (**C**) The larva at metamorphosis; the anterior structures of the larva are being replaced by adult head structures [redrawn from (*19*)]. Many more segments rapidly form, the prototroch degenerates, and the organism metamorphoses into a juvenile polychaete annelid.

structure is built and its terminal differentiations are installed. We think that a common upstream pattern formation mechanism underlies the development of the major morphological parts of all bilaterian body plans.

The initial mechanism in pattern formation is the installation of transient spatial territories of transcription factor expression. These foreshadow the various parts of the structure to be formed. The sole function of this process seems to be to establish different gene regulatory states in the sets of cells whose progeny generate different morphological components. The initial pattern-forming transcriptional regulators do not directly control the differentiation gene batteries that eventually will be expressed as the morphogenesis of the structure nears completion (*20, 28, 29*), although some of the same regulators may be in operation during later stages. Thus, whereas the specification of blastomeres in Type 1 embryos leads directly to differentiation, specification of the pattern elements of the adult body plan initially produces only a set of regional regulatory definitions. This is a more abstract, but also a far more powerful, process because an inextricable component of these pattern formation processes is growth. The initial transient pattern of transcription factor expression is typically imposed on the field of progenitor cells, whose far more numerous progeny will actually generate the structure. Regional control of cell multiplication as the structure is patterned is an essential function that is intimately linked to the morphological realization of the initial design (*30*).

The patterned expression of certain transcription factors has been shown to constitute an initial necessary step in diverse morphogenetic processes (Table 1). Transcription factors of many different biochemical families may be involved in such processes, although in the particular examples shown in Table 1, homeodomain proteins predominate. The first two cases concern *Drosophila* imaginal discs, which are small patches of 25 to 30 cells set aside during early embryogenesis, from the progeny of which the adult wings and legs are formed (*31, 32*). As the wing disc grows from about 10^2 to 10^3 cells, transcriptional regulatory processes occur that define the region of the disc epithelium that will give rise to the distal, dorsal, ventral, anterior, and posterior portions of the final structure. Differentiation begins only later when the discs contain about 5×10^4 cells. The vertebrate examples (Table 1) are in essence similar. The subregions of the respective embryonic domains that give rise to the parts of the hindbrain of the mouse and of the wing of the chicken are developmentally defined by the spatial expression of specific sets of transcription factors. The expression of these vertebrate pattern formation regulators occurs transiently, long in advance of downstream morphological features, but it is essential: Gene knockouts, or spatial misexpressions, result in duplications, homeotic transformations, or deletions of the structures formed from the domains where normally these regulatory factors are transiently present (*33, 35*).

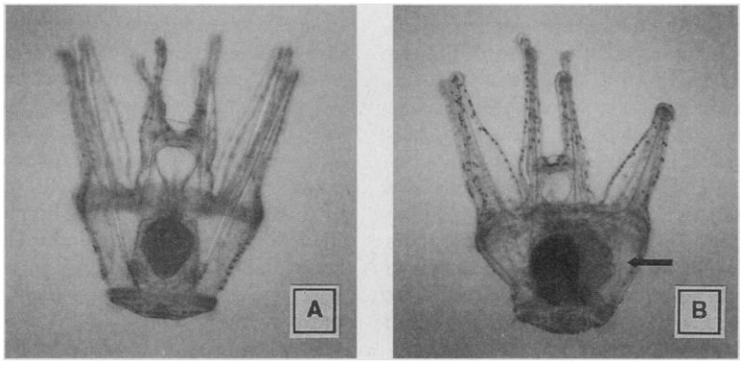

Fig. 4. Identical sea urchin larvae with and without an imaginal rudiment. (**A**) Genetically defective *S. purpuratus* larva of a strain discovered during a screen of inbred lines (*27*). No imaginal rudiment can be seen. However, all structures of the larva itself appear normal and the larva is feeding successfully, as can be seen from the appearance of its stomach. (**B**) Normal control larva, including the rudiment (arrow).

Table 1. Upstream pattern formation. Examples of transcription factors (TXF) that demarcate the progenitor fields for morphological elements of the organism.

Structure	Progenitor field and TXF*	Subregions and TXF
Drosophila		
Wing	Wing imaginal disc (*31, 32*); *vg*	Dorsal-ventral boundary; *vg* Distal; *dll* Posterior; *en* Dorsal; *ap, vg* Ventral; *sd, vg*
Leg	Leg imaginal disc (*32*); *dll*	Distal; *al, dll* Posterior; *en*
Larval stomach	Midgut (*53*); *hkb, srp*	Gastric caeca; *scr* Midgut constrictions; *AbdA, lab, Ubx, Antp*
Mouse hindbrain	Region of neural tube (*33*); ?	Rhombomeres 3 to 8; nested combinations of *HoxA1* to *-A5* and *HoxB, -C*, and *-D* paralogues define each rhombomere Rhombomeres 3 and 5; *Krox20*
Chicken wing	Limb bud field (*34, 36*); *HoxC6*	Individual digits; nested combinations of *HoxD9* to *-D13* define each digit

*TXF abbreviations: *vg, vestigial; dll, distalless*, homeodomain; *sd, scalloped*, TEA domain; *ap, apterous*, LIM homeodomain; *al, aristaless*, paired homeodomain; *en, engrailed*, homeodomain; *AbdA, HOM-C*, homeodomain; *Ubx, HOM-C*, homeodomain; *Antp, HOM-C*, homeodomain; *lab, HOM-C*, homeodomain; *scr, HOM-C*, homeodomain; *Krox20*, zinc finger; *srp, serpent; hkb, huckebein*, zinc finger.

Two of these same examples can be used to illustrate another point, one of evolutionary importance. The primordia from which the forelimb buds derive are initially demarcated in the lateral plate mesoderm by the expression of the *Hox* C-6 gene, as shown for the wing of the chicken (Table 1), the teleost pectoral fin, and the mouse and frog forelimbs (*36*). The development of these structures differs obviously among fish, birds, and mammals in their downstream morphological outcomes. Yet they all begin with the same molecular pattern formation process. This regulatory expression pattern thus correlates with a higher level clade, namely the teleosts plus the tetrapods. The forelimb bud progenitor field can be thought of as defining a morphological space, that is, a set of possible morphologies that can be generated from this particular precursor field.

A similar implication derives from comparative studies of pattern formation processes in insect imaginal discs. The region of the disc epithelium that is to give rise to the distal portion of the *Drosophila* leg is that where the homeodomain regulator *distalless* (*dll*) is expressed (Table 1); but in certain butterfly wings, the same patterning mechanism is used instead to determine the location of the "eyespots" that are a prominent feature of the scale design in this species (*37*). Furthermore, although the large wing of the butterfly is morphologically different from the tiny *Drosophila* wing, many of the other pattern-forming regulatory functions operating in the *Drosophila*

wing disc are also used in the butterfly wing disc (*37*). The morphological space is defined by these upstream transcription factor patterns, and it is filled in various ways that have lower level taxonomic significance.

Evolutionary change in the regulatory DNA sequences controlling the spatial expression of pattern formation transcription factors that control development could result in changes that affect body plans. Paleontological evidence indicates several episodes of dramatically fast organismal diversification, for example, in bilaterian body plans before and during the Early Cambrian (*1*) or in tetrapod forms later in the Paleozoic (*38*). This evidence suggests that once the basic mechanism exists, both the appearance of new genetic pattern formation systems, and the genetic changes in pattern formation processes needed to fill the morphological space created when these new systems appear, can occur very rapidly in evolutionary time. This argument is not concordant with microevolutionary, or gradual, change in adult body plans, and means that intermediate or transitional fossil forms of body plan are unlikely to be found (*39*, *40*).

Evolutionarily Novel Regulatory Mechanisms

We have concluded from the paleontological evidence that the origin of bilaterian body plans and hence the pattern formation mechanisms used in their developmental construction must predate the Ediacaran fossils. The mechanisms of Type 1 embryogenesis and the small larvalike metazoans that are its outcome must then have originated even earlier. This, in turn, implies an enormously greater antiquity for the structural gene batteries and genetic regulatory control systems that program the differentiated cell states common to all multicellular animals than for any higher metazoan morphological feature.

We conceive the most important evolutionary novelty to have been the developmental use of yet undifferentiated set-aside cells, which retain indefinite division potential, as a substrate for the morphogenesis of large structures. Thus we must understand origin of cellular domains in organisms similar to Type 1 larvae, domains that function similarly to imaginal discs, limb bud progenitor fields, or the imaginal anlagen from which adult echinoderms derive. Among the genetic regulatory changes required to produce set-aside cells are the disconnection of the cell division controls that are a prominent feature of Type 1 embryos and the disconnection of whatever differentiation gene batteries the lineages giving rise to the imaginal cell sheets earlier expressed. Many observations of mammalian cells show that these kinds of change occur relatively easily. Thus genetic misexpression of various oncogenes may result in release from cell replication controls and loss of differentiated phenotypes, as in retroviral tissue culture transformation. Whatever their origin, the evolutionary "invention" of developmental programs for the generation of set-aside cells was among the primary causes of the appearance of the higher Metazoa.

The appearance of pattern formation processes would have required a hierarchical regulatory network reorganization. Abstract upstream pattern formation functions would have to precede activation of regulators controlling differentiation. A new morphogenetic world was then created, one that was freed of the developmental constraints of quantal cell lineage, immediate embryonic specification, and intrinsic size limitations; and as history shows, one that is capable of great variety in the use of morphological space. Eventually, as in modern indirectly developing bilaterians, the new adult structures came to supplant entirely what we now consider larval structures, in each life cycle.

The *HOM*-C gene cluster may well be used in A-P pattern formation processes in all bilaterians (*41*). However, there is no reason to suppose that the origin of the *HOM*-C cluster was the single event that potentiated higher metazoan developmental processes. This event could indeed have been important for organizing these processes into the bilateral form that is the shared primitive character of the adult body plans of most modem metazoan groups. But many different biochemical classes of transcription factor beside those encoded by the *HOM*-C genes, including a large number of other, unlinked homeodomain proteins, participate in upstream pattern formation processes in the development of modern animals. It is the use of regionalized upstream transcription factors that is the key event, irrespective of their class.

Phyletic Origins and the Cambrian Explosion and Implications

Not all modern bilaterian phyla can reasonably be regarded as having arisen directly by the diversification of pattern formation processes, superimposed on a platform of preexistent micrometazoans. A test for such phyla is to ask whether they share specific larval rather than adult, characters with phylogenetically related groups. A clear example is afforded by the echinoderms and the hemichordates (Fig. 1). These taxa have remarkably similar embryonic and larval forms, although their definitive adult body plans are indeed different. Similarly, sipunculan worms and molluscs share the "molluscan cross," a particular arrangement of animal blastomeres of certain lineages. Yet, sipunculan worms and molluscs share few, if any, adult morphological characters. There are additional examples that are not so obvious because often what appear to be adult characters shared between sister taxa are in fact larval in origin, although they are carried through metamorphosis (*42*). In contrast, by the same test, other phyletic sister groups certainly share adult characters, rather than larval characters. We consider below the origins of the most important of such phyla, the chordates and the arthropods.

The advent of developmental processes capable of generating diverse assemblages of macrometazoan morphologies clearly must have sharpened selective pressures. With the extinction of most micrometazoan platforms except the "winners" in the pattern formation competition, the possible range of large metazoan morphologies may have been limited. These winners probably included all of the current phyletic body plans plus some others that did not survive to the present ("Problematica") (*43*). Enhanced selective pressure drove the rapid exploitation of the morphological space blocked out by the patterns of upstream transcription factor expression in the winning phylogenetic lineages. This is what we see as the Cambrian explosion of phylotypic body plans.

Maximal indirect development is a rather peculiar process. Our scheme renders the prevalence of indirect development no longer inexplicable; the many species developing by this means are simply continuing to use the original mechanism by which large metazoans first arose. Direct development is a derivative, and almost all bilaterian taxa begin life with Type 1 embryogenesis, with the important exceptions of vertebrates and insects (*21*). In the indirect development of modern animals, the larva serves only as a life support system for the imaginal set-aside anlagen within which the adult body plan develops.

The foregoing concepts lead to specific interpretations of the developmental evolution of both chordates and arthropods. Arthropods share adult body plan characters with their sister group, the annelids, such as metamerism (*44*, *45*). Yet basic annelid groups such as the polychaetes develop indirectly by way of trochophore larvae, whereas arthropods develop directly and do not produce trochophore larvae. Arthropods then generate their characteristic appendages by means of imaginal discs or the equivalent. Within some arthropod clades, new already-metameric forms of secondary larvae (such as the nauplius larva) were generated. Arthropods, in our terms, use set-aside cells twice in their development:

once when they form metameric structures from teloblast progeny and again in the specification of their imaginal anlagen.

Chordates are also primitively a directly developing phylum (*46*). They share only adult characters with their indirectly developing sister group, the enteropneust hemichordates (Fig. 1). For example, the notochord of adult larvaceans, the primitive urochordate class (*47*), and of all other chordates, is homologous with the stomochord of adult hemichordates (*40*, *48*). Another example is provided by the U-shaped pharyngeal slits, a character shared by enteropneusts and chordates; although this is a feature of larval and adult chordates, it appears in enteropneusts only after metamorphosis (*49*). No living chordate displays a larva of the "original" deuterostome type (the dipleurula larva), such as is still used in most modern indirectly developing echinoderms and hemichordates. Therefore, chordates arose through the imposition of their particular phyletic pattern formation functions on what were essentially directly developing hemichordates.

Deuterostomes and protostomes share specific pattern formation processes, of which the most prominent example is regional A-P specification by homologous genes of the *HOM-C–Hox* gene cluster, but there are many other examples. We argue that homologous pattern formation processes that have been discovered in insects and chordates (*28*) are processes involved in the formation of the adult body plans of these organisms. Because both insects and chordates develop directly, this is a difficult inference. However, an indirectly developing sea urchin larva, for instance, has no A-P or dorsal-ventral axis homologous with that of the adult body plan. Furthermore, pattern formation processes, in the sense used here, do not seem to be used in Type 1 embryonic processes. If the shared pattern formation processes of deuterostomes and protostomes are indeed aspects of adult body plan formation, this would require that the set-aside progenitor cells and the hierarchical regulatory systems required for the ontogeny of adult bilaterian body plans antedate the protostome-deuterostome divergence. Protostome and deuterostome embryos are in certain ways very different. For example, they organize the blastomere lineages that give rise to their embryonic mesodermal derivatives differently and they form their embryonic guts by different mechanisms. Furthermore, the imaginal rudiments that give rise to the adult forms of indirectly developing deuterostomes and protostomes arise by entirely different embryonic pathways (Figs. 3 and 4). Thus we need to ask how the ancestral forms from which modern deuterostome and protostome taxa evolved could have shared specific mechanisms used in the formation of the adult body plan. What was inherited from the latest common ancestor were the genetic programs specifying essentially abstract, upstream, regionalization mechanisms assigned to the generator of given aspects of the adult body plan, such as the A-P axis. These mechanisms could be so abstract that their function is simply to distinguish relative positions along an axis (the *HOM-C* genes) or to distinguish the center from the circumference of a progenitor field (the *dll* gene). Such regulatory program elements could be applied to embryonic fields of set-aside cells, however these might be generated in the development of the larva and wherever they might be situated. In conclusion, the lineages leading to deuterostomes and protostomes must have shared the genetic programs required to specify set-aside cells. The latest common ancestor of deuterostomes and protostomes used these genetic programs to generate some form of adult body plan, although this is unlikely to resemble any modern bilaterian form.

Some predictions that follow from our premises include: (i) Differentiation gene batteries, and their immediate regulatory apparatus, should be found to be essentially pan-metazoan. This should be true for a variety of canonical differentiated cell states. (ii) Individual *HOM-C–Hox* genes should be found to be used in Type 1 embryos as cell type–specific or lineage-specific regulators that directly control cell differentiation, but should not function during the process of Type 1 embryogenesis in the same way as do transcription factors that control regionally expressed upstream pattern formation (*20*). However, *HOM-C–Hox* genes should be required for the organization of the adult body plans that are ultimately generated by the larvae of organisms using Type 1 embryogenesis. (iii) Specific genetic regulatory systems should exist, the function of which is to define developmental set-aside cells, that is, the imaginal cell sheets from which adult phylotypic structures develop. Such mechanisms would account for two obvious properties of these cells: their undifferentiated states and their growth potential that persists in advanced larval stages or the equivalent. These regulatory systems can be studied in the set-aside cells of modern animals. The regulatory programs specifying set-aside cells may have been one of the revolutionary Precambrian genetic inventions that potentiated the appearance of the higher metazoans.

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