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The evolutionary origin of development: cycles, patterning, privilege and continuity

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SUMMARY

A scenario for the evolution of a simple spherical multicellular organism from a single eukaryotic cell is proposed. Its evolution is based on environmentally induced alterations in the cell cycle, which then, by the Baldwin effect, become autonomous. Further patterning of this primitive organism - a Blastaea, could again involve environmentally induced signals like contact with the substratum, which could then become autonomous, by, perhaps, cytoplasmic localization and asymmetric cell division. Generating differences between cells based on positional information is probably very primitive, and is well conserved; its relation to asymmetric cell division is still unclear. Differentiation of new cell types can arise from non equivalence and gene duplication. Periodicity also evolved very early on. The origin of gastrulation may be related to mechanisms of feeding.

The embryo may be evolutionarily privileged and this may facilitate the evolution of novel forms.

Larvae are secondarily derived and direct development is the primitive condition as required by the continuity principle.

Key words: evolution, embryo, cell patterning

INTRODUCTION

Now this is the next tale, and it tells how the camel got his big hump

Just So Stories, Rudyard Kipling

The evolution of the cell can be regarded as the 'big bang' of biological evolution even though it took a very long time. The origin of embryonic development from cells can be regarded as the 'little bang' since the cell was already there. So the general question is, what was required, given the eukaryotic cell, for development to occur (Wolpert, 1990)? How did the egg, patterning and change in form originate? Since embryonic development requires the formation of a multicellular organism from a single cell, the origin of the egg is a central and sadly neglected problem.

The evolution of development is intimately linked to the origin of multicellular forms. The earliest eukaryotic organisms are thought to have been present some 1400 million years ago, while the earliest evidence for metazoans is some 800 million years ago. Metazoan origins are generally thought to be monophyletic (Willmer, 1990; Slack et al., 1993). It may thus seem that the transition from single celled organisms to multicellularity was a difficult one, requiring hundreds of millions of years. However, the fossil record for such delicate organisms is undoubtedly fragmentary and incomplete but, as I have suggested, given the eukaryotic cell with its ability to replicate and move, all the basic elements required for development were already present, and the transition to multicellularity relatively easy. Nevertheless it took another few hundred million years, until the Cambrian, before fossils of recognizable animals can be found.

I will try to present a scenario whereby the eukaryotic cell could have evolved multicellular embryonic development. In doing so a central requirement is that each stage is required to have a selective advantage and that there is continuity between stages (Horder, 1983). Big jumps – hopeful monsters – are not allowed. Even so, I recognize that my scenario is only slightly better, perhaps, than one of Kipling's Just So Stories, like how the leopard got its spots, or the camel its hump.

There are two main theories as to the origin of the Metazoa (Salvini-Plawen, 1978; Willmer, 1990). The first proposes that there was a coming together of two or more cells to form a colony while the second proposes growth of a cell with nuclear division followed by the later establishment of cell boundaries. These theories are extended in various ways to account for the evolution of simple invertebrates and so invoke various sequences leading to Cnidaria, Porifera and Spiralia. In most of these scenarios a Gastrea-like organism appears either earlier or later. However, none of the theories give any attention to the evolution of developmental mechanisms.

ORIGIN OF THE EMBRYO

Of the two theories I shall pursue a modification of the one based on repeated division of a single cell. Plausible though the others may be – the cellular slime mould is an excellent example of aggregation and the early *Drosophila* embryo one for nuclear division, followed by cellularization - they do not

easily give rise to what is characteristic of all animal development: the generation of a group of cells by cleavage of a largish cell. This process had to originate at some stage or another so I prefer to take it as my starting point. It is also the only way of reliably obtaining a set of genetically identical cells.

I suggest a mechanism based on a process that is somewhat similar to the Baldwin effect, which was proposed in the early years of this century and extended by Waddington into what he called 'genetic assimilation' (Waddington, 1957). (For a recent review in relation to the evolution of animal behaviour see Bateson, 1988). In essence it involves an environmentally produced effect becoming part of the developmental programme. An environmental signal is replaced by a developmental one.

Several changes are required in order for a single cell to give rise to a multicellular group by cell division (Nurse, personal communication). Firstly, the cell has to grow larger than its normal size and this requires a transient block to mitosis. Secondly, in the enlarged cell, the block over mitosis has to be released and the cell must then divide several times. And thirdly, the cells have to remain together.

The first two requirements involve modification of the cell cycle and it is relatively easy to propose mechanisms based on knowledge of controls in modern cells; but then our basic assumption is that the cell cycle was present in our original eukaryotic cell. In fission yeast, cell size at mitosis is enlarged in a good medium and reduced in a poor medium. This works by modulating when the p34 cd2 kinase is activated, activation in poor media being at a lower cell mass. Thus it is very possible that environmental cues could affect when the transition occurs. For example a single cell growing in a good medium could become enlarged and on a shift to a poor medium undergo two divisions without further growth. This actually occurs with *Chlamydomonas*: during fast rates of growth, cells divide into 4 or even 8 at each cell cycle.

We can thus imagine a cell increasing its diameter 2.5-fold and then dividing 4 times to give 16 cells (Fig. 1). Let us further assume that the third requirement is satisfied and the cells remain together, and, moreover, the cells form a hollow sphere. The latter may require oriented cell divisions or the maintenance of junctions between cells on the outer surface but that may not be too difficult.

The net result of such changes would be an increased cell size in good medium leading to a multicellular sphere in poor medium. It would be a selective advantage if the cells were ciliated so a sphere might swim faster and so find an environment with good food. Now there would be, for the first time, a positive selection for the multicellular state in a poor medium and the multicellular state would be environmentally induced. By the Baldwin effect we can imagine mutations such that no matter what the medium the cells grow large and divide many times. An environmental signal would have been taken over by the genes: the signal has in a sense become constitutive. The selective advantage for multicellularity could now be speed of swimming, sharing of metabolites, protection from predators.

Just one more step is required for the evolution of the embryo. The individual cells need to separate and start the programme again. This could occur when the cells grow and became too large to remain in contact. Each individual cell could now go through the same programme. Embryonic development had evolved. It could well have taken a long time to reach this simple, but crucial stage.

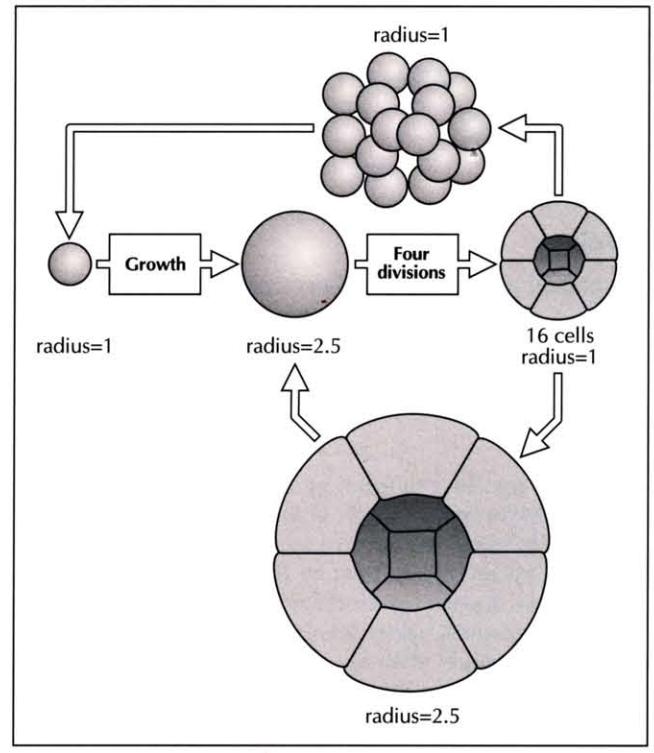

Fig. 1. The origin of the embryo. A single cell is assumed to have increased in size without dividing and then divided four times to form a Blastaea. This may have reflected external signals such as the nature of the medium in which it is growing. If this process became constitutive then the process could be repeated either by the cells separating and repeating the process, or growing in contact with the other cells and then separating.

In this primitive embryo, which, following Haeckel, we call a Blastaea, each cell becomes an egg and there is no spatial organization. Patterning is the next step.

THE ORIGIN OF PATTERNING

Given that the mechanism of altered cell cycles could have generated a hollow sphere formed by a single sheet of cells, we can now consider the origins of patterning, the origins of spatial organization.

The key to all development is the generation of differences between the cells, that is, making them non-equivalent (Lewis and Wolpert, 1976). Only if the cells are different can the organism be patterned so that there are organized changes in shape, and cells at specific sites differentiate into different cell types. How could this have evolved?

Consider that the primitive Blastaea came to rest on the ocean bottom (Fig. 2). At the site where it made contact with the substratum it is not unreasonable to assume that these cells will be differentially affected. It could alter metabolism or affect surface receptors. In time, this environmental signal could result in a cascade of activities starting at the point of contact, affecting both the cells at the contact site and signals that influence more distant cells. For example, there could be

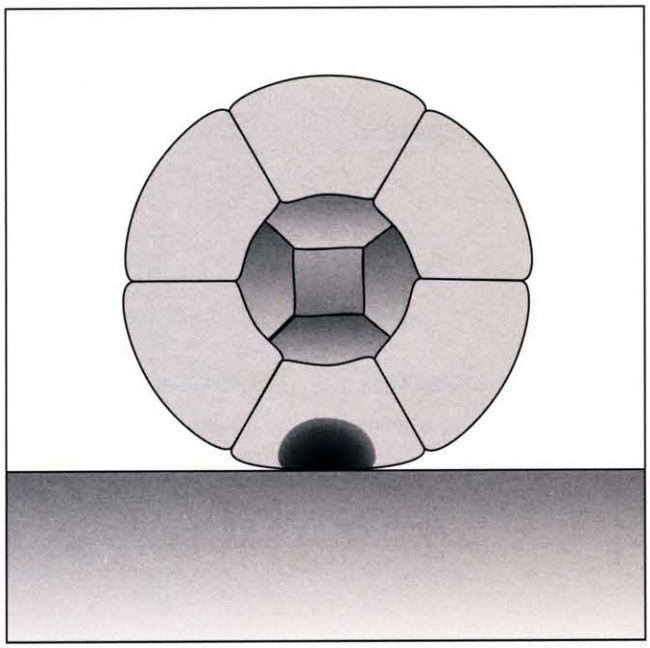

Fig. 2. The origin of an axis. If the Blastaea came to rest on a substratum the site of contact could induce a change in cell state which could specify an axis.

a selective advantage to the organism becoming attached to the point of contact. Or it may be an advantage for the cells to invaginate at the site of contact. Whatever the advantage, an environmental signal brings about a localized change in the organism which becomes elaborated with time. It could even lead to suppression of growth of adjacent cells and so the restriction to reproduction of cells at the opposite end of the embryo. An embryonic axis could be specified. Evolution of patterning has occurred.

There is of course the problem of the nature of the signal. Even now the identification of signals involved in development is restricted to a very few cases. However, one can imagine cells secreting a variety of molecules and by chance some of the cells responding to such signals. There would be little evolutionary pressure against such developmental explorations (see below). It is worth noting that most patterning occurs in sheets, that is in a two dimensional array of cells (Wolpert, 1992).

This initial patterning was entirely environmentally induced by an asymmetric external signal. Even today there are many such examples: light polarizing the *Fucus* egg; sperm entry determining the dorsoventral axis in amphibians; unknown environmental signals determining the anteroposterior axis in Hydrozoa and mammals.

Invoking again, the Baldwin effect, we can see how this process might be constitutive, that is the cells at the contact site could be genetically specified. This illustrates the advantages and economy for evolution of development that the Baldwin effect provides. In its absence it would be necessary to first genetically specify one group of cells as being different but without there being any selective advantage. Only then could there be changes in these cells similar to those described above. This sequence of events is unlikely in the extreme. The reason for invoking the Baldwin effect is that an environmental signal provides an initial basis for a developmental alteration, which could have a selective advantage.

CYTOPLASMIC LOCALIZATION AND ASYMMETRIC CELL DIVISION

Using the Baldwin effect we can imagine how the specification of an environmentally induced axis could be incorporated into the developmental programme. All the machinery for specifying the axis was now present and all that was required was to replace the environmental signal by generating special cells in which the signal would be constitutive. One way of doing this would be to have cytoplasmic localization in the egg so that only one cell or a few cells acquired this special cytoplasm following cleavage (Fig.3). This cell could then specify the axis. Thus asymmetric cell cleavages could be involved in defining a single or small group of cells originally specified by an environmental signal. Moreover, this cell could then become the only cell capable of development and a distinction between soma and germ cells would be established.

It remains unclear why today some organisms like nematodes rely quite heavily on highly determinate patterns of cell cleavage which are often coupled to asymmetric cell divisions, while others, like vertebrate and insects, rely almost exclusively on cell interactions. Why should these two different strategies have evolved? One possibility is that asymmetric cell divisions provide a more reliable means of specifying cell identity on a cell by cell basis, whereas cell signalling is preferable where groups of cells are being specified.

But the puzzle is compounded by the fact that organisms that are specified by determinate cell lineages, which probably involve asymmetric divisions, can also reproduce asexually and have considerable powers of regeneration. Such animals include Cnidaria, Platyhelminthes, and polychaete Annelida (Barnes et al., 1988). Since regeneration and asexual reproduction both absolutely require cell interactions - there is a problem of the relation between the mechanisms of early development and interactions in the adult. It seems that cell interactions represent the primitive condition and that the requirement for asymmetric cell divisions for specification was secondary. Both processes would set up a body plan in which interactions were dominant, and it is very difficult to see how cell interactions could evolve from asymmetric cell divisions. A general principle is that early development is very variable and the phylotypic stage is the more constant. It is worth noting that regeneration is essentially a meta phenomenon related to either asexual reproduction or the fortuitous maintenance of the embryonic state in which cell interactions dominate. Morphallactic regeneration is quite a common mechanism and this, as in *Hydra*, involves the specification of new boundary regions, and the respecification of intermediate values (Wolpert, 1989).

The specification of one axis imposes radial symmetry on a spherical larva (Fig. 4). In principle this only requires the patterning of one small group of cells which could then act as a boundary signal. It seems that bilateral symmetry is a very primitive character among invertebrates. Unfortunately we understand little about how bilateral symmetry is established in present day organisms though it can be related to the first cell cleavage and the specification of the dorsoventral axis. In principle it only requires the specification of another single

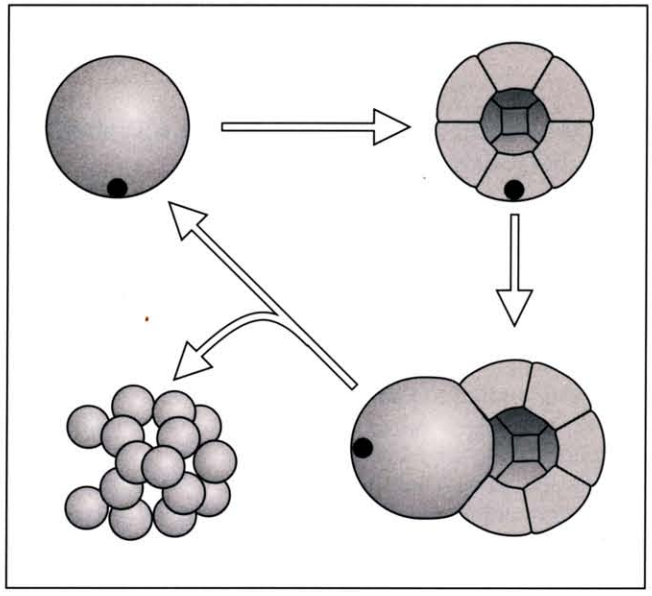

Fig. 3. Cytoplasmic localization and asymmetric cell division could specify a germ cell. Cytoplasmic localization of some factor could specify that only one cell could grow without dividing and so establish a difference between somatic and germ cells.

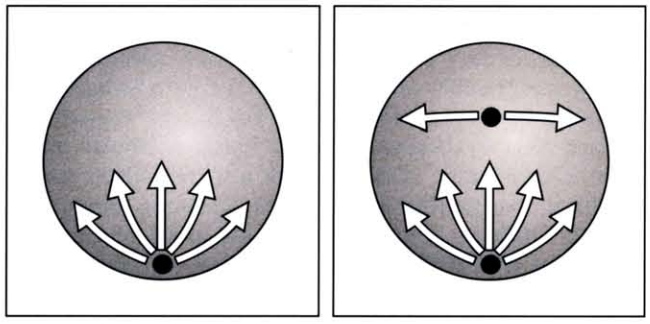

Fig. 4. Specification of axes. Specification of one group of cells could define the anteroposterior axis with radial symmetry; specification of another could define the dorsoventral axis and so produce bilateral symmetry.

location on the surface of the spherical embryo and anteroposterior, dorsoventral, and bilateral symmetry are defined

POSITIONAL INFORMATION AND CELL DIFFERENTIATION

A central feature of positional information is the dissociation of the specification of differences from the ultimate fate of a cell; that is, how it differentiates (Wolpert, 1989). For example, if cells acquire positional identities along the anteroposterior axis then how they differentiate further is almost unconstrained. The concept of the zootype (Slack et al., 1993) illustrates this nicely since apparently all animals have a set of positional identities along the antero posterior axis specified by similar homeobox containing genes. This very strongly suggests that this mechanism for patterning is very primitive.

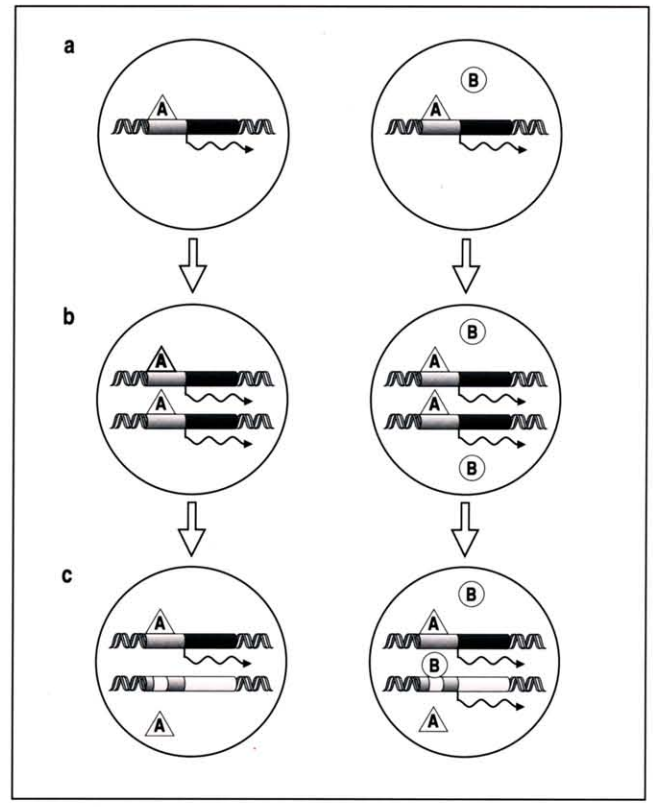

Fig. 5. (A) Two cells express a protein since both contain factor A, which activates the gene. Factor B is only present in the right hand cell. (B) The gene is duplicated and both cells express double amounts of the protein. (C) Changes in both the promoter and structural region result in the expression of the altered duplicated gene only in the right hand cell, because of the presence of B.

Presumably it was established when the first primary axis evolved, as suggested above.

Sternberg and Horwitz (1984) have suggested that cell lineages may evolve by a process of cell duplication followed by a modification of one of the duplicated cells, the analogy being with gene duplication. There are mutations in nematodes that give an extra cell division. Lineally equivalent cells with multiple potentials may thus be an intermediate step in the evolution of cell diversity. They may express different potentials as a result of extracellular signals and then later the potential for diversity might be lost. This requires either, that the two cells are exposed to different signals, or that they are made non equivalent at cell division, that is, the extra cell division is asymmetric.

Another way that new cell types can arise is by the cells originally being non equivalent and then diverging in development because of gene duplication (Fig. 5). Consider two adjacent cells containing transcription factors A and A, B respectively, both expressing a protein, since A activates the gene. If now the gene and its promoter are duplicated then initially both cells will produce double the amount of the protein. However, because of the non equivalence of the cells, and due to the presence of B in only one, small changes in the promoter region of the duplicated gene could result in it only being expressed in the cell with B, which offers the possibility of divergent differentiation.

PERIODICITY AND SEGMENTATION

As Francis Crick once observed, embryos seem to be very fond of stripes. By this he presumably meant that it is very common to find periodicity in embryos and animals, whether it be segmental arrangement of somites in vertebrates, segments in arthropods and worms, or even tentacles in Cnidaria. Development of periodic structures was undoubtedly primitive. Again we do not in general have a good understanding of the mechanisms whereby periodic structures are generated so it makes it difficult to envision an ancestral mechanism. The periodicity of parasegments in *Drosophila* comes from the positional information provided by the gap genes specifying each parasegment separately; in the leech, segmentation seems to be based on a temporal mechanism; and in the spacing of ommatidia in *Drosophila*, or feather buds in chick, it seems to be based on lateral inhibition.

The combination of a mechanism for making periodic structures like segments, together with each acquiring a positional identity opened the way to generating a very wide variety of structures. Using the same basic plan individual segments could diversify, rather like gene duplication.

MORPHOGENESIS AND THE GERM LAYERS

Morphogenesis involves cellular forces bringing about change in form of the embryo. Mechanisms for generating these forces were already present in the primitive eukaryotic cells: the contractile filaments in the furrow bring about cleavage, and the microtubules in the mitotic apparatus could be used for cell elongation.

A very important evolutionary step was gastrulation, which transformed the embryo from a single layered structure into a two-layered and later a three-layered system. I have considered elsewhere the possible origin of the gastrula from the Gastraea as originally suggested by Haeckel (Wolpert, 1992). It could have involved a simple invagination to assist with feeding. As already mentioned, patterning most often occurs in two dimensions, that is within sheets of cells, and gastrulation provides a mechanism for establishing the third dimension; this may be why the basic mechanism is so highly conserved.

It is conventionally thought that there are three basic germ layers, two of which, the mesoderm and endoderm, move internally during gastrulation. However, as Lawrence (1992) points out one must distinguish between the observed movement of layers during gastrulation and their actual fate. While the concept is useful and one can easily envision its evolutionary origin with the endoderm forming the gut, and mesoderm migrating in as single cells, their fate need not necessarily be fixed. For example, neural crest can give rise to 'mesodermal' cells like cartilage. Thus, while early patterning of the embryo usually divides it up into the three germ layers, their fates are not fully restricted.

Another important evolutionary step was the development of a further opening that could fuse with the primitive blind ending gut and so provide a genuine gut tube with mouth and anus that is characteristic of almost all animals apart from Cnidaria and flatworms. The formation of this second opening could originally have resulted from the invaginating gut making contact with the ectoderm and thus providing a signal for making these cells different – possibly the first inductive event in development. This description side steps the problem of whether the invagination occurs in the region of the future mouth as in Protostomes or the future anus as in Deuterstomes (Willmer, 1990).

THE EVOLUTIONARILY PRIVILEGED EMBRYO

What selection pressures will act on the developing embryo? Unlike the adult organism or larva, the embryo seems to be rather privileged. It need not, for example, seek food or mate, and so is not in direct competition for ecological niches. Its primary function is to develop reliably and this reliability is the main feature on which selection will act. This in no way excludes selection for aspects of development that relate more to reproduction and life cycles than structure. These modifications include yolkiness, rate of development, and the evolution of larval forms. Moreover, these can have considerable impact on developmental mechanism, for example, yolkiness can affect the mechanics of gastrulation (Wolpert, 1992). Again the mechanism of segmentation in short germ and long germ insects may be different and related to the rate of development. *Drosophila* with its long germ band develops very rapidly.

If the main selection pressure is for reliability, there is the possibility that the embryo is evolutionarily privileged in that invariants in development that do not affect reliability will not be subject to negative selection. For example, the expression of a gene in cells other than where it is required, the secretion of a variety of molecules, or a transient invagination will escape negative selection provided they do not interfere with development. There is, for example, no evidence that such processes are energetically costly and so not subject to negative selection. It is striking how costly it is just to be alive, the sodium and calcium pump alone using about 25% of total ATP and protein synthesis and breakdown another 50% (Wolpert, 1990). This lack of negative selection may offer the embryo the possibility to explore developmental pathways.

Consider for example the primitive Blastaea or Gastraea before the mesoderm had evolved. Imagine a small number of cells moving into the interior by a chance mutation. The cell could continue to be present for many generations and so could themselves differentiate in a variety of ways. Some will be selected against, while others may, for example, generate internal muscles. The selection is on the adult. Again the widespread secretion of signals and variety of gene expression offer greater opportunities for useful combinations to emerge.

An important consequence of the privileged position of the embryo is that different pathways of development, leading to a similar end result, are to be expected. No one way of gastrulation is 'better' than another, only the end result matters. And there are, indeed, many variations, for example, in the pathways leading to the simple two-layered planula of Cnidaria or to the patterning of the blastula in invertebrates (Wolpert 1992).

LARVAL EVOLUTION

A most remarkable feature of many lower invertebrates including annelids, molluscs and flatworms is the widespread

presence of a larval form known in various modifications as the trochophore. The trochophore is rather simple with an outer ectodermal layer with ciliated bands, a gut with mouth and anus, and mesodermal tube like muscles. Unlike embryos, larvae are subject to quite different selections as they have to both feed and disperse before metamorphosis. At metamorphosis there is a profound morphological change – one only has to compare a mollusc with an annelid.

Could the larva be the ancestral form, which gave rise to a variety of invertebrates? This has been suggested by several authors (see Willmer, 1990) who regard the larva as primitive and direct development as a secondary phenomenon. A similar view is taken by Wray and Raff (1990) in relation to echinoderms and their larvae. However, from a developmental point of view this seems improbable, if not impossible.

The difficulty arises because of the continuity principle. How could a larva evolve the ability to metamorphose into the complex morphological forms of the adult? The intermediate steps required are completely improbable. By contrast, if the larval form is a secondary adaptation of development then it is far easier to see how it could have evolved. The similarity of larvae would then reflect convergence rather than a primitive condition.

How larvae could evolve is most easily seen with amphibians and insects. The tadpole is clearly a modification of the early embryo for swimming and feeding. It is not possible for a tadpole to evolve legs. Primitive development was direct and leg development delayed in the larva. Similar consideration applies to insects. Insect larvae could never evolve wings. Thus direct development is always the primitive condition and larvae evolve by modification of embryonic stages.

CONCLUSIONS

Given the eukaryotic cell it is not too difficult to imagine scenarios in which multicellular organisms develop by cleavage of a single cell. It is also possible to imagine how eggs became specific as distinct from body cells and how axes and patterning evolved. The evolution of these processes may have initially involved environmental signals which then became autonomous via the Baldwin effect. Gene duplication and segmentation involving duplication of structures, together with nonequivalence, based on positional identities and asymmetrical cell divisions, opened up pathways for the development of divergent cell patterns and structures. Larval evolution is an adaptation of embryonic forms. It is possible that the evolutionary privilege of the embryo made new developmental pathways easier to evolve.

The future of such evolutionary studies lies in greater understanding of the extent to which changes in single genes can alter cellular behaviour in development.

I am indebted to Matthew McClements for drawing the figures.

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