Evolution of gastrulation

Gastrulation and the evolution of development

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Summary

The original eukaryotic cell may have possessed the key processes necessary for metazoan development - cell differentiation, patterning and motility - and these are present in the cell cycle. Protozoa also possess key patterning processes. It remains a problem as to why there should be two main modes of development - one based on asymmetric cell division and the other on cellular interactions. The latter may be related to asexual reproduction.

The morphogenetic movements of gastrulation - as distinct from specifying the body plan - are highly conserved in a wide variety of organisms. This may reflect the requirement for patterning being specified in two dimensions, sheets of cells, and a third dimension being created by cell infolding.

The origin of the gastrula can be accounted for in terms of Haeckel's gastrea theory - an early metazoan resembling the gastrula. Gastrulation in Cnidaria may resemble the primitive condition but there is nevertheless considerable diversity. While this may reflect, for example, yolkiness, it seems that there is little selection on developmental processes other than for reliability.

Thus it is possible that the embryo is privileged with respect to selection and this may help account for the evolution of novel processes like the origin of the neural crest.

Reliability is the key demand made on development. This may be provided by apparent redundancy. Since many developmental processes involve switches and spatial patterning reliability is provided by parallel buffering mechanisms and not by negative feedback.

Key words: gastrulation, evolution, pattern formation, selection, neural crest.

Introduction

By the evolution of development, I mean the evolution of development itself: the way the various developmental mechanisms and embryonic stages evolved and the selective pressures that were acting. Thus one wants to understand the origin of the key process in development - patterning, morphogenesis and cell differentiation, the origin of embryonic stages like gastrulation, and how developmental novelty, like, for example, the neural crest could have arisen. One also needs to understand variants in developmental processes, like different modes of gastrulation and patterns of cleavage, radial and spiral. In considering selective pressures, it is also necessary to try and understand redundancy. We may never know the true answers but that is no reason not to attempt to try and answer such questions, for they lie at the heart of the whole of evolution. In a sense all evolution of multicellular animals is the brilliant result of altering developmental programmes.

If we consider the three basic processes in development - differentiation, spatial patterning and change in form - these are already, it can be argued, well developed in the eukaryotic cell (Wolpert, 1990). One may assume that the original eukaryotic cells had all the basic structures that characterise all cells - membranes, nucleus, mitochondria and so on, and in fact it is remarkable how similar the basic components of all cells are. Given these characteristics little more was required to generate multicellular animals, and so development.

The cell cycle can be taken as a paradigm for several of the key processes in development. Every cell cycle involves a temporal programme of gene activity, a spatial differentiation that assigns the results of growth to the daughter cells, and cell motility, which ensures that the chromosomes are distributed to daughter cells and in animal cells brings about cell cleavage by active constriction. The evidence for a temporal programme of gene activity is well documented (Alberts et al., 1989) and it does not seem unreasonable to consider G_1, S, and G_2, as being homologous with different differentiated cell states.

Two further features of the cell cycle merit attention. The first involves the decision whether or not to enter the cycle following mitosis. This decision is closely linked to growth and the nuclear/cytoplasmic ratio. It also involves some sort of threshold event. In more general terms, it involves intracellular signalling and a switch of a kind that is very similar to many developmental processes.

The other feature meriting attention is mitosis and cleavage. Here there is highly organized spatial patterning within the cell which is also linked to motility. The development of the plane of cleavage at right angles to the spindle foreshadows the orthogonality that is fundamental to embryonic development. In addition, mitosis provides the opportunity for unequal distribution of components to daughter

cells that could be the basis for the origin of divergence in cell fate and differentiation. This autonomous generation of differences is clearly present in the division of yeast cells in relation to mating type (Wolpert, 1989a).

It requires little imagination to derive other properties from the primitive cell for development. Cell adhesion and cell-to-cell signalling may be novel but require little modification of a system in which there is flow of material to the membrane with an external coat. Even the provision of an extracellular matrix would seem to present little difficulty given an endoplasmic reticulum and Golgi. There is, perhaps, one cellular process that may require a novel evolution and that is cellular memory. The inheritance of the differentiated state through a cell cycle might require new methods for controlling gene action.

The single cell protozoa show complex patterning with very precise spatial location of organelles (Frankel, 1991). Many of the mechanisms required for the evolution of metazoan development are present in the protozoa (Goodwin, 1989), their presence suggesting that they represent fundamental biological processes possibly linked to the cytoskeleton that arise, as it were, easily and naturally in evolution. Protozoa clearly show polarity in form. The rows of cilia suggest that a mechanism for generating spacing patterns - stripes - is well developed. In the ciliated protozoa, fission involves considerable reorganization of existing structures. For example, in *Tetrahymena*, a new oral apparatus, which is normally located at one end of the animal, begins to develop near the zone of fission - in fact two new cell surface organizations are formed within what was originally one, the two now being in tandem alignment along the anteroposterior axis. This reorganization is essentially complete before fission occurs.

Thus it is clear that single cell organisms have many of the mechanisms required for multicellular development. But these mechanisms must now be used for multicellular patterning. With a mechanism for spacing patterns and positional fields, many patterns are possible (Wolpert, 1989b).

The evolution of early differentiation and patterning

The simplest conceivable multicellular organism would consist of two cells that differ from one another (Wolpert, 1990). This unlikely ancestor would have manifested two key processes of multicellular development, diversity in cell state, or cell differentiation, and spatial heterogeneity; for it was essential that the two cells be different. From the beginning, there needed to be a mechanism to ensure this difference and either cytoplasmic differences or a signal could be the basis (Fig. 1). Both became firmly established. Almost all eggs - mammals providing a major exception - have a well-defined polarity due to cytoplasmic differences, but in most embryos at least some later differences arise not because of cytoplasmic localization but because of cell-to-cell interactions.

It is a major problem in the evolution of development to understand why some animals have very well-defined cell lineages, the asymmetric behaviour of daughter cells being a key feature of their development, whereas others rely
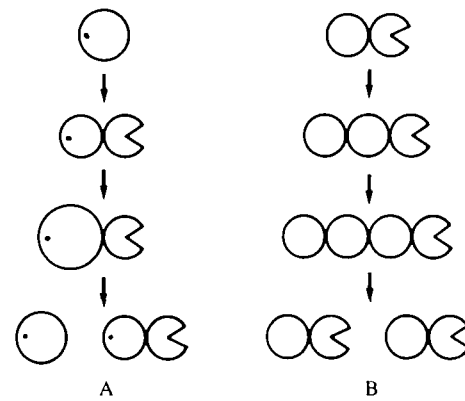
Fig. 1. Possible modes of development and reproduction in primitive metazoa, made up of just two cell types, one of which can be thought of being specialized for feeding. In A, development and reproduction is based on lineage and a stem cell. The dot represents some determinant that maintains the non-feeding cell as a stem cell. In B, development and reproduction is based on cell-to-cell interactions. One of the cells divides and the organism then reproduces by fission, a new feeding cell developing in just one of the two cells that have split off. This requires both cell signalling and polarity.

largely on cell-to-cell interactions. What does this diversity reflect? One possible scenario for thinking about the difference is to relate it to how cells are specified. In the former, cell fate is specified on a cell by cell basis, whereas, in the latter, groups of cells are specified, and this may be related to asexual reproduction.

Again, what underlies the differences between spiral and radial cleavage? Metazoans arose from protozoans that were almost certainly ciliated. All known protozoa are asymmetrical with no known examples of bilateral symmetry. This may provide the clue to spiral cleavage. For the asymmetric structure of protozoa may have led inevitably to spiral cleavage, the essence of which is cytoplasmic asymmetry. It also seems reasonable to think of some primitive organisms relying on this asymmetry to generate differences between cells by asymmetric distribution of some component. Once, however, cellular interactions are involved then asymmetrical divisions are no longer required. This provides a plausible explanation for the rather good correlation between spiralean development and cytoplasmic localization and its loss in systems based on cell interactions. While this in no way implies that in spirally cleaving embryos there are no cellular interactions, it is quite impressive that in vertebrates and insects, which are not spirally cleaving, there are no good examples of autonomous asymmetric cell divisions.

Gastrulation: conservation and dimensionality

In the evolution of gastrulation and its conservation in different animals, there are at least two separate processes that need to be considered - the setting up of the main body plan and the morphogenetic movements. Curiously, it seems that it is the latter as distinct from the former that has been most widely conserved. There is nothing equiva-

lent to the action of the organizer of vertebrates or the micromeres of sea urchins in spirally cleaving embryos. By contrast, in a very wide variety of animals as pointed out a long time ago by Haeckel, the formation of the endo-mesoderm involves invagination or at least a related process (Willmer, 1990). As far as is known, there is no case in which a metazoan develops its three germ layers without undergoing a substantial rearrangement of the cells, usually of the type characterized by invagination. Indeed the similarity of invagination in *Drosophila* and sea urchins is remarkable, a picture of one could easily be mistaken for the other (compare Leptin and Grunewald (1990) with Gustafson and Wolpert, 1963).

A possible explanation for the remarkable conservation of gastrulation movements throughout the animal kingdom is that it is a fundamental requirement for specifying pattern in just two dimensions in the early embryo. Patterning occurs in cell layers, often only one cell thick. Given this requirement, then gastrulation movements are the inevitable result. And in a way the 'layer' requirement makes biological sense for it reduces the problem of specifying pattern in three dimensions to specification in two dimensions, which is a considerable simplification. In this way, the third dimension is acquired or specified by cell movement.

The origin of the mesoderm and endoderm in a very wide variety of animals is rather similar, namely the movement of cells from an outer sheet into the hollow interior. Often variants reflect the yolkiness of the egg as in birds. The timing of such events - that is whether the endoderm or mesoderm moves in first, and when they enter accounts for much variation but the same basic mechanism is involved. However, a major variant is seen when the inner layers form by delamination, that is tangential planes of cleavage giving rise to inner cells from the outer layer. Many of these variants can be seen in cnidarian gastrulation (see below).

There have been attempts to classify animals into two major super-phyla on the basis of gastrulation - the Proto-stomea and the Deuterostomea. In prostostomes the blasto-pore is supposed to become the mouth, whereas in deuterostomes the mouth is supposed to form secondarily, at some distance from the blastopore which may become the anus or close up. However, classification on this basis has been questioned, since in almost every phylum there are animals that form their blastopores into anus when their classification requires they should be forming mouths there (Willmer, 1990). It seems rather that the relationship between mouth and anus to the blastopore should be seen as a continuum.

The origin of the gastrula

The origin of gastrulation is linked to the origin of the meta-zoa. Haeckel's views on metazoan origins have had a dominant influence: he supposed that some protozoan became colonial and gave rise to the 'Blastea', a hollow sphere of cells, the ancestor of all metazoa. Further development of the Blastea gave rise to the 'Gastrea' which was a two-layered structure, the inner layer entering through invagination
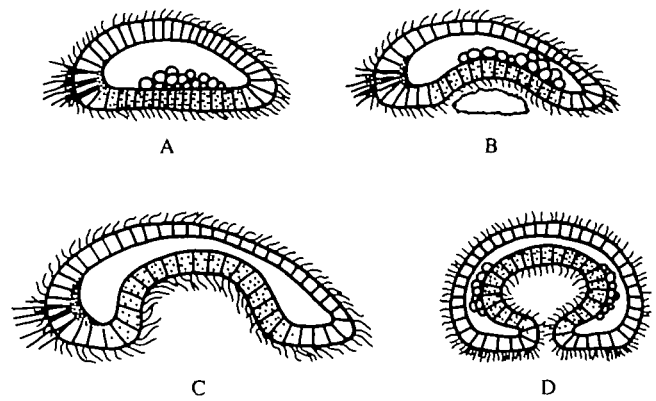
Fig. 2. A possible scenario for the development of the gastrula based on Jaegerstern (1956). In A, the 'Blastea' may have sexual cells inside. Phagocytosis may have become limited to the ventral side and this could have led to a small invagination to assist feeding and decomposing larger food particles. (B) Further development led to the development of a primitive gut with ciliary activity aiding the movement of food particles (C,D).

at the posterior pole. A somewhat different explanation was offered by Metschnikoff in 1886 (see Jaegerstern, 1956) and has been promoted by Hyman (1942). Invagination is seen as a secondary process, multiple ingression of cells being primary; in essence the idea is that phagocytosis would be carried out by outer cells and digestion by the inner cells, while in Haeckel's 'Gastrea' the invagination provides a primitive gut with a mouth. As Jaegerstern points out, there is much to support the view of the origin of gastrulation as being linked to the evolution of an animal - the gastrea - whose form corresponds to a very large number of animals at early stages of development. In support of the Gastrea theory, Jaegerstern has provided quite a detailed scenario for the transformation of the Blastea into a Gastrea based on its settling on the ocean bottom and ingesting food particles from the bottom (Fig. 2). In his view the primitive intestine retained the cilia and that these may have assisted feeding. He also proposes that this bottom dweller had dorsoventrality and was bilaterally symmetrical.

The Cnidaria provide a valuable phylum in which to try and understand the evolution of early stages of development (Campbell, 1974). The phylum present some of the simplest animal structures which are made up of only two germ layers but which develop into a variety of forms characteristic of other metazoa phyla. There are clear signs of segmental and spacing patterns and moderately complex spatial differentiation. Lacking a mesoblast they are less complex internally than other phyla. Most Cnidaria give rise to a ciliated larva known as a planula. This is essentially a two-layered hollow cylinder. An extraordinary feature of Cnidarian development is the apparent diversity of pathways from fertilization to this rather simple structure (Fig. 3). Cleavage, which is somewhat variable depending often on the yolkiness of the egg, is usually radial to begin with, the first few cleavages being at right angles to one another. In some cases, by contrast, cleavage is anarchic. Cleavage leads to two common blastula types - the stere-oblastula, which is a solid mass of cells, and the coeloblas-tula in which there is a sheet of cells surrounding a hollow
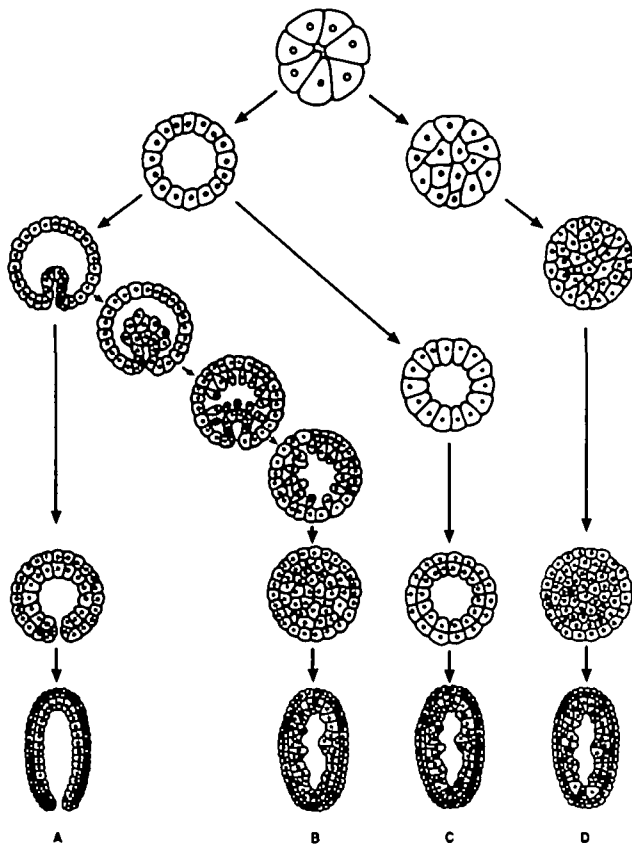
Fig. 3. Some different modes of development leading to the formation of the two-layered planula in Cnidaria (after Tardent, 1978). This may occur by gastrulation of the cells as a sheet (A) or by multipolar ingresssion (B). A quite different mode is by delamination (C). A yet further variant starts from a solid, rather than a hollow blastula, which then develops a cavity (D).

interior. There is, at first sight, no obvious correlation between these two kinds of blastulae and either the yolkiness or size of the egg (see Table 1, Campbell, 1974) but it may correlate with relative cell size (see below).

Gastrulation, the formation of a two-layered structure, comprising ectoderm and endoderm, is no less diverse. Even within one class there is great diversity. Gastrulation may occur by invagination of the sheet from one pole, or by ingression of cells from one pole, or by ingression from multiple sites, or by delamination by tangential cleavage. This last mechanism seems to be quite different from the others, being based on a specific plane of cleavage such that one cell remains at the embryo's surface and the other is effectively placed in the interior.

The other modes of gastrulation might be thought of as having a common theme. Studies on sea urchin gastrulation have shown that cell movement and change in cell adhesion are key processes (Gustafson and Wolpert, 1967; Hardin, 1990). Initially the mesenchyme cells enter at the vegetal pole and this is followed by invagination of the gut:ingression and invagination are rather similar. Since most the different forms of gastrulation in Cnidaria involve movement of cells from the surface layer towards the

centre, the differences between them may merely reflect spatial differences as to where this occurs and differences in the adhesion of the cells to one another. Multipolar ingression is not hard to understand, though there is the problem of specifying which cells will go in; unipolar ingression localizes the process which involves motility and loss of adhesion. Invagination will occur if loss of adhesion in unipolar ingression is delayed, a feature that can be directly observed in sea urchin morphogenesis when, in some embryos, the entry of the primary mesenchyme cells is delayed and a small invagination occurs. It is but a small step, no loss of adhesion, that might lead to invagination proper. A quite different theory about the origin of the gastrula based on competition between cell lineages has been proposed by Buss (1987) and critically discussed by Wolpert (1990).

So many aspects of the variability can be seen as variations on a basic theme. But the problem then, is, why should there be this variability? What, if any, is the selective pressure? And why should delamination sometimes occur?

Berrill (1949) has analysed development of scyphomedusae and claims a correlation of egg size and type of gastrulation. In general, he claims, the smaller eggs gastrulate by unipolar ingression and the largest by invagination alone, the intermediate sizes combining both methods. He rightly draws attention to the relationship of blastula wall thickness and blastula size. The most relevant correlation may lie here - polar ingression and invagination correlate with a thin wall and many cells, and multipolar with thick wall and few cells. Thus differences in gastrulation may relate to the mechanics of gastrulation, which are determined by the number of cleavages prior to gastrulation. This, I would suggest, might be related to selection for the appropriate amount of yolk to support development (see Elinson, 1987, for vertebrates).

From his consideration of the embryology of the annelids and arthropods, Anderson (1973) has concluded that "The blastula, or its equivalent stages of embryonic development, has a greater stability of functional configuration than any stage that precedes or follows it, no doubt because this is the stage at which the fundamental framework of bodily organization is established". Many of the differences may again be accounted for by changes in the amount of yolk and the manner in which it is used.

Nevertheless it is far from clear that the divergent modes of gastrulation give a functional significance. It simply may not matter to the embryo which way it gastrulates provided the end result is appropriate. There may thus be no selection for 'improving' gastrulation providing that it is reliable. Variants may reflect unimportant differences in developmental terms.

Selection on developmental processes: the privileged embryo

Selection on development will act to ensure reliable generation of reproductive organisms. Reliability will be at a premium. But that selective process is not so much on development but on the organism itself. For the evolution of development the more interesting question is whether

there is selection on the developmental processes themselves. Is economy of energy of one developmental process over another to be preferred? Will irrelevant development pathways be lost? Is there a tendency to shorten a developmental process? These are not only questions of interest in their own right, but they relate directly to the variable pathways embryos use to achieve similar results. Are all these variants merely drift or the result of selection for something else, like yolkiness or rapid development, with a knock-on effect on development (Sander, 1983; Dickinson, 1988).

It is possible that the embryo is evolutionarily privileged; that is, the main selective pressure is for reliable development. When one compares an embryo to an adult, this privileged status is evident: the embryo need not seek food, avoid predators, mate and reproduce, all functions for which there is such strong selective pressure. There is not even any evidence that conservation of energy is important and cell death, which is clearly wasteful, accompanies many developmental processes. If this is the case, it may facilitate the evolution of novelty in development.

Evolution of developmental novelty: the neural crest

A feature of developmental systems is that there are examples of apparently different pathways leading to a similar structure. Gastrulation in cnidaria has already been described; another example is the different ways of forming the neural tube in vertebrates - folding of the neural plate and hollowing out of a solid rod. Both occur in mice and chicks, the former in anterior regions and the latter in posterior ones. It is possible that there is no selective advantage of one mechanism over the other and there are merely variants that have arisen by chance. And indeed examination of the timing of the stages of gastrulation in sea urchins shows a considerable variation within embryos from the same species. Such variations may be neutral since the embryo is privileged.

If morphological variants that do not affect the later stages in development are not subject to selection, then they will persist and can provide the basis for the evolution of novelty. Over long periods, new variants will arise and this greatly increases the possibility that some of these will alter later development in a positive way. The variants, because of their neutrality, offer the opportunity for further diversity. They provide, in a sense, an opportunity to explore new developmental modes, some of which will be deleterious and so selected against, but others will be advantageous. These ideas can be illustrated with respect to the origin of the neural crest.

The origin of the neural crest is an important and unexplained event in the evolution of vertebrates. I propose the following scenario that naturally links it to the folding of the neural tube. The development of the neural tube by folding, involves two main processes. In the first process, the cells in an epithelial sheet change shape so that a sharp curvature results and the two ridges come together in the midline. In the second process, the cells in the sheet fuse across the midline and the outer sheet, the ectoderm, separates

from the inner sheet, the neural tube. This second process involves rearrangement of cell contacts. It does not seem unreasonable that during this process, in the primitive condition at least, there were errors in the fusion process and some cells were left in the space between the ectoderm and neural tube. These cells would have provided for the origin of the neural crest.

These primitive neural crest cells would not have been subject to selection since they would be neutral to the development of the animal. They would have persisted for many generations and during this period new variants could have arisen to give rise to Schwann or pigment or sensory cells. The key point is that the primitive population persisted long enough to, as it were, explore new possibilities.

This view of the evolution of development makes it much easier to understand how novelties could have arisen in development. Mutants, for example, that caused local invagination or ingression of cells may have been treated as neutral and so further variants generated until one provided the basis for a new structure and became subject to positive selection.

Selection on developmental processes acts primarily on reliability and this requires consideration of buffering and redundancy in developmental processes.

Buffering, redundancy and precision

The idea that redundancy may be quite common in cell and developmental biology probably has its modern origin in Spemann's (1938) idea of double assurance, a term taken from engineering "The cautious engineer makes a construction so strong that it will be able to stand a load which, in practice, it will never have to bear." Spemann's observations on lens induction led him to this view. In *Rana esculenta*, a lens will form without being induced by the eyecup even though the eyecup can induce a lens. Two different processes "are working together, either of which would be sufficient to do the same alone." Thus redundancy could provide a mechanism for ensuring reliability and precision.

More recently there have appeared an increased number of reports of redundancy in both cells and developmental systems (Wolpert and Stein, in preparation). To many workers' surprise, the complete loss of certain specific genes, like some actins, has had remarkably little, if any, effect on the phenotype of the cell or embryo. Another example in development is provided by the specification of the spatial pattern of expression of the gap gene *Krüppel* in early *Drosophila* development. This pattern is controlled both by *bicoid* and *hunchback* (Hülskamp and Tautz, 1991).

A number of different types of redundancy can be imagined; an example would be two very similar genes coding for two very similar proteins which fulfil the same function. Here I will focus on the case where there are two different genes controlling the same process but in different ways, as in the case of *Krüppel* or the three cyclin genes in budding yeast, any two of which seem to be dispensable. By buffering, is meant the control of some process in the face of variations like ambient temperature, pH and other

molecules. If the process is the production of a defined concentration of some biochemical species then negative feedback is the control mechanism that is almost invariably used. In such systems, there is no evidence for redundancy. And it is hard to see how a redundant system would be better and so have a selective advantage.

By contrast in those many systems in cells and embryos where a particular molecular species is constrained to vary in space and time to give a defined spatiotemporal profile, negative feedback cannot be the mechanism for buffering, precisely because things are not kept constant. Moreover such spatiotemporal profiles are usually themselves the result and cause of on/off switches, like gene activity. Such switching mechanisms for gene activity in eukaryotes, at least, almost never involve negative feedback but positive feedback is often present to keep a gene switched on by autocatalysis. In many systems, the amount of protein made is directly related to the number of gene copies as in amplification of chorion genes or when additional copies have been involved.

How can buffering be achieved in a system with spatiotemporal switching? An answer would seem to lie in providing multiple parallel mechanisms with differing - appositely directed - responses to environmental variations. In this way, buffering and hence precision could be achieved. Such a mechanism has been referred to as canalization when applied to developmental systems.

As an example, consider the specification of the activity of the *Krüppel* in the early *Drosophila* development. *Krüppel* is a gap gene, which has a broad band of expression about half way along the early embryo. Its pattern of expression is controlled by both activation and repression by proteins which have graded concentration. Activation is by low levels of both *bicoid* and *hunchback* and repression by high levels of these same two genes, and there are indeed separate binding sites adjacent to the *Krüppel* gene, both of which specify its expression in the same region. Two further genes *tailless* and *giant* control its final expression. There is apparent redundancy since the pattern of expression of *Krüppel* is very similar in the absence of either *bicoid* or *hunchback*. So why should specification require this apparent redundancy? We propose that this is required for precision in the expression of *Krüppel*. In general terms, precision will be improved if there are multiple parallel mechanisms for the specification, and this will be particularly so if the effect of environmental perturbation, like temperature, move the region of activation in opposite directions. We have, however, no evidence for this. On the other hand, it seems very unlikely that two or more behaviourly identical parallel processes, such as would be provided by two identically redundant genes, would improve buffering or precision.

The same arguments would apply to other systems where it is claimed that redundancy is present. We assert that in all such cases the redundancy is only apparent and the presence of more than one system in parallel has a selective advantage ensuring the precise buffering of the process. Where absence of a gene has no clear phenotype effect, we believe this merely reflects that the system has not been tested in the appropriate environment.

We do however have to recognise that some cases of

redundancy may reflect evolutionary relics, since genes coding for processes that no longer have a selective advantage but for which there is no selective disadvantage, may persist for millions of years.

Precision in specifying spatiotemporal patterns is a major problem for the developing embryo. Waddington's theory of canalization was to account for this (Falconer, 1964). He suggested that selection would operate against the causes of deviation from optimal shapes, such as, for example, that of the insect wing. Apparent redundancy thus provides the mechanism for such a process.

Conclusion

Gastrulation can be thought of as one of the most important processes in early development. Its evolution probably did not require any significant mechanisms not already present in the eukaryotic cell. The striking conservation of the formation of the endo-mesoderm by ingression may reflect the fact that it enables patterning to occur in cell sheets - that is two dimensions - and the three-dimensional structure to be created by gastrulation movements.

Because of its importance, it is necessary for the end result to be reliable. However, the precise pathway by which gastrulation takes place may be quite variable. Reliability and precision probably require apparently redundant processes.

Gastrulation may represent a very primitive metazoan whose development has been elaborated and extended in evolution. It is one of the few examples, possibly, of the discredited theory of recapitulation.

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