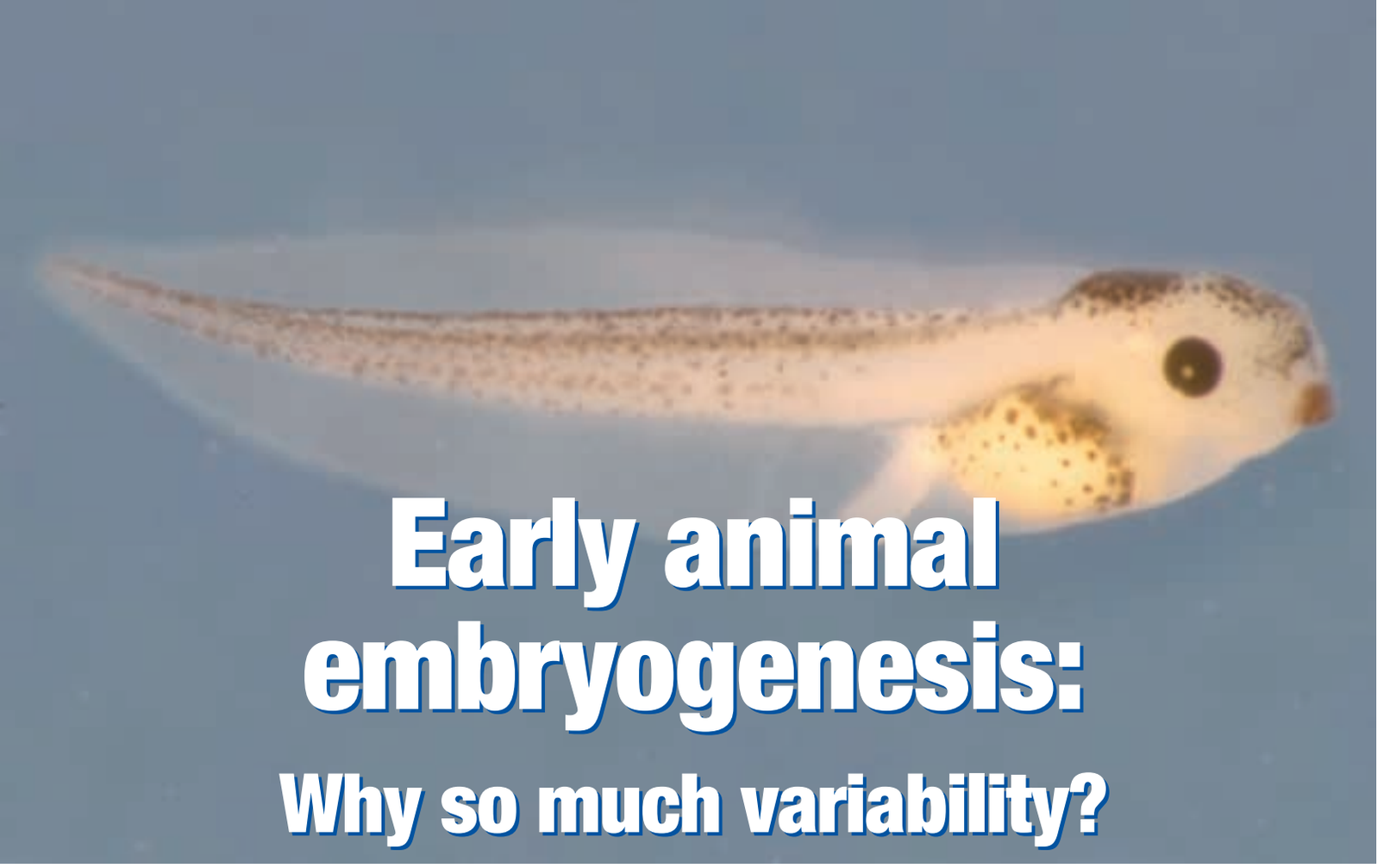

Early animal embryogenesis:

Why so much variability?

Patrick Lemaire and Sylvain Marcellini

LGPD/IBDM, CNRS Université de la Méditerranée, Marseille, France

Since the late nineteenth century, embryologists have probed how individual cells of a young embryo decide which fate to adopt. In the embryos of some animals, most cells appear to 'know' how they should develop. In others, most cells need to be 'told' by their neighbours how to behave.

Embryonic development leads to the formation of a multicellular organism from an egg, via a complex process integrating cell division, fate determination and terminal differentiation. During the process, the large majority of embryonic cells, also called blastomeres, progressively lose their differentiation potential until they terminally differentiate at the appropriate time and place in the embryo or larva. From the second half of the nineteenth century, biologists developed experimental strategies to decipher how these 'decisions' are taken (reviewed in Gilbert, 2000). Two major approaches were used. Chabry and Roux pioneered the specific ablation of early embryonic cells. With this approach, embryologists asked how the deletion of blastomeres affected the remaining embryo. Complementary experiments involved culture of selected blastomeres in isolation from the embryo. With this design, Driesch and his followers probed the capacity of a given blastomere to differentiate normally outside of the embryo. These experiments revealed that metazoan embryos make use of two major strategies to allocate cell fates, namely mosaicism and regulation.

Mosaic and regulative development

Ascidians are immobile marine invertebrates. In early ascidian embryos, posterior vegetal blastomeres give rise mainly to muscle. Laurent Chabry's pioneering experiments showed that loss of particular posterior blastomeres from the early embryo caused the absence of muscle in the larval tail. Hence, ablation of a blastomere can lead to specific suppression of the territories that this blastomere would have normally given rise to (Figure 1A). Remaining blastomeres cannot compensate for the loss. Conversely, many early ascidian blastomeres in the muscle, endoderm, ectoderm and neural lineages, differentiate according to their normal fate when cultured in conditions that prevent cell communication. Thus, the majority of early ascidian blastomeres appear to 'know' which tissue they should form and do not need to communicate with their neighbours to differentiate (Nishida, 2002). How is this possible?

In 1905, E G Conklin noticed that, during the first ascidian mitotic cell cycle, a yellow cytoplasmic domain was concentrated to the future posterior pole of the fertilised egg, and was subsequently inherited by muscle precursors (Figure 1A). Because Conklin suspected that this cytoplasmic domain contained a muscle determinant, he named it the myoplasm. Conklin's hypothesis was subsequently validated and, in addition to myoplasm, the position of localised maternal determinants for endoderm and ectoderm have since been mapped at the end of the first mitotic cell cycle (reviewed in Nishida, 2002). These experiments suggested that ascidian embryos consist of a mosaic of autonomously and independently differentiating territories. This mode of development is hence referred to as 'mosaic' and fits Weissman's theory (reviewed in Gerhart and Kirschner, 1997) that, during early embryogenesis,

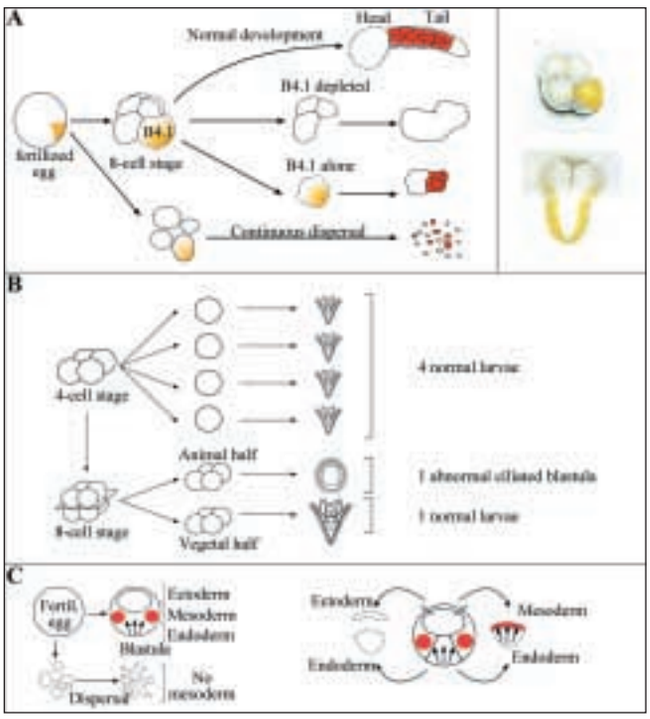

Figure 1. Examples of mosaicism and regulation in animal embryogenesis. ***(A)*** *Left panel: In ascidian eggs, the myoplasm contains maternal determinants (orange). Upon cleavage, these determinants are inherited exclusively by a blastomere called B4.1, whose progenitors will contribute to the primary muscle cells (red) during normal development. Analysis of blastomeres ablation reveals the mosaic nature of muscle formation. Right panel: Conklin's drawing representing the pigmented myoplasm in the ascidian* Styela partita *at the eight-cell stage, and its inheritance in the tail muscle cells of an older embryo.* ***(B)*** *Developmental potential of isolated sea urchin blastomeres at the four- and eight-cell stage.* ***(C)*** *In* Xenopus, *the mesoderm is located at the equator of the embryo. It cannot form in the absence of cell interactions and is induced by an endoderm-derived signal (arrows). The process can be reconstituted by placing ectoderm and endoderm in contact.*

cells inherit intrinsic maternal determinants that impose a given fate. In the case of ascidians, as in most other cases of mosaic development, the rigidity of the developmental program is associated with a small cell number and the existence of invariant cell lineages (Figure 2).

The urchin embryo appeared to Driesch to obey fundamentally different rules (Figure 1B). He showed that certain blastomeres up to the eight-cell stage were capable of generating a normally patterned, though smaller, larva when cultured in isolation (reviewed in Gilbert, 2000). As these blastomeres had the potential to compensate for the missing blastomeres and generate a complete embryonic pattern, this mode of development has been coined 'regulative'. By implying that the fate of a cell depends on the contacts it makes with its neighbours, this model places emphasis on cell communication rather than on the inheritance of determinants. It also highlights the idea that embryonic cells have greater potential than they normally exploit.

Subsequent experiments in other regulative embryos, such as those of vertebrates, provided a conceptual framework, which explained how cell interactions could shape early embryogenesis (Gilbert, 2000). This led to the concept of embryonic induction whereby two types of cells are needed to obtain a given induced fate: cells that emit the inducing signal and cells that are competent to respond to it. The latter are usually more abundant than needed and only those that are sufficiently close to the source of signal will adopt the induced fate, the others adopting an alternative 'default' fate. A classic example of such a process is mesoderm induction in amphibians (Gilbert, 2000), during which presumptive endoderm induces part of the overlying ectoderm to adopt a mesodermal fate (Figure 1C). Progressive patterning of regulative embryos is thought to proceed from a cascade of such inductions, part of an induced tissue becoming in turn inducer of a subsequent smaller territory. To initiate the process, it is sufficient to define, during the cleavage stages, a small number of localised sources of inducers, usually referred to as organising centres. This strategy is often found in embryos developing with a large cell number and indeterminate cleavage (Figure 2).

Scattered throughout the metazoan tree of life

Mosaic and regulative strategies appear to be so fundamentally different that one would expect to encounter them in animals with very different body plans and distant phylogenetic positions. Surprisingly, there are many examples of closely related animals sharing a common adult or larval bodyplan, but displaying very different early embryogenesis (Figure 2). For example, while ascidians and vertebrates di-verged over 500 mil-lion years ago, their larvae have retained a very similar tadpole organisation. Yet, they constitute classic examples of mosaic and regulative development, respectively. Likewise, all arthropods share a common body plan past gastrulation, the so-called segmented germ band.

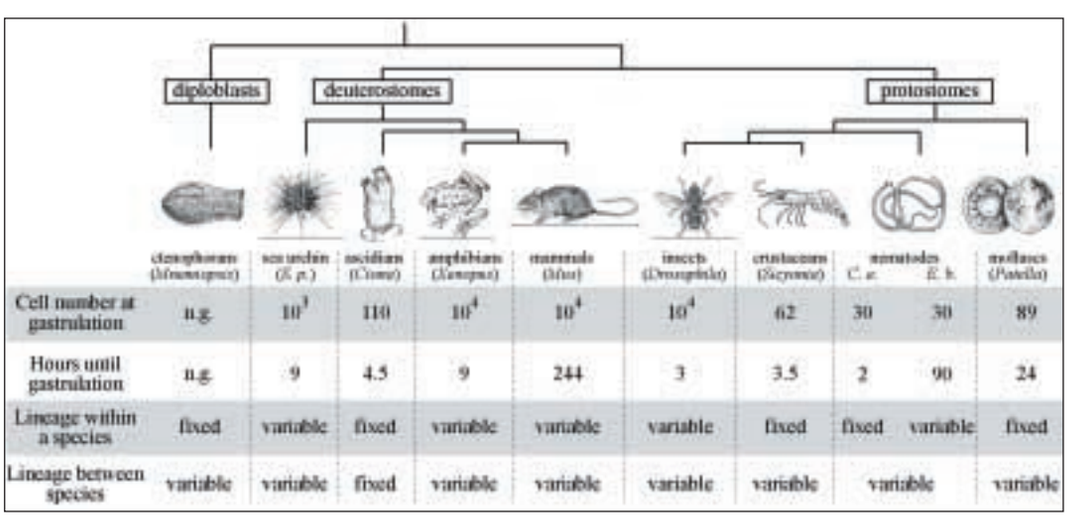

Figure 2. Relationship between animal phylogeny and embryological features. The tree shows the phylogenetic relationships and comparison of key embryological features between members of three major animal groups. The diploblasts are made of two germ layers: ectoderm and endoderm. The deuterostomes and protostomes are sister groups, which develop with three germ layers (ectoderm, endoderm, and mesoderm). The species chosen represent well studied laboratory models. n.g., no gastrulation; S.p., Strongylocentrotus purpuratus; C.e., Caenorhabditis elegans; E.b., Enoplus brevis.

But insects and crustaceans use a variety of early strategies to reach this common organisation (review-ed in Gerhart and Kir-schner, 1997)

Widely differing embryological strategies can even be found in closely related species as illustrated by the two nematodes *E. brevis* and *C. elegans* (Reviewed in Goldstein, 2001). Blastomere ablations and lineage studies reveal that the former has a typical regulative embryogenesis, associated with an indeterminate cleavage, while the latter is one of the most commonly cited examples of mosaic development. These few examples, chosen among many others, highlight that, whereas within a given phylum (chordates, arthropods, nematodes) all members share a common organisation at a later stage of their development (the phylotypic stage), their early embryogeneses are surprisingly diverse. This suggests that the very strong evolutionary constraints that act to keep the larval bodyplan constant are much reduced at early embryonic stages (Raff, 1996). This has recently been illustrated mathematically. On the basis of identified genetic networks and cell interactions in Drosophila, von Dassow and colleagues (2000) mathematically modelled the formation of the regularly spaced stripes of gene expression found along the antero-posterior axis of the fly embryo. Consistent with reduced constraints on early development, they found that many values for the biological parameters considered in the model led to the same repetitive stripe pattern.Yet, even if the system is robust and can compensate for alterations in the early developmental programme, the fact that closely related species can adopt either strongly mosaic or regulative strategies suggests that these may differ less than initially proposed.

Mosaic development does not preclude cell communication

In addition to the presence of localised maternal determinants, several ascidian cell types, including the notochord and the brain, develop as a result of inductive processes (Nishida, 2002). While localised maternal determinants appear sufficient to pattern the embryo up to the 24-cell stage, further refinement of the pattern involves cell interactions within vegetal cells to induce the notochord, and between vegetal and animal cells to induce the brain (Figure 3A) (reviewed in Nishida, 2002). Hence, one of the classic examples of mosaic development also makes use of inductive processes as a relay to the action of localised maternal determinants. This can be generalised to all systems in which the requirement for cell interactions has been tested carefully, including *C. elegans* (reviewed in Rose and Kemphues, 1998), ctenophores (Martindale and Henry, 1997), and spiralians (Lartillot *et al.,* 2002). In all cases, truly cell-autonomous differentiation is restricted to some tissue types, which often act as sources of inducing signals for the remaining tissues. Furthermore, these inductions are initiated before the onset of gastrulation, and as early as the four-cell stage in *C. elegans* (reviewed in Rose and Kemphues, 1998). Interestingly, the inducing signals used in ascidians, *C. elegans* and regulative embryos belong to the same families (including TGFß, FGF, Notch and Wnt) and activate very similar signal transduction pathways.

Localised maternal determinants in regulative embryos

While Driesch's experiments established that several blastomeres of sea urchin embryos could develop into normal larvae, this is not the case for all blastomeres. From the eight-cell stage onwards, it is possible to define animal blastomeres, which are close to the polar body of the embryo, and vegetal blastomeres, which are away from this structure. Subsequent experiments (reviewed in Ettensohn and Sweet, 2000) established that the vegetal and animal blastomeres had different potency at the eight-cell stage (Figure1B). At the 16-cell stage the vegetal blastomeres are of unequal sizes. The small blastomeres are called the micromeres. They are fated to form skeletogenic mesenchyme, and also play an important role in the induction of the overlying meso-and macromeres (the terms meso- and macro- refer to the respective intermediate and large size of these blastomeres). Blastomere culture experiments revealed that the micromeres are autonomously determined, presumably as a consequence of inheriting localised maternal determinants (Figure 3B). Hence, the largely regulative development of sea urchins is preceded by an initial use of localised maternal determinants to specify which cells will act as an early source of inducers.

This can be extended to vertebrates. In amphibians (reviewed in De Robertis *et al.,* 2000), for example, the first embryonic induction by vegetal cells leads to the specification of the mesoderm in the equatorial region of the embryo. The inducing properties of these vegetal cells depend on the inheritance of a localised maternal determinant, here the transcription factor VegT (Figure 3C). A second localised maternal determinant, of unknown molecular nature, acts to activate ß-catenin in the dorsal cells of the embryo and is necessary for the formation of Spemann's Organiser. The same general principles apply

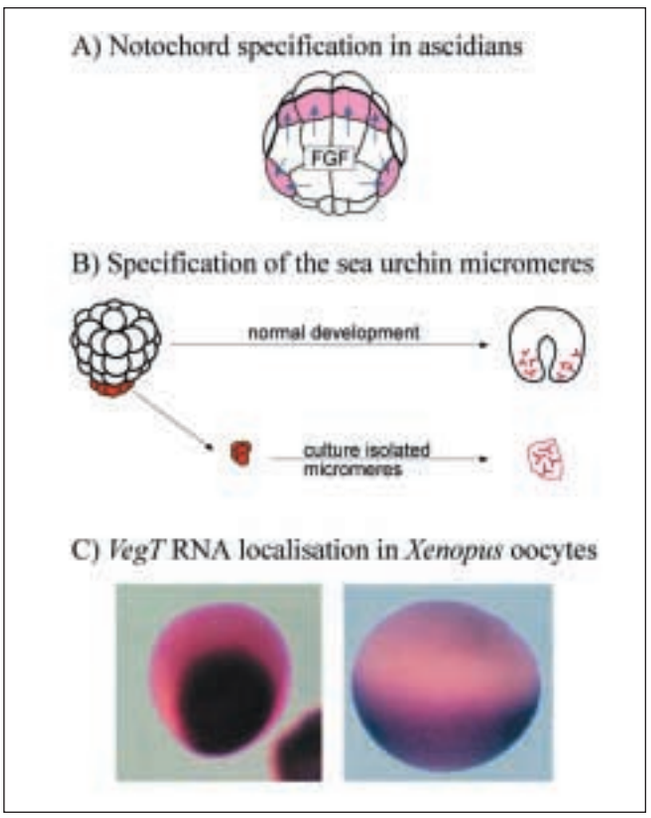

Figure 3. Inductive events in ascidians, maternal determinants in regulative embryos ***(A)*** *At the 32-cell stage, the notochord (pink) is induced only if some cells receive an FGF-like signal from their neighbours.* ***(B)*** *In sea urchin, the micromeres (red circles) can develop into skeletogenic mesenchyme (red lines) even if they are isolated from the rest of the embryo.* ***(C)*** *The* VegT *mRNA shows a very strong vegetal localisation in* Xenopus *eggs and oocytes .*

to fish embryos. In the mouse (reviewed in Zernicka-Goetz, 2002), elegant experiments indicated that removal of either animal or vegetal poles of fertilised eggs or early embryos does not prevent full-term development, arguing against the necessity of localised maternal determinants for the establishment of embryonic polarity during normal development. Yet, several proteins are asymmetrically distributed during early cleavages and two embryonic axes, bearing some relationship to the post-implantation body plan, are defined as early as the two-cell stage in the mouse embryo. One of these axes is defined by the polar body, located at the animal pole of the fertilised egg, the second by the sperm entry point. Thus, even mouse embryos display hints of cell autonomy in their axis specification strategy.

Different logics?

Mosaic and regulative embryos both make use of inductive processes and localised maternal determinants. Yet, they obey different logics. How is that possible? The answer lies in a different use of these strategies.

Observations of early blastomeres cultured in isolation from the rest of the embryo reveal that, in mosaic embryos, inheritance of a localised maternal determinant imposes, cell-autonomously, its fate to a cell. In vertebrates, the best studied regulative embryos, maternal determinants are usually not sufficient to do this. For example, VegT and ß-catenin, which are required for the formation of the early inducing centres, turn on the expression of members of the TGFß/nodal family of secreted factors required for the stabilisation and maintenance of the initial bias imposed by the maternal determinants (Yasuo and Lemaire, 1999). This constitutes an example of a general strategy referred to as 'community effect' and is largely used by embryos developing with many cells (Gurdon *et al.,* 1993). In these embryos, cohorts of cells that are identically fated need to communicate to differentiate, presumably to coordinate their behaviours. The use of inductive signals also differs.

In regulative embryos, inductive signals are thought to act at a distance of several cells from the source, thereby patterning large embryonic domains, or fields (reviewed in Gilbert, 2000). Because many more cells are usually competent to respond to an inductive signal than is required during normal development, the ablation of some responding cells brings other competent cells within reach of the inducer and leads to regulation.

The situation is different in mosaic embryos. In ascidians, ctenophores or *C. elegans,* an inducing cell can generally only influence the fate of the cells with which it establishes direct contacts during normal development. The local character of these inductions is achieved in two ways.

First, the inducing signals act at short range. In the extreme case of the induction of the ascidian notochord, the inducing signal acts by polarising the induced cell so that, upon cleavage, only one of the daughters adopts the induced fate (reviewed in Nishida, 2002). Little is known about the mechanisms underlying the difference in signalling range between the two strategies. It could be intrinsic to the inducers, due to their capture in the extracellular space, or simply due to the more rapid development of a majority of mosaic embryos, which may not leave sufficient time for the signal to diffuse before the next cleavage. The fact that the inducers characterised in regulative and mosaic embryos usually belong to the same families suggests that their range may depend on the systems rather than on their molecular identity.

Second, in mosaic embryos, the loss of the induced fate in response to the ablation of a particular induced cell indicates that the number of competent cells is much more restricted than in regulative embryos. In ascidian embryos, for example, the only cells that can be induced to form notochord are the notochord precursors (reviewed in Nishida, 2002), and they can only do so in a narrow time window. This indicates that spatial and temporal competence to respond to signals, a process generally poorly understood in animal development, is tightly regulated in these embryos.

In summary, mosaic embryos use autonomously acting maternal determinants, short range inductions, and show a tight regulation of cellular competence to the inducing signals. In contrast, the action of maternal determinants in regulative embryos is frequently relayed by secreted factors. Also, embryonic inductions in these embryos tend to act at a range of several cell diameters, and the domains of cellular competence to the inducers are broad.

A general strategy for animal embryos?

In spite of these differences, two common themes emerge when comparing the logic used by mosaic and regulative embryos. With a few possible exceptions (such as amniotes), all studied embryos make use of localised maternal determinants to break the initial symmetry of the egg, thereby initiating the definition of the embryonic axes. In many cases, there is evidence that these determinants, or their mRNAs, are localised in a restricted position of the egg cortex following fertilisation (Sardet *et al.,* 2002). In the cases where this has been carefully studied, the determinants activate zygotic inducing factors, thereby defining the initial embryonic organising centres. This is clearly demonstrated for the endoderm in ascidians and amphibians, the sea urchin micromeres, Spemann's organiser in fish and amphibians, the posterior cell in *C. elegans,* the D blastomere in molluscs such as *Ilyanassa obsoleta* and the comb-inducing cells in ctenophores. The wide distribution of these examples throughout the metazoan tree of life suggests that this scenario, depicted in Figure 4, reflects an ancestral situation.

The question we then have to answer is how this ancestral scenario became biased towards the preferential use of either mosaic or regulative strategies, which we observe in present-day animals. As the balance between the use of these two modes of development can differ significantly in closely related animals, the use of one or the other strategy

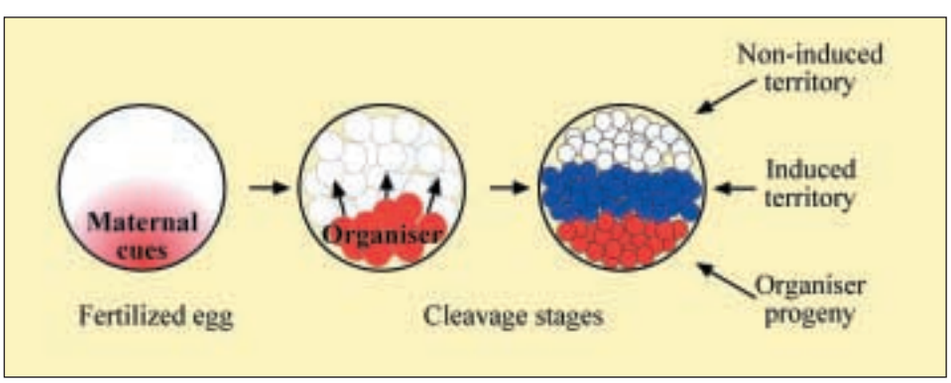

Figure 4. A general strategy for early animal development.

is unlikely to be due to ancestry. It more likely reflects the adaptation to different environmental influences. Also, with some exceptions (such as *E. brevis*), regulative development is found in animals with a slower developmental rate and larger cell number than tends to be found in mosaic embryos (Figure 2). This may suggest that a largely mosaic development, as seen in ascidians or nematodes, is an adaptation to rapid development with few cells.

The mechanisms that lead to a modification of early development as a consequence of an environmental change remain very mysterious. A comparative analysis of the relationship between ecology, genotype and development of closely related species, or even of different isolates of the same species, may help (Delattre and Felix, 2001). It is also worth remembering that computer modelling of *Drosophila's* early development has led to the idea that the value of a given early parameter can change substantially without altering the final body plan (von Dassow *et al.,* 2000). Hence, many early changes may be neutral or nearly neutral. This may explain why the frequent correlation between fast development, small cell number, invariant cleavage and mosaic development is not always observed (Figure 2). If many solutions can lead to the same result, a certain randomness in Nature's choices can be expected.

Acknowledgements

We thank Martin Scaal for critical reading of this manuscript. PL is supported by the CNRS, SM by a PhD fellowship from the French Ministry of Research. We apologise to the many authors we could not cite for lack of space.

References and further reading

De Robertis E M, Larrain J, Oelgeschlager M and Wessely O (2000) The establishment of Spemann's organizer and patterning of the vertebrate embryo. *Nat Rev Genet,* **1,** 171–81.

Delattre M and Felix M A (2001) Microevolutionary studies in nematodes: a beginning. *Bioessays,* **23,** 807–19.

Ettensohn C A and Sweet H C (2000) Patterning the early sea urchin embryo. *Curr Top Dev Biol,* **50,** 1–44.

Gerhart J and Kirschner M (1997). *Cells, Embryos and Evolution.* Blackwell Science Inc.

Gilbert S F (2000). *Developmental Biology.* Sinauer Associates, Inc.

Goldstein B (2001) On the evolution of early development in the Nematoda. *Philos Trans R Soc Lond B Biol Sci,* **356,** 1521–31.

Gurdon J B, Lemaire P and Kato K (1993) Community effects and related phenomena in development. *Cell,* **75,** 831–4.

Lartillot N, Lespinet O, Vervoort M and Adoutte A (2002) Expression pattern of Brachyury in the mollusc *Patella vulgata* suggests a conserved role in the establishment of the AP axis in Bilateria. *Development,* **129,** 1411–21.

Martindale M Q and Henry J Q (1997) Reassessing embryogenesis in the Ctenophora: the inductive role of e1 micromeres in organizing ctene row formation in the 'mosaic' embryo, *Mnemiopsis leidyi*. *Development,* **124,** 1999–2006.

Nishida H (2002) Specification of developmental fates in ascidian embryos: molecular approach to maternal determinants and signaling molecules. *Int Rev Cytol,* **217,** 227–76.

Raff R. (1996). *The shape of life.* Chicago. University of Chicago Press.

Rose L S and Kemphues K J (1998) Early patterning of the *C. elegans* embryo. *Annu. Rev. Genet.,* **32,** 521–545.

Sardet C, Prodon F, Dumollard R, Chang P and Chenevert J (2002) Structure and function of the egg cortex from oogenesis through fertilization. *Dev Biol,* **241,** 1–23.

von Dassow G, Meir E, Munro E M and Odell G M (2000) The segment polarity network is a robust developmental module. *Nature,* **406,** 188–92.

Yasuo H and Lemaire P (1999) A two-step model for the fate determination of presumptive endodermal blastomeres in Xenopus embryos. *Curr Biol,* **9,** 869–79.

Zernicka-Goetz M (2002) Patterning of the embryo: the first spatial decisions in the life of a mouse. *Development,* **129,** 815–29.

Websites of interest

www.ucalgary.ca/UofC/eduweb/virtualembryo/
This site and its links provide a comprehensive introduction to developmental biology and its concepts.

www.wormbase.org
This site hosts the major *C. elegans* database. It provides useful information on the worm and its community, including a genome browser and an anatomical atlas.

Dr Patrick Lemaire *is a Researcher with the French National Centre for Scientific Research (CNRS).* ***Sylvain Marcellini*** *is a graduate student. Both work at the Institut de Biologie du Développement de Marseille, Laboratoire de Génétique et Physiologie du Développement, CNRS Université de la Méditerranée, Case 907, Campus de Luminy, F-13288 Marseilles, France.*
lemaire@lgpd.univ-mrs.fr,
marcellini@lgpd.univ-mrs.fr