are displayed prominently in intraspecific interactions, such as aggressive or mating behavior. These animals thus use patterns of polarized light in the same way that other species use color patterns, providing a unique and therefore unmistakable appearance to visual systems capable of analyzing polarization patterns. Also, since the polarization pattern is independent of the spectrum of illuminating light, the signal looks the same in a great variety of lighting conditions.

***Is there anything else unusual about their visual signals?***

Naturally — they seem to have discovered how to enhance their color signals by adding fluorescence. Water absorbs long-wavelength light (that is why it looks blue), so visual signals based on long-wavelength colors like yellow or red are not very useful at depths greater than 15 or 20 meters.

Some stomatopod species that inhabit these depths, however, still look yellow. They do this using fluorescence: light in the blue spectral range is absorbed by pigments that re-emit it in the greens and yellows.

Fluorescence allows animals like these to have a similar appearance over a large depth range, reflecting yellow light in shallow water and fluorescing it in deep. It is possible that fluorescent signals might be more common among aquatic animals than previously thought.

***Where can I find out more about stomatopods and their vision?***

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**Primer**

# Mosaic and regulative development: two faces of one coin

**Peter A. Lawrence**[1] **and Michael Levine**[2]

"*There seems to be no more a completely mosaic egg than a completely regulation one*" Waddington, Principles of Embryology 1956, Allen and Unwin, p63.

From the mid 1800s and for about a hundred years, mainstream embryologists ignored genetics and tried to understand the mechanisms of animal development without it. The attempt was a brave one, but it became increasingly foolish. In the early 20th century, the gifted embryologist Thomas Morgan realised the importance of genes and took what he thought would be a temporary diversion into genetics. But it was a long detour; only at the end of his life was he able to return to his beloved embryos. But most other embryologists continued to work as if genes were irrelevant (and some have carried on like this into modern times!). The most original and resourceful of the old school, such as Hans Driesch, Sven Hörstadius and Hans Spemann, approached embryos by transplanting or combining pieces. But many of their results were so counterintuitive and conflicting that their hypotheses became abstract and ornate. The philosophical and the whimsical found this attractive. By contrast, it was the mathematical and the rigorous who joined the new science of genetics. Naturally enough, the two types of scientist failed to understand each other and embryology drifted off into metaphysical swamps while genetics explored the dry savannahs of statistics. For lucid and entertaining insights into these times we recommend Klaus Sander's essays: "*Landmarks in Developmental Biology*".

Embryology developed a rich and impenetrable terminology. Some hypotheses were mutually exclusive and thus the terms came in opposing pairs. For example: the information needed to drive development could be either fully executed in the egg (preformation) or progressively elaborated from simpler beginnings (epigenesis); development could be driven by a vital force (entelechy) or by a chemical and structural process (an ontogenic machine); embryonic cells could be preprogrammed and have a limited fate (determined) or they could be unrestricted and able to contribute to any organ (totipotent); embryos could, as we discuss below, be either mosaic or regulative. Embryology courses and text books still feel it necessary to give students a sense of these debates, and we can understand this — we too grew up with them. However, there has been a revolution brought about by genetics and molecular biology and it is time to bury some of the old arguments. Here we look at the classification of embryos into those with 'mosaic' or 'regulative' development and ask if we should still preserve these concepts.

## Mosaic and regulative embryos: the concepts

As students we were taught that embryos fall broadly into two classes: *Regulative* embryos were thought to be characteristic of the vertebrates. Regulation was defined by Driesch in 1909 as an embryo adapting to interference, such as removal of a part, by restitution to or towards the normal. In 1971, Sander argued that the concept of regulation should also encompass cases where parts of embryos respond to experiments by changing their fate away from the normal; it is the flexibility itself that is diagnostic, not the direction of any change. Intrinsic to the regulative process are interactions between embryonic

cells, interactions that specify cell fate. This type of cell interaction has helped bring intercellular signalling to the fore in current fashion.

*Mosaic* embryos were thought to be characteristic of many invertebrates, for example annelids and arthropods — indeed, the style of development became a criterion in the grand classification of animals into subkingdoms and superphyla. The key test was to isolate blastomeres of embryos; if each blastomere went on as it would have done in situ and only made part of the whole, the embryo was said to be mosaic. Mosaic embryos were thought to derive from eggs that are a patchwork of determined territories. They were supposed to develop according to a program, each cell having a predetermined and restricted fate. A lack of interaction between cells and rigid patterns of cell lineage are parts of this concept. From these ideas came a research emphasis on maternal determinants and cell lineage.

Generalisations like these develop their own momentum and can become dogmas. Consider the case of insects: text books described insect embryos as mosaic for much of the 20th century, yet as early as the 1920s Friedrich Seidel did clear experiments showing that insect embryos exhibit regulation. The same is true of nematodes; when Sydney Brenner chose the nematode *Caenorhabditis elegans* to be his experimental muse, he was impressed by the precise number of cells and rigid cell lineage. These observations led to a widespread belief that nematode development is programmed, and offered the promise of deciphering secret codes that control cell fate. One of the consequences was the conviction that cell interactions would not be important to cell determination in nematodes. Later on, many cases of cell interactions were discovered in *C. elegans* and these placed nematode embryos where they belong, that is not so far away from other embryos.

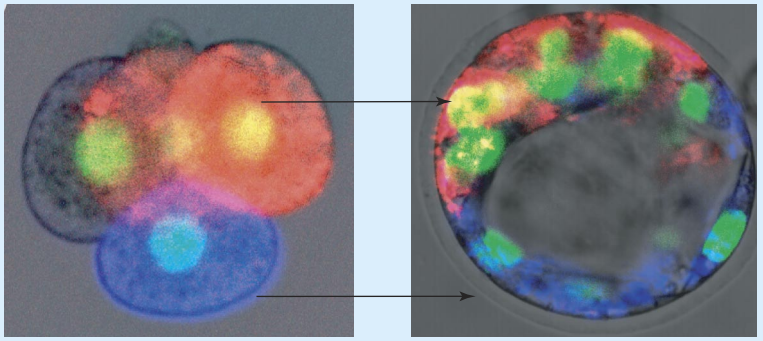


Figure 1. Two and four cell mouse embryo blastomeres, marked with dyes according to their division pattern, differ in their fate — contribute to different regions of the blastocyst — and have differing developmental potential when combined with cells of the same origin. (Images courtesy of Magdalena Zernicka-Goetz.)

Thus it became clear that the 'mosaic embryo' is an abstraction that does not exist. Nevertheless, the concept embodies important principles: there are determinants that are placed in the egg by the mother; the fate of cells is sometimes restricted by localising proteins to a limited part of an egg cortex or the cell membrane. Selector genes can permanently become turned on or off by special mechanisms, for example those implemented by genes of the *Polycomb* group.

But what of a regulative embryo, is that also an abstraction? Two-headed tadpoles and siamese twins are living proofs of the flexibility of vertebrate embryos. Mouse zygotes and early embryos were traditionally considered to be completely unspecified, rather like eggs of the brown alga *Fucus*, where the main axis is fixed by the environment (for example by light). In mammals, however, careful studies are now uncovering signs of a bias, so that, already by the first cleavage, the two blastomeres are not equivalent. At the four cell stage, and in embryos cleaving in a common but particular way, each cell has a different and largely predictable prospective fate (Figure 1). In these embryos, the four cells can be distinguished and experiments show that they are different — those originating from the animal half of the egg are totipotent, while those from the vegetal part of the egg are not. So although mammalian embryos are remarkably flexible, they are not truly regulative. The regulative embryo probably exists only in our imaginations.

**Mosaic and regulative development: the molecules**

The molecules that underlie these concepts are becoming more defined and understood. To oversimplify: mosaic development depends on agents, such as transcription factors, being placed locally in the egg by the mother. Regulative development depends in part on long-range gradients of positional information, such as that provided by the Hedgehog protein, that can pattern many cells at once. Regulative development can also be driven by short-range signals that trigger changes in cell identity in nearby neighbours.

The first example of a localized metazoan determinant came in 1887 from what now, in our hierarchical times, would be seen as an unlikely source — a medical student, Laurent Chabry. Chabry studied embryos of the sea squirt, an ascidian. He used a needle to destroy two individual blastomeres — the so-called B4.1 blastomeres — at the eight cell stage. The remaining cells made partial tadpoles which lacked tail muscles. When he took these same two blastomeres and cultured them on their own, they formed tail muscles, but nothing else. These studies launched the hypothesis that ascidian embryos are highly mosaic, with localized

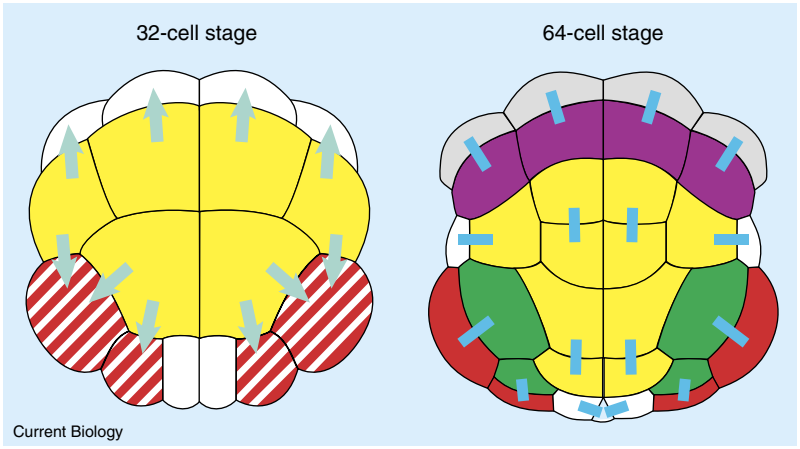


Figure 2. Cell signaling modifies the action of a localized determinant in the ascidian *Halocynthia roretzi.*

The localized determinant Macho-1 initiates a cascade of gene activities leading to the differentiation of tail muscles. This cascade operates in the blastomeres denoted by the red and white stripes at the 32 cell stage. An asymmetric division leads to the segregation of distinct red and green blastomeres (the blue lines indicate daughters arising from the same blastomere). The red cells form tail muscles, while the green cells follow a modified fate due to the receipt of FGF signals from the endoderm (yellow cells). Removal of these signals causes both the red and green cells to form tail muscles. The blue arrows in the left panel indicate the movement of FGF signals from the presumptive gut. The blue bars in the right panel show the orientation of cell division from the preceding stage. An asymmetric division distinguishes presumptive notochord (purple) and nerve cord (gray) at the 64-cell stage. (Adapted from Kobayashi *et al*. 2003, Development *130*, 5179–5190.)

determinants rigidly specifying the fate of individual blastomeres. Chabry was a committed socialist and was discomforted to find his embryos seemed to be under totalitarian control.

This same approach was taken further by Edwin Conklin, who made use of a yellow pigment in the ascidian *Styela* to follow the tail muscle lineage during development. Prior to fertilization, the pigment is uniformly distributed throughout the ooplasm of the egg. After fertilization, the pigment is first localized to the vegetal cytoplasm and then becomes confined to the B4.1 blastomeres, the progenitors of the tail muscles. Conklin did not believe that the yellow pigment was itself the muscle determinant but he thought it might be telling him where a determinant was.

Just five years ago, nearly 100 years after Conklin's first observations, Hiroki Nishida and colleagues identified that determinant as the zinc finger transcription factor Macho-1. Macho-1 is at the top of a regulatory cascade that drives localized expression of a group of genes to initiate muscle differentiation. After the maternal *Macho-1* mRNA is inherited by the B4.1 blastomeres, it is translated into an active protein during the next two cell cycles. At the 32 cell stage, Macho-1 switches on production of Tbx6, which in turn activates MyoD and Snail. MyoD is essential for muscle differentiation, while Snail is a transcriptional repressor that blocks notochord formation.

This behaviour is all straightforwardly 'mosaic'. But, after the next cell cycle, a subset of these mesodermal cells come into direct contact with the presumptive endoderm, itself secreting fibroblast growth factor 9 (FGF9), a short-range signal. FGF9 modifies the action of Macho-1, so that this subset of cells now form mesenchyme rather than tail muscles. And to confirm this, it is found that, when FGF9's action is inhibited, all the mesodermal cells follow the Macho-1 'default' pathway and form tail muscles (Figure 2).

In another sea squirt, *Ciona*, FGF9 also induces the CNS by locally activating both a ubiquitous, maternal Ets1,2 transcription factor and GATAa. Indeed, overdoses of FGF9 cause the CNS to expand and take over other territory, illustrating the flexible nature of tunicate development. So, even in the embryos that have the mother of all determinants, cell–cell communication guides cell fate. This paragon of mosaicism is no more.

Now let us turn to the vertebrates. The *Xenopus* egg contains a number of localized determinants, including Vg1, an activin signalling molecule, and VegT, a T-box transcription factor. These cause restricted expression of *Xnr*, which encodes a signalling molecule related to Nodal. After fertilization, cortical rotation leads to nuclear transport of β-catenin along the future dorsal side of the embryo. The dorsal mesoderm, which includes the venerable 'Spemann's organizer', is defined by where Xnr signalling meets activated β-catenin. Active β-catenin interacts with Tcf-3, a ubiquitous transcription factor, and drives the dorsal activation of the homeobox gene *Siamois*. Siamois protein interacts with defined regulatory elements in its target gene, *goosecoid*. But Siamois cannot act alone; expression of *goosecoid* also depends on activated Smads and these are triggered by Xnr signalling in vegetal regions. Siamois and activated Smads are found together only in the presumptive dorsal mesoderm, and there they activate production of Goosecoid protein to define, at least in part, Spemann's organizer. If only the authoritarian Spemann were still here to enjoy the knowledge that his favourite and so flexible embryos do, after all, contain determinants!

What about *Drosophila*? In the early embryo, longitudinal stripes of cells are sent towards different developmental destinations, the most ventral to mesoderm, the nextmost ventral to mesectoderm and the more dorsal to neurogenic ectoderm. These allocations are largely the responsibility of the maternal gradient of the Dorsal protein that is set up by determinants

in the egg; however, pattern formation thereafter depends on interactions between groups of cells.

At the highest concentration of Dorsal, the *snail* gene is activated and Snail helps direct the most ventral cells towards mesoderm. These cells invaginate at gastrulation, moving inwards and spreading across the inner surface of the neurogenic ectoderm. As the mesoderm cells enter the embryo, the neurogenic ectoderm moves downwards to meet at the ventral midline. The mesoderm cells migrate more dorsally and the lateralmost cells come into contact with ectoderm that expresses the BMP signalling molecule Decapentaplegic (Dpp). Dpp then induces that part of the underlying mesoderm to express the regulatory gene, *tinman* (related to *Nkx2.5* in vertebrates), which drives differentiation of the heart.

A lower concentration of Dorsal leads to the localised expression of *single-minded* in the mesectoderm, the ventralmost cells of the neurogenic ectoderm that form on either side of the new ventral midline. Single-minded protein coordinates the localized expression of *rhomboid* and other signalling components required for the processing and release of the EGF-like ligand Spitz from the midline. Secreted Spitz helps pattern the ventral neurogenic ectoderm, perhaps in a similar way to the patterning of the vertebrate neural tube by Sonic hedgehog.

Thus, in embryos previously classified as either mosaic or regulative, many-sided intercellular conversations lead to progressive elaboration. Localized determinants and signalling molecules are agents in these conversations and all embryos have both. Localized patterns of gene expression depend on the combinatorial action of transcriptional activators and repressors. These activators and repressors together determine which cells are set up to respond to longer range signals, such as the BMPs, Wnts, Hedgehog and the FGFs. Evolution has had fun tinkering with the relative contributions of signalling and transcription in the establishment of cell fate. But, as we have seen, these processes are intimately linked and interconnected, so that, working together, they drive development forwards. In the past, because of a tendency to compare and then contrast, an apparently stronger reliance on signalling would shove the embryo into the regulative category, while the occurrence of localized transcription factors made the embryo a mosaic. But all embryos employ both mechanisms. They work as a team, and, with exquisite precision, define cellular identities progressively. Cell identity is first partly defined within broad zones of competence, but these then become refined and subdivided as organs and tissues are built. It is time to move on and donate mosaic and regulative development to the archives.

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## Correspondences

# The prospect of sexual competition stimulates premature and repeated ejaculation in a mammal

**Brian T. Preston* and Paula Stockley**

When faced with the prospect of female promiscuity, males have evolved diverse strategies to limit reproductive loss to competitors through the sperm competition that would follow [1]. Although striking variation exists between species, mammalian copulatory behaviour is often complex and protracted, and could serve both to curb female re-mating, and enhance male fertilization success if sperm competition occurs [2,3]. Here, we demonstrate that male wild house mice, *Mus musculus domesticus*, adjust key components of their copulatory behaviour when there is an elevated risk that females will mate with a rival, showing that dynamics in male copulatory behaviour have evolved in the context of female promiscuity.

Wild house mice are an ideal model for studying evolutionary adaptations to occasional female infidelity, because although dominant males establish and aggressively defend territories in which females nest, females do sometimes mate promiscuously and produce mixed-paternity litters [4,5]. Like many other rodents, house mice engage in multiple bouts of intra-vaginal thrusting — called intromissions — during each copulation, between which males will dismount and move away [2]. The genital stimulation caused by such protracted copulations induces a neuroendocrine