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THE EVOLUTION OF CLEAVAGE IN METAZOAN

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Abstract

Cleavage is the earliest developmental stage. In it, the fertilized oocyte gives rise to a cluster of smaller cells (blastomeres) with a particular spatial pattern (a cleavage pattern). Different metazoan species have different cleavage patterns, but most of them fit into a small set of basic types.

The relationship between the phylogeny of a given species and its cleavage pattern is far from direct, but most taxa seem to use the same basic cell processes (such as directed cell division or cell adhesion) to build their cleavage patterns. We assess which are those mechanisms in the first section of this chapter. In a second section, we explore how the combined action of these mechanisms can account for the emergence of particular cleavage patterns in different metazoan taxa and the evolutionary transitions between them.

Keywords: early development, cleavage pattern, cell processes

Introduction

The fertilized oocyte contains all the information to, given the appropriate environmental conditions, build a functional adult organism by means of a complex process called development.

The first stage of development is called cleavage. Cleavage starts from a single cell, the oocyte (or egg), that despite being more or less spheric is far from being homogeneous. Normally, oocytes have internal gradients (maternally inherited) that are oriented along one axis called animal-vegetal axis (Gilbert and Raunio, 1997). During cleavage, a series of fast cell divisions partition the oocyte into a set of smaller cells called blastomeres. The spatial distribution of blastomeres observed when cleavage finishes (at the onset of gastrulation) is what we call a "cleavage pattern" (Gilbert and Raunio, 1997). As a general rule, cleavage proceeds without an overall growth of the embryo (the volume of the embryo is roughly equal to that of the oocyte). In many organisms, this is because the oocyte is surrounded by a protective eggshell (also involved in selective metabolite exchange) that limits the space available for the developing embryo).

A major driver of the diversity observed in metazoan cleavage patterns is yolk: a nutritive substance normally concentrated in the oocyte's vegetal pole. Yolk interferes with the cytoskeletal processes involved in cell division, and therefore blastomeres in the vegetal part of the embryo tend to divide more slowly than blastomeres in the animal part of the embryo (Gilbert and Raunio, 1997). Moreover, if yolk is dense enough it cannot be pierced by the cleavage furrow when blastomeres divide, often resulting in incomplete cell divisions in which blastomeres are not totally separated by the cytoplasmic membrane. This type of cleavage is called meroblastic. According to the distribution of yolk within the blastula, meroblastic cleavage occurs in eggs that are either telolecithal (yolk is distributed throughout most of the blastula) or centrolecithal (yolk is located in the center of the blastula). Blastulae with a low or moderate amount of yolk display holoblastic cleavage (the furrows of cell divisions traverse the whole blastula, whereby blastomeres get individualized). In addition, many cleavage patterns exhibit geometrical regularities in their blastomere arrangement, which allows for a general classification into a few major cleavage types.

Distantly related taxa can exhibit similar cleavage patterns, while species belonging to the same taxon can have different cleavage types (Valentine, 1997). These discrepancies between the cleavage patterns and the phylogenetic position of metazoan taxa are difficult to explain and seem very counterintuitive unless we gain more knowledge on how the different blastomere arrangements can be generated during early development. This means, how the spatio-temporal combination of different cell processes can generate the different cleavage patterns. The aim of this chapter is to gain an overview of such cell processes, and to review how they are combined in the early development of the major metazoan groups. For the taxa with enough available data, evolutionary transitions between different cleavage patterns are also addressed.

Cell processes involved in cleavage

Compared to later developmental stages, only a single kind of relatively undifferentiated cells exists during cleavage, and the number of cell processes that they can display is relatively small. These cell processes are:

Cell division

Cell division can occur in a specific direction (the plane of cell division can be oriented in different ways in space), at a specific moment in time, and with a specific degree of size asymmetry between daughter cells (Gillies and Cabernand, 2011).

Direction of cell division: Cells can be polarized and this polarization can determine the direction in which cells divide. Ultimately, cell polarization results when one or several sources of spatial information are translated into a spatially asymmetric distribution of some specific molecules within the cell. In many cells under division this asymmetry promotes a differential attachment of the astral microtubules to the part of the cortex with the highest concentration of these molecules, thus tilting the mitotic apparatus and biasing cell divisions to occur perpendicularly to the direction of cell polarization. The sources of spatial information can be located either within the cell itself (autonomous mechanism) or in the cell's surroundings (inductive mechanisms, see chapter "Evolution of development").

The autonomous mechanisms do not require any physical or chemical interaction with other cell(s) to determine the direction of cell division. That means that cells use asymmetries that are already present in the intracellular environment to polarize themselves. These asymmetries are usually inherited, and usually consist in the heterogeneous distribution of some factor(s) in the cytoplasm. In other cases, this heterogeneity is attained when some factors (if dense enough) are attracted by gravity to the lower part of the embryo, creating new spatial information. These factors can interact with the mitotic spindle in such a way that the spindle always tends to point towards the place where the factors are most abundant.

When spatial cues are absent, the cell shape itself (specifically, its longest axis) is able to determine the direction of cell division. This is commonly referred as Hertwig's rule, and it occurs because of the differential tension in astral microtubules, which depends on the contact angle between the microtubule tips and the cell's surface. By simple geometry, this angle is smallest at the cell boundary at the most distant points of the cell, causing that the astral microtubules attached there exert a stronger tension than the microtubules attached elsewhere, thus leading to the alignment of the mitotic spindle along the longest axis of the cell (and then to cell division to occur perpendicular to that axis).

Finally, it exists a phenomenological rule (Sachs' rule) by which the direction in which a cell divides tends to be perpendicular to the direction of the division that gave rise to it (that is its mother cell division). This has been proposed to arise from the stereotypic (90°) duplication of the centrioles between cell divisions, that in turn biases the position of the mitotic spindle towards perpendicularity.

Cues in the cell's surroundings can also provide external spatial information by means of short-range or long-range diffusible signals between neighbouring cells (inductive mechanisms). A special case of "short range" signals is the physical contact between cells. In this case, cells tend to divide towards (or against in some cases) the part of the cell making contact to adjacent cells. This has been suggested to occur because physical contact in a cell region would modify the underlying cell cortex so that the astral microtubules are stabilized in this region, increasing the local traction of the mitotic spindle during cell division.

Differential growth: During cleavage, cells in a blastula can divide at the same time (synchronous cell divisions) or not (asynchronous cell divisions). Synchronous cell divisions give rise to different cleavage patterns than asynchronous cell divisions. In general, the resulting cleavage patterns depend on the relative rates of cell divisions between the different regions of the blastula. Assuming that some factors can trigger (or inhibit) cell division, this asynchrony can be achieved by a heterogeneous distribution of those factors in different regions of the blastula.

One of these factors is yolk (which usually forms an animal-vegetal gradient), that is known to delay (or even prevent) cell division. As a consequence, cells close to the vegetal pole divide at a slower pace, and remain bigger than those close to the animal pole. In some groups, cell division is inhibited just in a single specific blastomere, which becomes larger than the others.

Specification of daughter cells' size: In general, when a cell divides, the resulting daughter cells are equally sized (symmetric cell division), but in many embryos some cell divisions are asymmetric: one daughter cell (macromere) is significantly larger than the other (micromere). Different mechanisms can result in asymmetric cell division. In some cases, the relative size of daughter cells is regulated by the asymmetric concentration of intra-cellular factors (e.g. PAR proteins in *C. elegans* embryos). The microtubules of the mitotic spindle get more stabilized in the regions of the cell where these factors are more abundant, generating asymmetric pulling forces during cytokinesis. Since the concentration of these factors often varies along the animal-vegetal axis, cell size gradually increases along the animal-vegetal axis.

Alternatively, asymmetric cell division can result from an inherently asymmetric spindle. In these cases (e.g. *Tubifex* worms), one centrosome is inactivated, so as only a half of the mitotic spindle is plenty developed and exerts a greater traction force than the other, degenerated, half of the spindle, thus displacing the cleavage plane to one side of the cell.

Asymmetric mitosis may play a role in generating variation between cleavage patterns. This is because when the cells are tightly packed (e.g. by increased cell adhesion), the resulting blastomere arrangement may depend on the relative size between blastomeres (e.g. small blastomeres may occupy the furrows between big ones). Cell division

can also be asymmetric if the mother cell has some kind of internal polarity (e.g. an mRNA gradient), which the two daughter cells inherit in a differential manner, even if they are equally sized. That way one daughter cell can incorporate different molecules than the other, which may cause differential gene expression between sister cells.

Cell processes not related to cell division

Cell adhesion: During cleavage, cell adhesion keeps the blastomeres together, thus maintaining the physical integrity of the blastula as a whole. Moreover, cell adhesion increases the contact surface between adjacent cells, which can lead to cell shape changes that may affect the relative position and contacts between neighbouring blastomeres (Lecuit and Lenne, 2007), and even the direction of the cell divisions if Hertwig's rule applies. If adhesion molecules are expressed non-uniformly on the surfaces of individual blastomeres, complex spatial arrangements, such as embryonic cavities or cell chains can be formed. In some taxa, adhesion strength is not constant over cleavage time, but cells suffer cycles of increased cell adhesion coupled to cell division cycles.

Local variations in cell adhesion (and in the surface of contact between blastomeres) are important when the cell fate determination is controlled by inductive mechanisms. In these cases, cells are not induced below a certain area of contact, but are only induced above this area.

Cortical rotation: During the first cell divisions in certain taxa (e.g. *Xenopus*, snails), blastomeres rotate over themselves just after cell division around the rotation axis that links the two cells (Meshcheryakov and Belousov, 1975). Around this axis, rotation occurs in the same sense in all blastomeres (e.g. all counterclockwise respect to their sister blastomere). Whereas in some taxa this rotation does not seem to have any morphogenetic effect in the blastomere arrangement (as in *Xenopus*), it has been suggested that this rotation produces relevant changes in cell relative positions in other taxa (Brun-Usan et al. 2017; see section 3.4). The molecular mechanics of this rotation remains unclear, but it seems to be related with the chiral structure of the F-actin, a protein present in the cell cortex.

Packing constraints: When cleavage proceeds inside an eggshell, a compressive effect may be exerted by the limitation of the available physical space for the blastomeres (Kajita et al. 2003). Due to geometrical considerations, when a set of spheres (blastomeres) is packed within a limited three dimensional space, there are only a small number of optimal cell spatial arrangements.

Notice that many of the previously described mechanisms are not directly encoded genetically (Newman, 2011). Rather, they arise from the complex dynamics of the cell cytoskeleton and purely physical processes like membrane surface tension, volume displacement, gravity, and molecular diffusion. It is worth mentioning that some of these processes also apply to inanimate matter. Because of that, some non-living systems such as soap-bubbles or mineral aggregates share many geometric regularities with cleaving embryos. The “cleavage patterns” in these inorganic systems inform, thus, of which are these “default” cleavage patterns. These are the cleavage patterns that require less precise regulation and that are, thus, more likely to arise in evolution (since they require less mutational changes).

Moreover, most of the cellular processes described in here, as well as their molecular basis are not specific of blastomeres, but are also found in unicellular organisms, meaning that they were already present before the origin of multicellularity (Newman et al. 2003; Sebé-Pedrós et al. 2010, see also chapter “Origins of multicellularity”). Thus, these easy-to-arise patterns may have represented the raw material upon which evolutionary forces may have acted in order to build more complicated patterns later on (by using more cell processes and regulating their spatial and temporal location finely).

Selective forces need also be considered to understand why some of these patterns are evolutionary conserved while others are not. The adaptive significance of these conserved patterns may rely on two (not exclusive) facts. First, if cell fates are specified by cell-cell interactions between specific blastomeres, a constant relative position between these blastomeres is crucial for the appearance of functional adult organs. In these cases, variations in the blastomere positions within the blastula should be maladaptive and selectively suppressed. This is often referred as “internal selection” (Riegler, 2008; see also chapter “Evo-devo organs and body plans”). Second, adaptive modifications of some aspects of early development (e.g. an increase in the amount of yolk in order to nourish the embryo, or a hardening of the eggshell to provide it physical and biological protection) may, in turn, have an effect on the shape and arrangement of the blastomeres (Wray, 2000).

Evolution of cleavage in metazoans

In order to see how the described phenomena can account for the different cleavage patterns and their evolutionary transitions, we present an overview of the cleavage patterns among the extant metazoans (See Fig. 1):

Non-bilaterians

Despite their apparent morphological simplicity, the cell processes and the cleavage patterns deployed during the early development of non-bilaterian groups (Poriferans, Placozoans, Cnidarians and Ctenophores) are extremely diverse

(Adamska et al. 2011). In general, they show holoblastic cleavage patterns (even though some species have abundant yolk) that are characterized by their irregularity. Cell divisions are often asynchronous and random in direction, resulting in amorphous blastulae with low cohesion and no recognizable geometrical regularities (anarchical or chaotic cleavage). In many species, especially among cnidarians, the cleavage pattern is also variable between individuals (involving even transient syncytial stages by random fusion between blastomeres or by anomalous cytokinesis).

Interestingly, during these disordered cell divisions, some non-bilaterian taxa show transitory ordered patterns resembling those found in spiralian and deuterostomes (see Section 3.4 and 3.5). These are called, respectively, pseudospiral and radial-like patterns, but they are restricted to the very early stages (before 8-cell stage) and appear only in some individuals within a species. This suggests that they are likely to be produced by the mechanical stability of cell adhesion between blastomeres (best packing configurations).

The great spatio-temporal variability of the chaotic cleavage pattern prevents the determination of the cell fates during early cleavage: the cell fate of each blastomere cannot be unequivocally determined by its embryological context, since the relative position and identity of its surrounding blastomeres is far from constant. Some non-bilaterians also exhibit truly non-chaotic patterns. In some sponges cell divisions are perpendicular to the cell surface (polyaxial cleavage) or to the animal-vegetal axis (incurvational cleavage).

On the contrary, Ctenophores display a regular cleavage pattern in which the four nearly identical quadrants organized around the animal-vegetal axis correspond with those found in the adult organism. This pattern does not resemble any other one found in metazoans, which does not help to clarify the phylogenetic position of this controversial taxon.

Xenacoelomorpha + Chaetognatha

Xenacoelomorpha (Acoela, Nemertodermatida and Xenoturbellida) are thought to branch from the rest of the bilateria very early on. In general, their cleavage is holoblastic, and shares some characteristics with the spiral one (Wanninger, 2015). In Acoela, this cleavage pattern is called duet spiral. Mechanistically, it seems that the main difference to the quartet spiral cleavage (see section 3.4) relates to the timing in which the cell processes are deployed: in xenacoelomorpha, the “spiralizing” events leading to oblique cell divisions start one cell-cycle earlier (in the 2-cell stage) and the synchrony between cell divisions is lost earlier than in Spiralia. The mechanisms specifying the clockwise-counterclockwise alternation, as well as the cell fates and modes of cell fate determination differ substantially between spiralia and xenacoelomorpha. Thus, it is likely that both quartet and duet spiral cleavage patterns have evolved independently from an ancestral radial-like cleavage pattern.

In Chaetognatha, another bilateral phylum whose phylogenetic position is very controversial, the cleavage is holoblastic with equally sized and tightly packed blastomeres, making difficult to attribute their pattern to radial or spiral. However, some features like the left-right alternation of cell divisions respect to the AV axis and their cell fates suggest that their development is more similar to protostomes than to deuterostomes (Shimotori and Goto, 2001).

Scalidophora

In Priapulids (the only scalidophoran taxon whose early development has been accurately described), the cleavage pattern is holoblastic, synchronous, and sub-equal (slightly different sizes between micro- and macromeres) (Wennberg et al. 2008). Up to gastrulation, cell divisions tend to occur at right angles to each other, generating a symmetric pattern that resembles the radial one. After the 16-cell stage, the blastula is so compact (either by increased cell adhesion or compression from the eggshell) that the visible face of each blastomere acquires a polygonal shape. The directions of further cell divisions seem to depend on these shapes: cell divisions take place along the longest axis of the visible face of each blastomere (this is specially clear for “rectangular” blastomeres). This may imply that a Hertwig-like rule restricted to the outer faces of blastomeres is the main driver of priapulid cleavage.

Nematoda + Nematomorpha

Nematode development is in general holoblastic and shows relatively high variation, especially within the subclass *Enoplia*). Cell fates are specified very early in development through a variety of mechanisms (Gilbert and Raunio, 1997; Goldstein, 2001). In their predominant mode of cleavage (holoblastic rotational), the longest axis of the ellipsoidal egg corresponds to the future antero-posterior (AP) axis. The polarity of this axis (which part of it will become the anterior part of the body) is determined either by entry point of the sperm in the oocyte or by the relative position of the egg within the uterus (the mechanism is taxon-specific).

Several mechanisms, including cell adhesion, cortical rotation and specific cell-cell contacts controlling the direction of cell division determine the blastomere arrangement. Inter-specific variations in this arrangement can be explained by variations in the strength and timing of these mechanisms.

In the model species *C. elegans*, the first cell division is asymmetric along the AP axis, producing a large anterior blastomere and a small posterior one (the germ-line precursor). Just after the first cell division, cells divide by default along successive orthogonal axes, suggesting that Sachs' rule applies in this system. However, cells belonging to the posterior half of the embryo always divide along the same AP axis because just after cell division and spindle positioning, the centrosome and nucleus rotate as a unit 90°, counteracting the “orthogonalizing” effect of Sachs' rule

and leaving the mitotic apparatus oriented in the same axis as the preceding cell division. The compressive effect of the eggshell has also a pivotal role in the blastomere arrangement of nematoda. This is supported by the way the eggshell shape correlates with different blastomere configuration in different nematode taxa.

In Nematomorpha, a phylogenetically related phyla, cleavage is also holoblastic and all cell divisions are symmetric. Their cleavage pattern is highly variable, presenting transitory pseudospiral appearances that vanish after the 8-cell stage. Because of this high variation, the cell fate of each blastomere is not specified until later stages (Malakhov and Spiridonov, 1984).

Arthropoda

The early development of arthropods (including the two related phyla Onychophora and Tardigrada) is very diverse, but always results in a similar segmented body pattern. In general, the geometry of blastomere arrangement is fairly irregular and homologous structures cannot be unambiguously derived from individual blastomeres (Scholtz and Wolff, 2013). In very general terms, arthropod eggs have maternally provided asymmetries both in the antero-posterior and dorso-ventral axis that are essential for further development. These eggs are very yolky and display meroblastic cleavage. In many species, non-cellularized nuclei (energids) start dividing deep within the yolk and then get displaced to the periphery forming a monolayer around the egg called blastoderm (intralecithal cleavage). After this migration, energids get cellularized and the yolk remains in central position (centrolecithal cleavage).

In other cases, the cytokineses are almost complete but the yolk and the blastomeres start dividing in one (2D) side of the embryo (discoidal or superficial cleavage). In this case, and due to Sachs' rule, transient regular (squares of 2 or 4 cells in each edge) configurations are often visible.

Holoblastic or yolk-poor cleavage patterns appear in some arthropod lineages that are viviparous or have planktotrophic larvae. In general, these holoblastic cleavage patterns display mixed features and thus cannot be classified in the main categories. For instance, a number of crustacean groups exhibit a cleavage, called modified spiral, which loosely resembles the canonical spiral pattern (See next section). Their cell fate map, however, and the cell processes involved in the direction of cell division (cell contacts) are different from the one observed in spiralian, suggesting that the crustacean cleavage is not homologous to the spiralian one. Probably, these early claims of spiral-arrangement in crustaceans was a misconception driven by the goal of finding embryological characters supporting the taxon *Articulata* (Annelids + Arthropods), nowadays rejected. Rather the contrary, the similarities between the crustacean cleavage and those found in nematomorphs and scalidophorans points to a radial-like holoblastic cleavage (which can arise from basic cell processes) as the ancestral mode for Ecdysozoans.

Spiralia

Spiralia comprise almost half of the animal phyla. Most of them (Mollusca, Annelida, Nemertea, Platyhelminthes, Entoprocta and Gnathostomulida) exhibit a very conserved cleavage pattern called spiral or "quartet spiral" (Hejnl, 2010). The quartet spiralian cleavage begins with two meridional cell divisions giving rise to four large macromeres. These macromeres then divide towards the animal pole but at an oblique angle relative to the animal-vegetal axis, giving rise to four, normally smaller animal micromeres that are all displaced to the right (or all to the left depending on the organism) of its sister macromere. This tilt between macro- and micromeres can be explained by oriented cell division (the mitotic spindles are tilted prior to cell division) and/or by cortical rotation after cell division. The direction of this tilt is determined by maternally inherited factors and often correlates with the symmetry of adults (e.g. snails with a tilt to the right have a dextrally coiled shell). The ensuing cell divisions follow a right-left alternation (the reverse alternation applies if the third division is to the left), making that, when viewed from the animal pole, the new micromeres seem to spin clockwise or counterclockwise when they arise. Sachs' rule has been proposed to be the driver of this alternation (Brun-Uzan et al. 2017).

When cell fates are compared between different spiralian, it is often observed that the same adult or larval organs in different species arise from the same blastomeres (defined by lineage and relative position in the blastula). However, the mode of cell fate determination differs between quartet spiralian cleavers. Specifically, the so called "D-blastomere" (a mesodermal precursor) can be specified either by cell-cell interactions after the fifth cell division or by asymmetrical segregation of cytoplasmic determinants, which in turn is caused by asymmetric cell division at the 4-cell stage.

In some Spiralian, the quartet spiral pattern is total or partially lost by different causes. For instance, in lophophorates (Brachiopoda, Bryozoa and Phoronida), all cell divisions are symmetric, so that the distinction between macro- and micromeres does not hold as in canonical spiralian. In addition, their blastomeres are loosely attached, and consequently the mechanical interactions between them are weak. Under these conditions, the mechanism of cortical rotation lacks efficiency and is unable to produce any net cell displacement towards spirality (this mechanism requires enhanced cell-cell adhesion, see Section 1). Thus, the first cell divisions proceed perpendicularly (Sachs' rule) until the 8-cell stage, which exhibits a radial-like pattern. After the 8-cell stage, the cleavage pattern of the different phyla of lophophorates becomes less predictable and more idiosyncratic: in Brachiopoda the pattern becomes irregular mainly due to asynchronous cell divisions, whereas in Ectoprocta and Phoronida both spiral-like and radial-like cleavage have been reported (Pennerstorfer and Scholtz, 2012). This latter pattern (biradial pattern) leaves successively four and eight tiers of 4 blastomeres in line, but its symmetry axes are not always related to the larval body axes. Intra-specific

variations of the cleavage patterns of lophophorates have also been reported, including the coexistence of radial-like and spiral patterns within the same population. This fact, at least in this group, can be understood by considering that small differences in cell mechanics (e.g. population-level variation in cell adhesion) can lead to drastic effects in the resulting blastula configuration.

The remaining spiralian phyla display a variety of highly derived forms of spiral cleavage. Many of these deviations from the quartet spiral pattern involve the compressive effect of very elongated eggshells, which produces drastic blastomere rearrangements (Wanninger, 2015). This, in turn, prevents the relative twist between macro and micromeres (Rotifera) and/or even the formation of quartets, thus deleting any spiral appearance (Acanthocephala, Gastrotricha). Finally, a massive amount of yolk correlates with the loss of the spiral pattern (and a switch to a specific meroblastic cleavage) in cephalopod mollusks.

Deuterostomes

Deuterostome cleavage is holoblastic, with loosely attached blastomeres and typically radial. In radial cleavage, early cell divisions follow Sachs' rule: they are either parallel or perpendicular to the animal-vegetal axis, depending on their relative position along the animal-vegetal axis. Thus, along this axis blastomeres are always located one on the top of each other, not in oblique positions as in spiralia. In addition, some of these cell divisions are often asymmetric, yielding groups of cells of different size sorted along the animal-vegetal axis. Deuterostome taxa exhibit slightly different radial patterns, which arise from changes in the cell adhesion and in the timing and location of asymmetric cell divisions.

In ascidians (Urochordata), radial cleavage is replaced by bilateral cleavage, a remarkably conserved pattern, even between distantly related species. In it, the furrow of the first cell division establishes a plane of symmetry that separates the future right and left halves of the embryo. During most part of the cleavage, this plane keeps the right half of the embryo as the mirror image of the left one. First cell divisions proceed perpendicularly one to another (Sachs' rule), but other, more complex, forms of oriented cell division appear very early on, causing the blastula to depart more and more from radial cleavage. The evolutionary transition to this bilateral cleavage from the radial one is related to the presence of the centrosome-attracting body (CAB) in ascidians. The CAB is an actin-rich organelle that anchors the centrosomes of some neighbouring blastomeres, so that they remain attached one to another. This in turn has a double effect: on the one hand it reduces the ways in which blastomeres can move, and on the other hand it enables the asymmetric segregation of maternal determinants in one of the two daughter cells via asymmetric cell division (Munro et al. 2006). The asymmetric distribution of these determinants, combined with inductive signals between neighboring cells within the constant cleavage geometry, pave the way for a very early cell-fate determination. This allows ascidians to develop quickly a functional tadpole larva with a small number of cells.

Other important departures from radial cleavage are found within vertebrates, and many of them are driven by a great amount of yolk in the vegetal pole. In amphibians, this causes the equatorial cell divisions to be displaced towards the yolk-free animal pole (displaced radial cleavage). If the yolk is distributed over all the egg, as in fishes, reptiles and birds, only a meroblastic cleavage restricted to the surface of the animal pole can happen. In this case, first cell divisions still follow Sachs' rule as in radial cleavage, but the stereotypic pattern disappears soon. This kind of cleavage is called discoidal, and presents morphological commonalities with the one found in some Arthropoda.

In placental mammals, the early embryo develops inside the mother's body in a close metabolic dependence. Their eggs are consequently yolk-free, and the cleavage is holoblastic. The second round of cell division is not only perpendicular to the previous one, but also perpendicular between the two blastomeres (one is meridional, the other equatorial). Because of the resulting tetrahedral configuration, it is called rotational cleavage (notice the resemblance with the rotational cleavage of nematoda is restricted to the 4-cell stage, and the mechanisms involved differ). After that, cell divisions become asynchronous with cycles of increased cell adhesion, and their directions are determined by Hertwig's rule and the contacts between adjacent blastomeres, losing any radial (or even regular) appearance.

Conclusions

Despite their diversity, most cleavage patterns seem to have been built by means of the combination of a handful of similar and evolutionary old cell processes (Salazar-Ciudad et al. 2003). Among the many developmentally available patterns, most metazoans seem to use those exhibiting both mechanical stability and basic symmetry axes. These invariant cleavage patterns, in which the timing, orientation and symmetry of cell divisions are precisely defined for all cells, allow the early use of inductive mechanisms for cell fate and body axes determination. Conversely, inductive mechanisms can affect the timing and orientation of cell division, thus determining subsequent fate decisions in a dynamic interplay.

More irregular and variable cleavage patterns are also displayed by many taxa (especially early divergent groups). Many of these groups with a loose control of cell processes during cleavage exhibit similarities in the blastomere arrangement in the very early stages, which seems to be due to mere packing principles. In these cases, the variability in blastomere arrangement prevents the early cell fate determination via inductive cellular interactions (Salazar-Ciudad, 2010).

Evolutionary transitions between different cleavage patterns have happened many times. Some of them may

be explained by adaptive changes in their underlying cell processes (e.g. changes in cell adhesion, in the amount of yolk or the acquisition of a more rigid eggshell), but the developmental bases of these transitions are poorly understood (Brun-Usan et al., 2017). Much work remains to be done (involving experiments in model and non-model species, and computational approaches) in order to disentangle how the interplay between developmental and selective forces has sculpted the geometry of metazoan cleavage patterns.

Cross-references:

“Mechanisms of Pattern Formation, Morphogenesis and Evolution”

“Modeling Evolution of Developmental Gene Regulatory Networks”

“The Developmental Hourglass in the Evolution of Embryogenesis”

“The Evolution and Development of Segmented Body Plans”

“Twisted Shells, Spiral Cells, and Asymmetries: Evo-Devo Lessons Learned from Gastropods”

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Figure caption:

Figure 1. Example (holoblastic) cleavage patterns found in metazoans. Most of them may be explained by means of the combination of a few conserved processes (see text, section 2). All blastulae are displayed in lateral view with the animal pole on the top, and the small straight lines link sister blastomeres when both of them are visible. A) Chaotic (=anarchic) cleavage pattern characteristic of non-bilaterian taxa such as Cnidarians. B) The cleavage pattern of Ctenophores. C) Duet spiral cleavage pattern of Acoel flatworms. D) Rotational cleavage pattern of *C. elegans* (a model species representative of Nematoda). E) Quartet spiral cleavage pattern as displayed by many Spiralian taxa (the “pseudospiral” cleavage pattern found in other non-spiralian taxa is similar to this one until the 8-cell stage). F) Biradial pattern of some Lophophorates. G) Lost of spiralian features in the cleavage of Gastrotricha (class Macrotrichida). H) Radial cleavage pattern of a Deuterostome (sea urchin).

	2-Cell stage	4-Cell stage	8-Cell stage	16-Cell stage		2-Cell stage	4-Cell stage	8-Cell stage	16-Cell stage
A Chaotic (Cnidaria)					E Spiral (Spiralia)				
B Ctenophora					F Biradial (Lophophorata)				
C Duet Spiral (Acoela)					G Gastrotricha				
D Rotational (Nematoda)					H Radial (Deuterostomes)				