THREE-MODAL THEORY OF EARLY EMBRYO ASYMMETRIC CLEAVAGE DETERMINATION

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ABSTRACT: Developmental biology attempts to understand the most remarkable phenomenon of life, the process of embryogenesis, and trace back the common link between different groups of animals to analyze when and how the advantage of multicellularity and pluripotency first emerged. Gradual changes accumulated in animal genomes and the environmental influence on gene expression have led to the emergence of a higher complexity in body patterning and tissue diversity best seen in mammals. Although many questions have been answered, even more await to be elucidated. Presented is a developmental biology theory set into three main hypothetical claims that are collectively harmonizing at the molecular level of animal embryogenesis. Focusing on vertebrates and mammals, the three-modal theory of early embryo asymmetric cleavage determination attempts to explain how over 200 mammalian cell lines originate from a single zygote. Within the theory, biophysical interactions between first mammalian embryo cells are expected to develop a biochemical niche pivotal for further interblastomeric communication and asymmetric fate determination distribution, including those of the maternal origin. The theory builds on extensive research data from the past century and coherently fits in modern molecular understanding of a plethora of embryological processes.

KEYWORDS: Embryo cleavage; Embryo development; Origins of life

INTRODUCTION

German biologist, Wilhelm Roux, wrote that the complexity of animal embryo development is one of the most challenging fields in science, '*since every new cause ascertained only gives rise to fresh questions regarding the cause of this cause*' (Roux, 1894).One of the most frequently asked questions in developmental biology is how does a spherical mass of cells derived from only one zygotic cell give rise to an intricately complex animal embryo? A significant number of studies have presented biochemical and genomic routes through which embryonic cells

develop, organize into more complex structures, and communicate with each other. However, the question has not been fully answered yet and it is still the paramount unknown of biology.

Some researchers have attempted to change the main course of thinking and study the earliest embryological events from another perspective. Much less has been studied regarding how the extracellular environment affects the embryo development and what intracellular processes could be directly identified with the external influences. The ability to respond to the ever-changing conditions is the pinnacle of the multicellular world; including its simplest forms. The genome-wide association study of Volvocine algae, the simplest colonial multicellular eukaryotes, showed that only three gene families had originated in the multicellular organisms whereas all other gene groups are shared with the single-celled organisms (Featherston et al., 2018). However, those genes that had originated independently are, in many instances, involved in intercellular communication and external stimuli sensing.

Responsiveness is incredibly pivotal during embryogenic development in all multicellular organisms. Survival and the differentiation rate of the first embryo cells depend heavily on their external and internal stimuli sensing and how they adapt to the changes of stimuli. Edwards and his colleagues (2005) suggested that external stimuli may exert primary regulatory effects on the polarity formation in mammalian cells via contact receptors (e.g. cadherins, integrins) and soluble chemicals through the cellular sensing ability. In determining the mechanical interactions between the cell surface and an external stimulus, Vogel and Sheetz (2006) demonstrated that cells can respond to different environmental changes of local geometries, adjacent cells, and matrices through the three-step process: mechanosensing (discerning external physical stimuli), mechanotransduction (transducing physical force-induced signals into biochemical pathways), and mechanoresponse (cellular adaptation to the applied physical stress). The authors suggested that mechanosensitive ion channels attribute to the mechanoresponsiveness of the cells; for example, certain K^+ channels can be opened by a membrane convex curvature.

Local geometries, adjacent cells and surrounding matrices are nothing else but physical stimuli. Surprisingly, physical stimulation tends to be as significant as chemical signals, although the former has not gained even half the scientific

attention in the recent years. In order to comprehend how mechanical constraints affect the differentiating embryo cells in multicellular organisms, computational modeling has been implemented to quantitatively describe the influence of physical qualities of the cells, such as size, shape, convexity, and topology (Bassel et al., 2014; Tassy et al., 2006). The interplay between geometric and genomic inputs in one developing organism orchestrates all downstream intra- and intercellular processes of growth distribution, and this can be noticed on many examples in the animal kingdom.

Mollusks develop through spiral holoblastic cleavage. Larvae of the *Unio* family are free-swimming and hence are in danger of being carried downstream. In order to increase their survival rate, *Unio* have modified their cleavage pattern: the 2d micromere attains the greatest amount of the cytoplasm with leverage of the 2D macromere, which changes its developmental field and leads to developing a specialized gland (glochidium) responsible for producing a large shell that enables the mollusks to adhere to nearby fish (Freeman & Lundelius, 1992). This fascinating interplay is well illustrated by the cleavage pattern effect on the complexity of an organism. Cleavage of an embryo is defined as the first rounds of cellular division with no or little growth of the dividing cells. Cleavage patterns in every organism are heavily influenced by the yolk deposition, by the symmetry of the yolk deposition with respect to the oocyte polarity, and by the cell shape (Hasley et al., 2017). In the literature, embryo cleavage is described either as holoblastic or meroblastic. In holoblastic cleavage, the whole zygote is completely dichotomized with the evenly deposited yolk. In meroblastic cleavage, the egg is divided incompletely with a portion of the yolk always remaining. When the first multicellular organisms emerged, holoblastic cleavage had been employed as the default, while meroblastic cleavage had arisen independently several times in the evolutionary timeline (Collazo, Bolker, & Keller, 1994; Romer & Parsons, 1977). However, it is still striking how different organisms share the same evolutionary tool of embryo formation. The conspicuous example is portrayed by mammals and nematodes; they both share the same cleavage mode (holoblastic rotational), even though mammals are highly complex deuterostomes and nematodes are extremely simple protostomes. However, it is still considerate to denote relatedness between the degree of evolutionary development and cleavage pattern, at least in most animal groups.

The spatial cues the first embryonic cells reside in influence how these cells will develop. The spatiotemporal patterns of gene expression, ionic gradients, intracellular transport, and morphogenetic constituents distribution are in cross-talk with physical and geometric inputs from the external habitat of the developing embryo. Upon this premise, the following is a uniform theory of molecular embryology and developmental biology composed of three, well-harmonizing hypotheses which build on the main attributes of every embryological process: cleavage pattern and yolk distribution, community effect and signal processing of blastomeres, and maternal-to-zygotic transition relying on the distribution of maternal material. The theory delivers a possible and satisfying explanatory conduit of biological reasoning for the earliest events of embryogenesis in all animals demonstrating how these events synchronize to execute the "default" set of supramolecular, microscopic, and macroscopic commands evolved from the very first multicellular organisms.

POSITIONING HYPOTHESIS

Most animals are bilaterian with embryos having well-defined anterior-posterior (A/P) and ventral-dorsal (V/D) axes. Bilateria represent two main clades: protostomes and deuterostomes. The former includes animals with determinate cleavage (asymmetrical distribution of morphological determinants among blastomeres), mesodermal coelom formation, and the mouth developing from the blastopore. The latter is characterized by indeterminate cleavage (isolated blastomeres can develop into a whole organism) and the anus originating first from the blastopore. Some of the most relevant clades belonging to Protostomia are nematodes, arthropods, worms, and mollusks; echinodermates (sea urchin, starfish) and chordates (including fish, amphibians, and mammals) are the major phyla of Deuterostomia. However, the evolutionary differences in embryo development between protostomes and deuterostomes are as important as the similarities which both groups share, mainly in respect to cleavage.

Cleavage type is not directly related to the stage of evolutionary complexity of animals; more likely, it is a result of convergent evolution. For example, according to Colazzo, Bolker, & Keller (1994), only in craniates has meroblastic cleavage evolved five times. Nevertheless, most highly developed animals, including mammals, share the same mode of embryo cleavage which is holoblastic cleavage with phylum-specific pattern modifications (Table 1). This

may hint at the evolutionary advantage of holoblastic cleavage over meroblastic embryo divisions. Because even fine changes in early embryo development can result in grossly magnified effects in the later stages of gastrulation, the physical effects that cleavage has on the first stages of blastocyst formation are inestimable.

Organism	Development Type	EGA Timing	Cleavage Type	Autonomously Specified Cell Lineages	Maternal Transcripts
Sea urchin (echinoderm mates)	Slow	18-22 h	*Holoblastic* displaced radial	Mesenchyme	β-catenin/Tcf, Otx
***C. elegans* (nematodes)**	Fast	90 min	*Holoblastic* rotational	Mesenchyme, germ line, gut	PAR-6, PAR-3
***Drosophila* (arthropods)**	Fast	2.5 h	*Meroblastic* superficial	Pole cells, major pattern segments	Bicoid, Nanos, Caudal
Zebrafish (chordates)	Fast	4.3 h	*Meroblastic* discoidal	---	*radar, yobo, janus, foxH1*
***Xenopus* (chordates)**	Fast	6 h	*Holoblastic* radial	Gut endoderm, ciliated ectoderm	VegT, *Xbrachyury, Antipodean*
***Mus* (mammals)**	Slow	1-2 d	*Holoblastic* rotational	---	*DICER1, Ago2,* HR6A, *Zar1*

Human (mammals)	Slow	2-4 d	*Holoblastic* rotational	---	*Mater*, *ZAR1*, Dnmt-1[1]

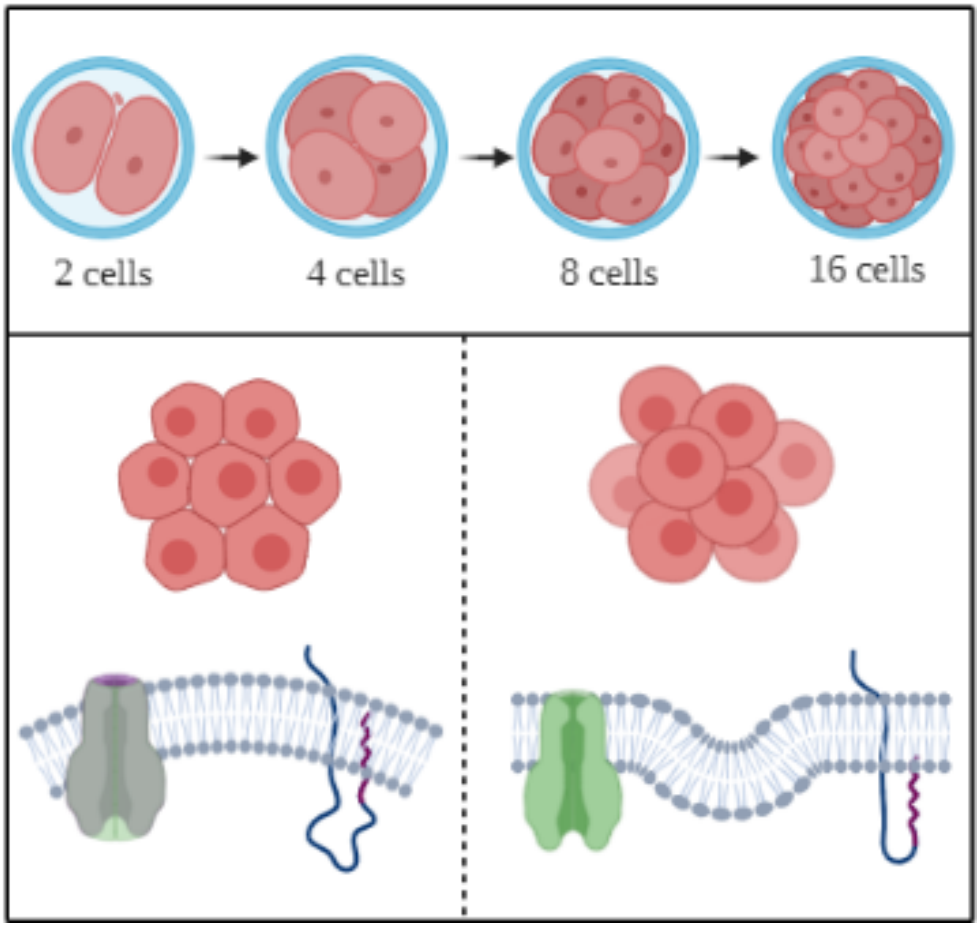
Because the complete set of genes is shared in its majority among the more complex multicellular *Eukaryota* and this set of genes determines body plan formation, only delicate mutations in particular regions of the genome play a key role in the shocking discrepancies among animals. On the other hand, physical barriers and interactions between the yolk and the fertilized egg, the zygote and its surroundings, and interactions among the first cleaved cells of the embryo matter as much as the genomic counterpart. The following Positioning Hypothesis builds on the geometric (physical) inputs of the interacting blastomeres.

Two main presuppositions are introduced.

1. Spatial positioning and an actual weight of one cell alters the shape, convexity, and surface over volume ratio (S:V) of other cells through various physical forces vectors. Depending on the cleavage type, the upper-tier cells "knead" the lower-tier cells (assuming that the top-bottom physical interactions are stronger than the sideways pressing) resulting in the mechanistic membrane strains and the direct intracellular rearrangements.
2. Interblastomeric (intercellular) communication influences the quality of cell-to-cell membrane interactions. Because the earliest gap junctions in mammals are established at about the 8-cell embryo stage (Kidder & Winterhager, 2001), the pure dialogue between the first blastomeres is conveyed through their intermembrane synergy. In radial (sea urchin) or

[1] **Table 1. Relationship between the cleavage type, increasing biological complexity, and the developmental program in animals**. During holoblastic cleavage, the embryo is completely dichotomized with even deposition of the yolk, whereas in meroblastic cleavage, the embryo undergoes incomplete division with the unproportioned yolk deposition. Holoblastic rotational cleavage (the upper-tier cell covers the shared membrane surface of two lower-tier cells) is shared by both protostomes (Nematoda) and deuterostomes (mammals), with slight modifications in the mammalian embryo cells. Holoblastic radial cleavage (echinodermates) is characterized by an enormous furrow dividing the egg meridionally, whereas displaced radial cleavage (amphibians) represents the mesolecithal type of cleavage with the vegetal pole deposition of the yolk. Meroblastic discoidal cleavage is found only in deuterostomes with the furrow not fully penetrating the yolk. Meroblastic superficial embryos undergo the divisional stage with no cytokinesis, which results in a cellularized blastoderm surrounding the central yolk mass.

rotational (mammals) cleavage, the cells in the lower tiers of the blastula potentially have more chances to receive "inputs" (membrane contact) from the upper-tier cells and in greater number than a cluster of blastomeres on top of the yolk (Figure 1). The lower-tier blastomeres have a greater potency then to develop into the inner cell mass (ICM) cells and become the embryo-forming epiblast (what actually is the case in sea urchins and mammals) (Lawson, Meneses, & Pedersen, 1991; Tam & Loebel, 2007).[2]



[2] **Figure 1**. **The Positioning Hypothesis**. The figure illustrates two possible manners through which the spatial positioning of two (or more) blastomeres alters their chemical status quo. The left panel demonstrates a situation when clustered cells share their intermembrane synergy without the gravitational or other physical compression force other than the intercellular communication. The right panel represents another scenario when physical compression does occur. In this case, the change in the membrane convexity and its tension vector causes a mechanosensitive receptor to open and leads to the change in conformation of a transmembrane protein being a part of a signaling pathway which eventually affects genomic expression.

The first presupposition has extremely significant repercussions on the blastomeres through morphogenetic factors distribution, cytoskeleton rearrangements, and the linked mitotic spindle movement proceeding to consequential first axis formation. It is safe to conjecture that the same mechanistic forces model the fate constituents' map of an embryo if to infer that embryogenesis always proceeds the same route with the same exact geometric interactions.

The two presuppositions must be here properly discussed. It is necessary to include constant movement of the blastomeres and steady biochemical dynamics (Kaneko & Yomo, 1997) that cause the fluidic and solid vibrations and discrete spinning of the cytoplasm beneath the plasma membrane; these can impose their effects on the intermembrane synergy. This synergy must be 1) sensed by the cell (mechanosensing) and further 2) processed through a signaling pathway (mechanotransduction). The importance of mechanical (physical) force to cellular differentiation is already well established (Brezavšček, Rauzi, Leptin, & Ziherl, 2012; Lau et al., 2015; Schwartz & DeSimone, 2008; Yamashita et al., 2016). However, it is not yet clear how embryonic cells sense mechanical tension, but a multitude of molecules and mechanisms are suggested to perform such functions, including cell adhesion receptors , such as nectins, selectins, cadherins, caterins, or integrins (Chen & Gumbinger, 2012; Katsumi et al., 2004; Twiss et al., 2012), as well as cytoskeleton and cytoplasmic proteins (Collinet et al., 2015; Desprat et al., 2008; Hirata et al., 2015; Sawada et al., 2006), membrane ion channels (Guharay & Sachs, 1984; Ranade et al., 2014). In most cases, there is a subtle cooperation between cell adhesion molecules and the cytoskeleton that sense and transduce the mechanical stress onto the intracellular structures responsible for cell-cell adhesion stability and adaptability (Choquet, Felsenfeld, & Sheetz, 1997; Geiger & Bershadsky, 2001; Jiang et al., 2003). According to Engler and his colleagues (2006), undifferentiated cells respond to changes in matrix elasticity by pulling against the matrix and transducing a signal through protein pathway cascades equalizing the force applied to the stressed cell. The so-called focal adhesions (FAs) between interacting cells generate a signal which remodels the cellular cytoskeleton resulting in the forthright intracytoplasmic movement, nucleus positioning, protein-protein interactions, and Ca^{2+} flux gradient (Syeda et al., 2015). Calcium ions are particularly interesting in terms of their vast

biochemical activity during fertilization and embryogenesis: only to name calcium-dependent non-canonical Wnt signaling (Kohn & Moon, 2005), the sperm PLCζ-mediated calcium release in the egg (Miyazaki & Ito, 2006), and convergent extension and tissue contraction triggered by the calcium ions waves (Wallingford et al., 2001a). In recent years, a rapidly growing set of experimental results has demonstrated that pure physical stimuli become translated into strong biological responses navigating the embryogenesis process.

Even though cell adhesion molecules and cytoskeleton are pivotal in transducing mechanical stimuli, it is the mechanosensitive ion channels that may be the first line responders to the promptly changing embryo milieu. The inner and outer membrane leaflets have equal tension in a planar configuration and exhibit differential tension on bending. Thus, with a tension put on the membrane of a very "sensitive" undifferentiated cell, certain mechanogated ion channels open and trigger all subsequent events caused by the electric changes of the membrane (Vogel & Sheetz, 2006). Interestingly, focal linkages between the plasma membrane and the nuclear envelope exist; the plasma membrane once strained and bended lets the focally positioned paxillin (integrin-mediated focal adhesion protein) to be transported to the nucleus (Woods et al., 2002). Henceforth, physical forces may modulate the transcriptional network of the cell.

The mechanosensitive ionic channels and transcriptional network seem to be a rational explication of how physical forces translate into biochemical and genomic activity. Considering the blastula's incessant contact with the inner mucosal lining of the oviduct and the strong interblastomeric forces, the Positioning Hypothesis reinterprets recent findings and adjusts them to the first few blastomeres which influence each other's developmental program by exerting physical stimuli through spatial and geometric effects. This physical intercalation is enhanced through direct cell-to-cell cross talk facilitated through the gap junctions signaling whose intensity depends on the number of such junctions shared between singular blastomeres.

QUALITY AND QUANTITY OF INPUTS/ DISTRIBUTION – DIFFERENTIATION HYPOTHESES.

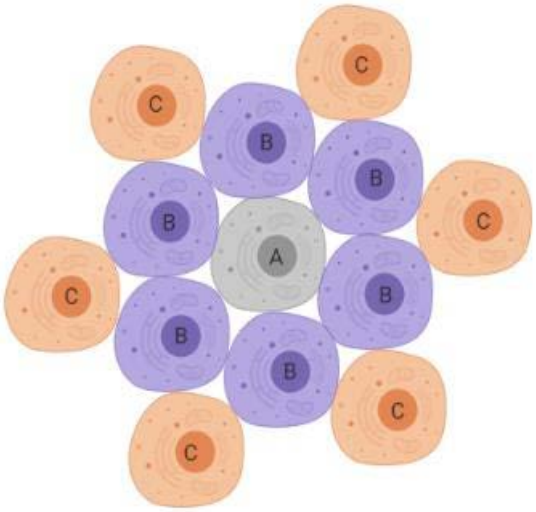
A specific number of inputs delivered by a group of cells become transduced by another cluster of cells as genomic outputs executed at the *cis*-regulatory nodes

which result in activation or repression of particular progenitor circuits in the developing embryo. The second presupposition of the Positioning Hypothesis is strictly affiliated with this Quality and Quantity of Inputs Hypothesis.

The best manner to imagine how the Quality and Quantity of Inputs Hypothesis could be applied to the developing animal embryo is to introduce neuronal activity as an explanatory example of the scheme. For a neuron, a potential signal emerges and is transmitted along the axon sheath if singular ion concentrations (Ca^{2+}, K^{+}, Na^{+}, Cl^{-}) compile to exceed the potentiation activation threshold. Likewise, the interconnected blastomeric cells distribute their signaling inputs shortly after the first gap junctions are established (or when the first ligand-receptor juxtacrine signaling begins) with the net of "positive" (activatory) and "negative" (inhibitory) inputs characterizing the desired output in a cell or a group of cells and determining how these cells will distribute their own signaling inputs.

There is no perfect existing mathematical model to describe the following hypothesis. However, the Moore neighborhood analogy best fits the biomechanical behavior of the embryo cells treated here as single entities. Neighboring cells share their outputs with other cells and the external environment, and proper cells (competent cells) respond to the right stimuli (inputs) at the right time establishing the very first spatiotemporal cues of

development. [3]



Given the range:

$$N^{M}_{(x_0 y_0)} = \{(x, y): |x - x_0| \leq r,\ |y - y_0| \leq r\}$$

Where (x, y) are possible neighbors of the central cell (x_o, y_o), and r is the neighborhood's dimension of the number of cells represented by the formula: $(2r + 1)^2$. The farther away from the central cell (the bigger the r), the more inputs must be incorporated into the system in order to help establish new cell identities. It is, however, not directly related to the quality of inputs or their characteristic gradient distribution (unlike with non-linear reaction-diffusion model of

[3] **Figure 2. The Quality and Quantity of Inputs/Distribution-Differentiation Hypotheses.** The interactions between inner and outer optic cup layers (of the neuroepithelial optic vesicle) and head ectoderm lead to the differentiation of the neural retina (Vogel-Höpker, 2000). Those interactions are an example of instructive interaction (Wessels, 1977) between an "inducer" (head ectoderm) and "responder" (optic cup). Wessels proposed three general principles of most instructive interactions: Cell A helps cell B develop in a certain way, but in the absence of cell A, cell B will remain in the same differentiated state; however, in the presence of cell C, cell B will acquire a different lineage fate. This concept aids the Distribution-Differentiation Hypothesis, which proposes that distribution of fate constituents from cell A to nearby cells changes not only these cells, but it also does change the identity of cell A. This distribution of fate determinants is possible due to interblastomeric communication: it depends on how many inputs each cell will receive (cell B will receive inputs from the founding cell A, synonymous cells B, and the new lineage of cells C) and what information the inputs carry.

morphogens), but this model refers to the quantity of inputs solely. Inputs accumulate, modulate and change the identity of any responding cell.

But it must be both the quantity and the quality of morphogenetic inputs to effectively implement their molecular function. As an example, the germinal hematopoietic stem cells (HSCs) establish their niches in the bone marrow by interacting with a specific cluster of differentiation (CD) molecules. There are two classes of HSCs: long-term (LT-HSCs) and short-term (ST-HSCs) stem cells. They occupy two different niches in the bone marrow: the osteoblastic (by the stromal cells) and vascular (by the endothelial cells), respectively, interacting with other HSCs within a group as well as with the cells building their niches (Yin & Li, 2006). Researchers have found that LIF-1 and BMP gradients control the stem cells' asymmetric division and differentiation, but also the interactions between the stem cells and the niches they reside in drive their progressive development into one of the two possible lineages (Swiers, Patient, & Loose, 2006). Not only is the quantity of inputs essential in regulating the cellular differentiation process, but the quality (the identity) of those inputs as every daughter blastomeric cell will receive this signaling which comes directly from its founder cell. As it is seen on the HSCs example, an explicit cross-talk between the differentiating cells and their surroundings play a significant role in how the inputs are translated on the genomic level. Henceforth, the Positioning Hypothesis aims to describe the asymmetrical blastomeres division and first developmental fields formation, whereas the Quality and Quantity of Inputs Hypothesis is the natural consequence of logical interpretation of the early developmental events.

On the other hand, the Distribution-Differentiation Hypothesis posits that the central cell contributes to the change in differentiation state of the neighboring cells through four major mechanisms: autocrine signaling, juxtacrine signaling, environmental sensing, and direct transfer of cytoplasmic fate determinants. This is in agreement with the model of inductive interaction proposed by Holtzer (1968) and with the distinct instructive interaction type described by Wessel (1977): a proper signal produced by one cell induces the corresponding change in the other cell's expression identity. The Distribution-Differentiation Hypothesis expands on those models indicating that the cell which interacts with its neighbors by distributing the fate determinants ("inputs") must change its own cellular identity and alter its fate determination potential in

favor for the neighboring cells and thus transform itself into the cell with changed competence and/or functional potential ("self-output").)

Hematopoietic stem cells lose their "stemness" identity to progenitor cells which further lose their potential in favor for the fully determined blood cells. The chordamesoderm cells induce specification in the nearby mesoderm and epithelial cells by losing their own instantaneous competence and turning into notochord, whose cells ultimately die by apoptosis at the end of the gastrulation stage (Eimon et al., Stemple, 2005). These are only a few examples which demonstrate that every "input" cell modifies the "output" cell, while altering its own differentiation identity. It is a remarkable system of signaling molecules, morphogens, direct cell-to-cell communication, and environmental influences that regulate the default embryo developmental program. The Quality and Quantity/Distribution-Differentiation Hypotheses help explain the phenomenon of multiple cell lineages derived from the single zygote in a relatively short span of time and with imbalanced concentrations of proteins non-randomly distributed among the first embryo cells.

MATERNAL AND ENVIRONMENTAL CONTROL HYPOTHESIS

All protostomes exhibit autonomous specification, which means that most cell lineages are already specified before gastrulation. This is due to the highly specific fate determinants distribution in the early blastomeres (Anderson & Nüsslein-Volhard,1984; Ruth & Nüsslein-Volhard, 1991; Liu et al., 1991; Wood, Laufer, & Strome, 1982). It is the maternal effect that is responsible for the phenomenon: a number of pivotal mRNA transcripts present in the egg and transferred to the fertilized zygote activating the zygote's genome, enabling it to rapidly express its own genes, and guiding normal development (Davidson, 1990; Kaletta, Schnabel, & Schnabel, 1997). Maternal effect is distinguished from maternal anisotropy, another embryological phenomenon, where maternal products are distributed in different concentrations across separated egg's cues, thus leading to establishing local developmental fields representing future cellular fates (Davidson, 2010).

Many deuterostomes develop at least some of their tissues via autonomous specification, hence experiencing the maternal effect, however, most of them establish the developmental fields through conditional specification, which is a cell-extrinsic mechanism of tissue patterning involving interblastomeric

communication (community effect), cell-environment interactions, and the activity of extracellular signaling molecules (morphogens) (Peterson, Cameron, & Davidson, 1997). An indeterminate cleavage (early blastomeres have equal potencies to develop into the embryo), demonstrated by most deuterostomes, requires from the early embryo cells to have even distribution of fate determinant hence even though the maternal effect is observed in the conditionally developing embryos (including mammals), the maternal anisotropy in deuterostomes is still a conundrum(Davidson 1990; Davidson 2010).

There is no direct evidence of maternal anisotropy in vertebrates nor is there for any differential distribution cues in the first vertebrate blastomeres; there is significant evidence for maternal transcripts stored in the eggs of vertebrates (De Robertis et al., 2000; Dosch et al., 2004; Marlow, 2010; Moody et al., 1996). Moreover, transcriptome and genomic analysis data of mammalian eggs and embryos reveal a strong contribution of maternal control of development in mammals (Howell et al., 2001). Several maternal factors have been identified and characterized in mammals, including BRG1 (a chromatin-remodeling protein) (Bultman et al., 2006), TIF1α (transcription intermediary factor) (Torres-Padilla & Zernicka-Goetz, 2006), Mater (a leucine-rich maternal antigen) (Pennetier et al., 1006; Tong et al., 2002), and ZAR1 (an oocyte-to-embryo transition protein) (Wu et al., 2003). Surprisingly, Vinot and her colleagues (2005) showed that the PAR proteins complex (Figure 3), which in *C. elegans* is responsible for cell polarity and unequal distribution of fate determinants, is also present in mammals as maternal products (to some extent) whose homologs are expressed in the preimplantation mouse embryo and modulate cell adhesion of blastomeres, but their distribution is not asymmetric until compaction and are most likely not of maternal heritage. It has not been shown that any of the above mentioned maternally inherited transcripts are unevenly distributed across the mammalian eggs.

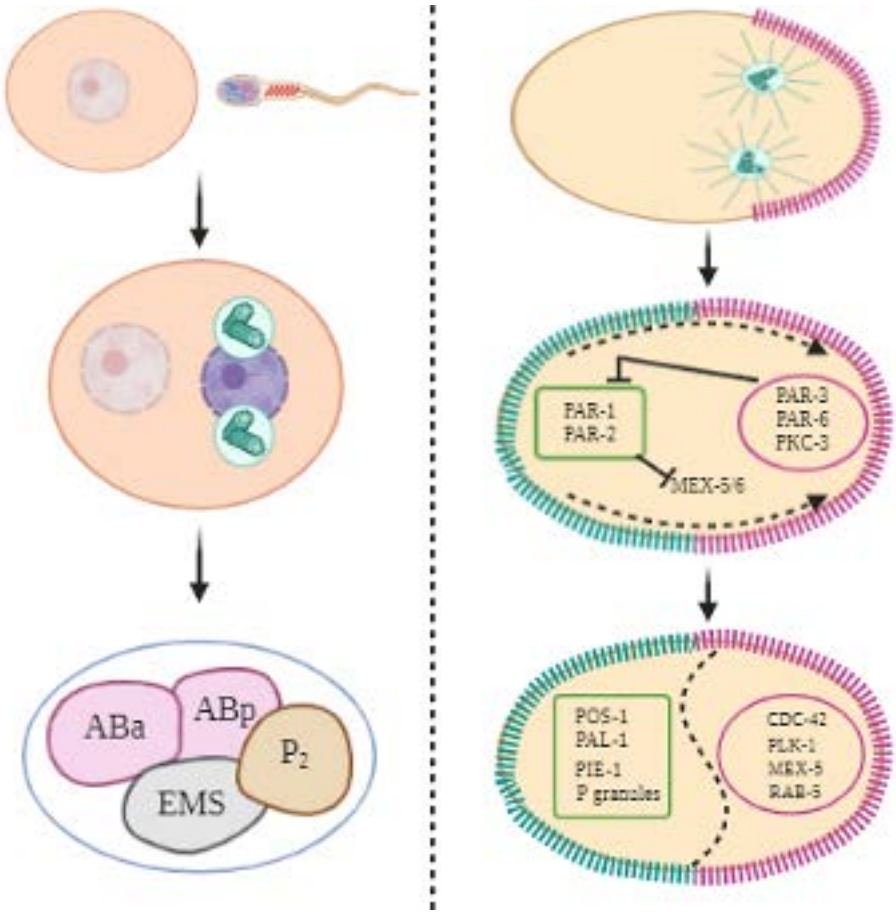
Maternal anisotropy occurs by three distinctive means: the extracellular 1) injection of transcription factors and signaling molecules (Toll-related signaling particles, BMP proteins, NOTCH signaling transcripts) by nursery cells, the intrinsic 2) distribution of maternal proteins and transcripts in the ooplasm, and by 3) activation of the external receptor particles on the egg surface by extrinsic signaling (inputs) which downstream activate or deactivate particular genomic

regions (Davidson, 2010). These mechanisms are ubiquitously employed by most protostomes and deuterostomes, thus a very intuitive question arises: Is it safe to expect maternal anisotropy to occur in the mammalian egg?

The Maternal and Environmental Control Hypothesis suggests two following presumptions:

1. The protostomic phenomenon of maternally inherited fate determinants and their predestined spatiotemporal distribution in special biological cues of the maturing egg can be applied to mammals, including humans.
2. The mammalian developing egg interacts with the environment through the means of supporting (stromal) cells in the ovary, external signaling particles, internalized biological molecules, and the downstream web of transcription factors.

The interplay between the external "influencers" and the internal "responders" of the egg might explain executing the unbelievable number of cell fate programs at the later stages of embryogenesis. The Maternal and Environmental Control Hypothesis suggests that basic developmental programs are carried on by the internalized anisotropic changes of fate constituents by interacting with external "influencers" or "modifiers." The more complex programs are executed with subtle transitions and changes in the developing system of transcription factors, morphogens, and signaling pathways.



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[4] **Figure 3. Maternal effect and maternal anisotropy in *C. elegans*.** *The left side* of Figure 3 illustrates the early worm embryo development starting from fertilization, going through the zygote's centrosome formation and duplication, and proceeding to further asymmetric cleavage rounds producing the posterior and anterior founding cells. That sequence of events is molecularly explained with the concepts of maternal transcripts (present in the ovum and the zygote) and their anisotropic (asymmetric) distribution inside the ooplasm (*the right side*). Breaking the egg's symmetry starts with the sperm entry and its delivery of the centrosome, which leads to the local cessation of cortical actomyosin contraction (Bienkowska & Cowan, 2012). The non-contractile cortical area (pink dash line) expands anteriorly (dash line arrows) causing the posterior-directed streaming of cytoplasmic proteins (Tostevin & Howard, 2008). The first embryo axis, the anterior/posterior (A/P) axis, is established by the asymmetric cortical movement, compensating cytoplasmic streaming, and the interdependent activity of the PAR proteins (Gönczy & Rose, 2005). PAR-1 and PAR-2 are located in the posterior pole (green dash line), while the PARA-3/PAR-6/PKC-3 complex is restricted to the anterior part of the egg (the nucleus is removed for clarity of the image). The dynamic mutual inhibition of the anterior and posterior cues establishes the A/P axis and segregates many other

Female gametogenesis is a dynamic process during which germ cells go through many developmental changes. Functional oocytes, developed through the late embryogenesis, are stored in an arrested state within primordial follicles until they are signaled to undergo further transitions, which require the surrounding stromal cells nurturing and external signaling. Vascularization, paracrine and autocrine growth factors (for instance, LIF, BMP, and bGFG), cytokines (including Il-16, Il-1β, and TNFα), and transcription factors (GDF9, c-Kit, SCF) guide the follicle cells through all transition phases (Feeney, Nilsson, & Skinner, 2014; Skinner, 2005). Interactions between the pituitary LH and FSH tropic hormones, the FSH receptors on the follicle surface, estrogen, and local ovarian growth factors (IGF-I, EGF/TGFα, APO-3) establish an immunocompetent, mature and fully developed ovarian egg apt to be secreted to the Fallopian tube and fertilized (Kaipia, 1997). There are studied pinpointing at the direct "modifying" effects of the surrounding oviduct cells (Xu et al., 2004) or indirect influence on the egg maturation and zygote transformations by more distant cells (Bauersachs et al., 2004).

The mammalian egg does contain a substantial amount of maternal mRNA transcripts and maternally inherited proteins which do not lead the first embryo cells through determinate cleavage, but instead they could constitute specialized egg cues along the animal-vegetal axis, thus determining the first cleavage sites, cytoskeleton movement, and the spindle formation. The Maternal and Environmental Hypothesis does not undermine the conditional specification of mammalian embryos, but only strengthens it: delicate and finely regulated distribution of a chosen group of fate determinants in the egg and first blastomeres would help establish specific developmental cues in different regions of the embryo and allow for precise influencer-responder cross-talk of late blastomeres.

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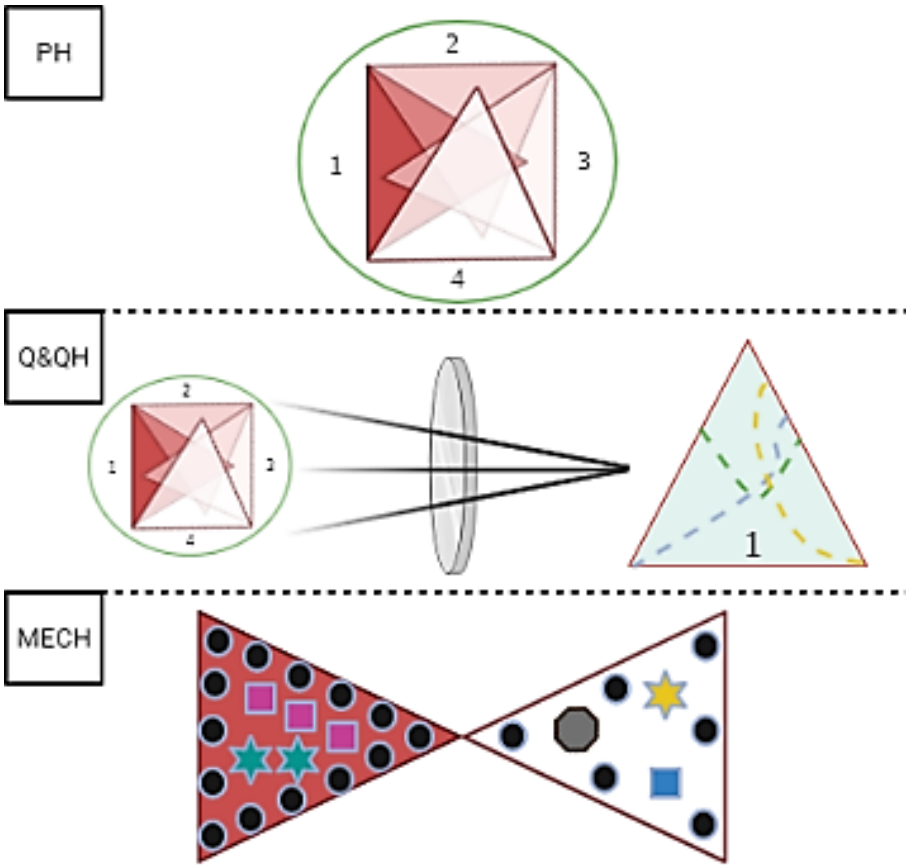
maternal determinants, including MEX-5/MEX-6 proteins, which play a major role in the unequal segregation of cell fate constituents responsible for creating all future cell lineages (Schubert et al., 2000). *PKC-3* (protein kinase C-like 3), *ABa* (anterior founder cell), *ABp* (posterior founder cell), *EMS* (endomesodermal precursor cell), P_2 (second posterior cell).



[5]

[5] **Figure 4. Summary of the Three-modal Theory of Early Embryo Asymmetric Cleavage Determination**. The Positioning Hypothesis (PH) suggests that the physical and geometric interactions between the first blastomeres have direct effects on the fate determinants distribution and alterations of genomic activity. The darkest cell (triangle 1) experiences the biggest force (compression) vector, while the transparent cell (triangle 4) experiences no physical compression. This observation expands further onto the Quality and Quantity/Distribution-Differentiation Hypotheses (Q&QH). The number and character of inputs (signaling particles, gap junctions, mechanosensing) delivered by one cell (inducer) to the neighboring cells (responders) shapes the genomic and developmental identity of both groups through 1) prompt effects of inputs and 2) their differential gradient fluctuations stemming from unequal distribution (triangle 1's area is divided into the regions influenced by triangle 2 (blue line), triangle 3 (green line), and triangle 4 (yellow line). Finally, the Maternal and Environmental Control Hypothesis (MECH) proposes to revisit the maternal anisotropy phenomenon in mammals, which have been already shown to be under maternal effect

CLOSING STATEMENTS

The question of what effect the geometric positioning and physical stimuli have on the early divisional patterning of the embryo has been asked several times. Two other theories attempted to answer similar questions asked in this article focusing on asymmetric division. Wroblewska & Tarnowski (1967) suggested the existence of cytoplasmic territories (cues) in the egg with already differently determined fates (Tarkowski & Wróblewska, 1967). They also concluded that as early as at the 8-cell stage human embryos, the cell position either on the outside or the inside the developing blastula decides on the trophoblast (TE) or inner call mass (ICM) lineage choice. Their Inside-Outside model claimed that it was the asymmetric flow of the particles that determined the further blastomeres generations. On the other hand, Johnson & Ziomek (1981) proposed the Cell Polarity model suggesting that the cell fate is established at the 8-cell stage embryo by establishing cell polarity along the early morula radius. The further cleavage leads to either symmetric (two TE cells) or asymmetric (a TE and ICM cell) divisions based on the angle of cell division (Johnson & Ziomek, 1981). In summary, the Inside-Outside model points that the cell position affects which developmental field the cell will establish; the Cell Polarity model predicts that the cell fate affects the cell position. There is evidence for both models, reviewed elsewhere by Yamanaka et al., however, the two models lack the biomolecular reasoning and leave many variables behind the final conclusions that had been drawn by the original researchers.

The presented theory, especially the Positioning Hypothesis, aims to help understand what natural processes lead to differential embryogenetic programs execution starting from a single cell. One developmental process comes to light in particular, the maternal-to-zygotic transition (MZT) helps the early embryo cells become independent from the maternal transcripts by clearing them off. In the meantime, the same cells begin their own mRNA synthesis, phenomenon known as the early genome activation (EGA) (Lee, Bonneau, & Giraldez, 2014).

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control, but the Positioning Hypothesis reinterprets how maternal anisotropy might be achieved in these animals (the community effect of blastomeres as well as through interactions with the reproductive tract). (*Lower part*) black dots represent uniformly distributed maternal proteins, while green stars and purple squares demonstrate putative asymmetrically distributed maternal products (triangle 1); the daughter cell (triangle 4) still has some remaining maternal products (black dots), but newly expressed proteins start to emerge.

The considered Positioning Hypothesis becomes a plausible explanation for how and why the MZT happens.

Slow-developing animals (including humans) present delayed cleavage and moderate independence from maternal factors, thus they activate their genomes relatively quickly. Fast-developing animals rely on the maternal (inherited) factors for considerably longer. They develop through autonomous specification, and activate their genome much later. Therefore, slow-developing animals are composed of only a few clustered blastomeres when they initiate the EGA, while fast-developing organisms complete at least 10-12 cell cycles before their EGA starts. In fact, the Positioning Hypothesis could explain the occurrence of the EGA in both groups, but there is no evidence yet for how many blastomeres it takes for the positional effect.

[6]	**Primary Event**	**Dependent Event**	**Event with the Main Contribution to Early Embryogenesis**
1	PH	EGA	EGA ∩ PH
2			EGA ∪ PH
3			EGA
4			PH
5	PH, EGA	---	EGA ∩ PH
6			EGA ∪ PH
7			EGA
8			PH

The introduced hypotheses will be experimentally validated in the nearest future. As much as they are novel, they are the outcomes of straightforward

[6] **Table 2. Relationship between the Positioning Hypothesis (PH) and the Early Genome Activation (EGA).** In slow-developing animals (including mammals), the EGA is the primary mechanism used to form the first development cues in the embryo (although the first asymmetry occurs not until compaction starts). Fast-developing animals rely on their maternal products, but the community effects still plays a key role in the developmental fields formation. The table summarizes the possible outcomes resulting from interactions between the effects of the Positioning Hypothesis and the effects of the Early Genome Activation. ∩ - intersection (both events contribute equally), ∪ (union) – separate contribution of two events.

reasoning based on the available literature and experimental results of outstanding world-class laboratories which have gathered enough pieces of evidence to support this article's daring, but still resolute conclusions. Embryogenesis is not an enclosed biosystem with fixed variables and specific conditions, but a multilevel network of all possible molecules, signaling pathways, morphogens, transcription factors, and cellular interactions.

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