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Getting *ab ovo* Developmental Processes Intelligible Using Metaphors, Microscopy and Paradigms

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Abstract

Developmental biology is a field fertile in metaphors and paradigms, and offers a playground rich of possibilities for various technologies. The underlying mechanisms for developmental biology processes have challenged science and imagination for centuries. Herein we propose a non-exhaustive incursion into three major concepts in the field: self-organization, pattern organization and mechanical forces. Observations and views issued from synthetic and systems biology are discussed to move towards a more comprehensive and accurate "landscape" of the very early steps of embryogenesis. Past and potential results from optical microscopy are considered, in their contribution to visualize the wholeness of the first cellular events from the early embryogenesis.

Keywords: Developmental biology; Photonic microscopy; Mechanobiology; Morphogenesis; Self-organization; Spindle morphogenesis

Introduction

Embryology to be put in the picture

Embryogenesis is a story that starts *ab ovo*. After fertilization is completed, the egg begins a set of rapid divisions, called segmentation, ending with an organized bi-layered structure. The so called blastula hosts a cavity (blastocœl(e)), and is ready to start the next developmental step, gastrulation. The latter will bring a third layer in the embryos. The three embryonic layers formed, the endoderm, the mesoderm and the ectoderm are at the origin of all the tissues within a given organism, with a cell-lineage that can be rigorously determined, according to the animal model considered. Soon after gastrulation, organogenesis is set with the induction and the initiation of the neural tissue. Cluster of cells may then organize themselves into distinct and functional units: the organs. The underlying mechanisms for these processes have challenged science and imagination for centuries. Thus, developmental biology has been a playground rich and fertile for metaphors and paradigms, i.e. canalization of developmental process for differentiation, creodes, hidden corpuscles within gametes (homunculus), epigenetic landscapes, morphogenetic fields organizers (Spemann organizer) or concepts such as cellular self-organization (Table 1) [1-3]. Some of the metaphors have been wiped out by microscopy improvement, like the hidden gametes corpuscles (from the spermist and ovist theories) to be replaced by a similar paradigm. In that particular case, the metaphorical paradigm of genetic program has replaced and revived the preformationist theories, sustained by the enthusiasm brought by molecular biology and genes discoveries. The elevation of genes as the key masters for species and individuality programming (metaphor of genetic program) led then to a genocentrism that was quite difficult to untie, placing the gene at the center of any phenomenon. Nevertheless, genocentrism failed to explain developmental process in its wholeness, and other metaphors and paradigms were seek for situation where genes appeared as "inert", leading to an increasing interest on the concept of epigenetic landscape and self-organization.

Creode: Paradigm used by Waddington (1905-1975) in his description of epigenetic landscape. Creodes illustrate developmental pathways followed by cells as they proliferate and differentiate into organs [63,67].
Epigenetic landscape: Concept imagined by Waddington, within which cells are represented by spheres rolling down in a landscape into deep and narrow valleys (canalization or fate determination, which cannot be escaped), until reaching a stable state. Changes of states are prevented by ridges (or energy barriers) that do not allow movement from one stable state (valley) to another one. Creodes materialize the trajectory of cells, in a succession of expression patterns. Waddington's epigenetic landscape also provided a metaphor for how development might be modulated [68].
Genocentrism: Neologism derived from the adjective, genocentric, to emphasize the overwhelming role taken by gene in cellular processes. Genocentrism at its climax would restraint any phenomenon under the exclusive control of genes.
Homunculus: Miniature human, integrally formed, also called animalcules, which is within eggs (ovist theory) or spermatozoon (spermist theory), according to preformationist theories. Organisms are preformed and embryogenesis is restrained to successive growth periods [69].
Self-organization: Emergence from an initially disordered system of a dynamic organization. The latter arises from the collective behavior of components, where the individual properties of the component cannot account for the final dynamic pattern. Thus, an organization in space and time leads to emergent properties, whose characteristics are qualitatively different from the components. Self-organization is considered as a dissipative phenomenon.
Spemann organizer: The concept of induction in developmental biology. In amphibian, it refers to a group of cells, positioned at dorsal lip of the blastopore, which induces the development of the neural tissue. Initially discovered by Hans Spemann and Hilde Mangold (1898-1924) in amphibian, this primary organizer is now admitted to exist in many animal models. This primary organizer is set up by the *Nieuwkoop Center, which* is the dorsal- and vegetal-most cell of the early amphibian blastula.

Table 1: Metaphors and paradigms in developmental biology.

The genesis of these paradigms and metaphors came in parallel to the improvement of microscopy and the redefining "life" into smaller entities. Indeed, the development of optical microscopy always had an immediate impact on the understanding of embryogenesis: human eyes can resolve objects separated by more than 0.1mm. It is not enough to allow a discrimination of the first steps of embryogenesis in small animal models, particularly if one wants to look more specifically at subcellular modifications. The first impact of optical microscopy was to provide a sub-micron resolution, and to discover what was going on at the cellular and sub-cellular level in space and time. The credit of the first microscopes invention is controversial. Zacharias Janssen claimed in the mid XVIIe century that he built the first microscope with his son in 1590, while Galileo developed an occhiolino (a microscope composed of a convex and a concave lens) in 1609. It was used to dissect biological events for the first time by Antoni van Leeuwenhoek (1632-1723) observing single cells microorganisms he called 'wee animalcules' (Table1) and embryos [4]. For the first time cells and organites were described, without reporting existence of fully pre-patterned and pre-formed animal in eggs or spermatozoon refuting the homunculus theory. This optical frontier crossed, entities were compared to Russian dolls: all cells appeared as new lands where macromolecules were exerting activities, which were thought to orchestrate cells lives in most if not any of their aspect. A hierarchy was established with DNA as the support of genetic data, acting as an inert modular set of informations, around which were dancing and swinging enzymes. In this picture transcription and translation of genes into proteins were involved in all the aspects of cell lives (migration, proliferation, differentiation, death). Behaviors of this molecular bricks were almost at hand, but they remained difficult to observe at work, either in fixed or living materials. After a descriptive phase, there was a call to understand how the molecular mechanisms where underlying and promoting cellular morphologies and fates. To this respect, one can go further with considering that these mechanisms does not rely only the sum of the intrinsic properties of molecules (as it could be postulated classically in biochemistry), but may rely partially, if not mainly, on properties emerging of a network of molecular. Once again, these emerging properties do not encompass the sum of molecules intrinsic properties but arise beyond these limits, opening the field of systems biology.

From the global imaging of cells and pluricellular organisms, another revolution came with the discovery of Green Fluorescent Protein family, awarded by a chemistry Nobel prize in 2008. These new tools enabled the creation of genetically-modified embryo and permit to follow the fate of specific proteins (protein tracking, activity or interaction reporters) and cells (cell-lineage). While embryo is a relatively thick object, it benefited from the optical sectioning provided by confocal microscopy and by deeper tissue imaging with the two-photon excitation microscopy. Other adaptation of Non Linear Optics, such as imaging of second (SHG) and third harmonic generation (THG, see Table 2 for glossary and references), were also major contributors to embryogenesis description with images of specific cell compartments without supplementary tags. Combined to automated analysis, these tools provided a deeper view of the physiological and genetically pattern of cell lineage.

Optical microscopy is also a player for the *in vivo* dissection of mechanical properties integrated in cell signaling pathways. An example, which will be discussed further, came with the mechanotransduction mediated by cadherin that emerged from evolutions in [1] molecular biology with the developments of genetically encoded biosensor to measure the tension force applied to the cadherin in space and time and with [2] the nanotechnologies with cells seeded on microneedles coated with cadherin. When the cell applies traction forces through cadherin-mediated adhesions, it causes detection of measurable deflections of the micro-needles [5] (Table 2).

Confocal laser scanning microscopy: CLSM is a technique for obtaining higher resolution images compared to classical video-microscopy with the ability to perform optical sectioning by adding a spatial pinhole in the confocal plane. It enables the 3D reconstruction of acquired objects from a vertical series of images. It was first patented in 1957 by Marvin Minsky and became continuously used in biological science from the 90th [70].
Fluorescence correlation spectroscopy: in FCS, a laser beam is focused on the sample. The measured intensity fluctuations are analyzed by temporal autocorrelation. This analysis can be applied to really low concentration (nM range) of proteins and gives quantitative concentration and diffusion coefficient of fluorescent particle as well as the abnormality of the medium [71].
Fluorescence recovery after photobleaching: FRAP consists in irreversibly inhibiting fluorescence in a restraint area of the sample by very fast exposure of this region to a high intensity laser creating two spatially distinct molecules population: fluorescent or bleached. If the molecules can move, there will be a redistribution of molecules until homogenization of population, and thus a fluorescence recovery in the bleached area. Analyzing this recovery give the diffusion coefficient and the mobile/immobile fraction of analyzed molecules [72].
Genetically encoded biosensors: are a protein sequence flanked by fluorescent proteins, whose peptide sequence are sensitive to the presence of small molecules or to proteins activity that will alter its fluorescence properties. They can be introduced in cells, tissues or organisms and permits in vivo long term investigation of signaling pathways in space and time [73].
Green fluorescent protein: GFP is an intrinsically fluorescent protein arising from a jellyfish (*Aequorea victoria*). Its gene can be fused in-vitro to the gene of a protein of interest. This recombinant gene can then be introduced in a cell. A fluorescent fusion protein will then be synthesized and can be localized in-vivo using fluorescence microscopy. Variant from the GFP with different excitation and excitation properties allows following several proteins at the same time [74].
Second harmonic generation: SHG is a second-order nonlinear phenomenon in which a fundamental wave is partially converted into a second-harmonic wave with half the initial wavelength. It appears under two photons excitation. It doesn't need any absorption process and no fluorescence labeling is necessary. It can be measured at exactly the half of excitation wavelength and the signal comes specifically from molecules which don't show any centrosymmetry. It is thus of major interest for probing structures with high degree of orientation and organization either from extrinsic or intrinsic harmonophore such has collagen or tubulin [75].
Two photons excitation microscopy: fluorescence imaging technique in which two photons carrying half the energy needed for traditional excitation can excite a fluorophore in one quantum event. From a practical point of view, it uses pulsed and red-shifted excitation sources, reducing scattering in the tissue and phototoxicity while increasing penetration depth compared to conventional fluorescence techniques [70,75]. It also benefits from optical sectioning capability while if the system is correctly set, the two photon effect only occurs at the focal plane.
Third harmonic generation: THG is a third-order nonlinear phenomenon in the fundamental wave is partially converted into a third harmonic wave with one third of the original wavelength. Contrarily to SHG, no geometrical rules are required and thus all material potentially can be used for THG. However, while THG signal is intrinsically weak, it is mainly used to image specific molecules such as lipids, myosins or collagen [75].

Table 2: Optical microscopy-related technologies.

The picture of developmental processes and the blossoming of metaphors were somehow intimately linked to the development of constructed perception for embryos as objects for microscopy. The first animal models used were those involving external medium fertilization and development (such as ascidians, echinoderms, amphibians). Secondly chicken and mammalian models like mice with

internal processes were studied. Finally, other models, such as nematodes and zebrafishes arose, due to their optical transparence and their amenability to genetic studies. Analogies in mechanisms were found and implemented works were done in the sense of evolutionary developmental biology.

More and more paradigms and metaphors evolutions have been linked to physics theories. Their application and their development lead to the exploration of sub-cellular components behavior in synthetic biology or *in vitro* approaches [6-9]. Cell-tracking has determined cell-lineage and "genealogy" within pluricellular organisms [10,11].

Self-organization begins within the female reproductive cells: oocytes and eggs

Self organization is a concept that derived from a reductionist approach and from the examination of individual components and from manner they interact with each other. Far from biology, self-organization had its origin in thermodynamics, with the observation of spontaneous dynamic patterns in liquid by Bénard rolls [6,12]. Both at the experimental and theoretical levels, Alan Turing (1912-1954) addressed the appearance of patterns and sets the chemical basis of morphogenesis, and in a way that could predict self-organization in embryos [13]. In biological systems, self-organization was mostly observed studying microtubules behaviors, mitotic and meiotic spindles formations and cytoskeleton architecture. Indeed, patterns and oscillations can easily been observed in pure microtubules solutions [14] and microtubules nucleation and aster-like structure formation in frog egg extracts [15]. The self-organization concept came the collective behavior of motors-proteins and microtubules, with i.e. type of patterns depending upon motors concentration [16,17]. The reaction-diffusion mechanisms often explain self-organization but another mechanism has been proposed: in fission yeast, it has been proposed that self-organized behaviors of motors during meiotic prophase rely on load-detachment/action mechanisms of motors to microtubules, thereby generating also oscillations [18].

Meiosis is an alternative mode for cell division, in which a diploid cell undergoes through two successive divisions (meiosis I and II), without replication, to generate haploid cells (called gametes) [19]. Meiotic divisions segregate the genetic material in absence of astral microtubules and in absence of functional centrosomes, acting as microtubules organizing centers in somatic cells. Morphogenesis of the first meiotic spindle appears in a self-organized manner. Studies in mammals have proposed a self-organizing mechanism for the meiotic divisions and the first two cleavages, depending upon the combined activities of motors dynein and kinesin 5 [20,21]. While the spindle is a relatively demanding structure in term of bipolarity, size and shape, the self-organizing properties of meiotic spindle appeared to be able of a certain plasticity since two meiotic spindles put in a close vicinity can be merged into a perfect single one [22,23].

More has to be determined on the mechanisms at the biochemical and the physical levels on the key players and mechanisms controlling organization of spindle morphogenesis; Current efforts are undertaken to provide more accurate model i.e. in vertebrates like frogs, which have taken their part in the exploration of self-organization of microtubules, cytoskeleton and spindle for decades [6,24]. Several questions remain: How do key players and mechanisms controlling organization of spindle morphogenesis react to perturbation? How do self-organization fit into the picture of rapid assembly and disassembly of the mitotic spindle apparatus? How do the key players behave in cooperation with cytoskeleton in context where cytoplasmic streaming drives the spindle into an asymmetric cytokinesis and symmetry breaking between the two daughter cells? Are other properties of the molecular network arising beside the intrinsic properties of the identified molecular actors, involved in the process?

One shall not also forget that genes are part of DNA, which structurally trigger the self-organization of the pattern segregating them: indeed, chromosomes play a crucial part in the triggering of the self-organization of mitotic spindle [25].

Roots of early determination: Molecular gradients and specific localization seed cellular fates

Embryonic cells progressively differentiate, being canalized according to the metaphor of Waddington's landscape, into developmental processes that establish axis of polarity and culminate during organogenesis. How cell types diversity and fates are generated from a common pool remain a fundamental issue and a permanent challenge in developmental biology. Cell fates and phenotypes diversity involve polarized intrinsic factors, inductive signals and competences to integrate all inductive signals.

A striking and extreme example of how one molecule expression may "seed" and determine an organism individual fate, was provided by studies in the parasitic polyembryonic wasps [26,27]. In these insects, polyembryony is a process through which several embryos arise from a single egg. In the case of the parasitic wasp *Copidosoma floridanum*, several thousand of embryos are obtained from a single egg. Another developmental particularity of this specie is that the offspring, which may be regarded as genetic clones, forms two morphologically different types of larvae, or castes: (1) precocious larvae and (2) reproductive larvae. The first ones exert functions analogous to workers and soldiers in social insects: they defend theirs "clones" from competitors and are involved in sex ration adjustment. The reproductive larvae initiate morphogenesis, consume their host and insure the maintenance of the specie by forming adult wasps. While the different casts are dependent upon environmental conditions in social insects, the different phenotypes arise in clonal embryos, being under the same environmental conditions in *Copidosoma floridanum*. Even if sex ratio and presence of competitor may influence the percentage of precocious larvae [28], reproductive larvae emerge from clones, which have inherited the maternal transcript of RNA Helicase Vasa, a germ-line marker. Asymmetric distribution of *Vasa* (*CfVas*) is observed at the 4-cells stages, and inherited in the primary morula, before the proliferative stage that lead to increase the cellular mass and the splitting into several morulae, ones that express *CfVas* (detection by *in situ* hybridation or by antibodies), the other ones, which don't [26,27]. The ablation of the first blastomere containing *CfVas* can be achieved by laser, under microscope, using a micropoint laser ablation system. Such ablation dramatically reduced the proportion of reproductive larvae by 95% while the precocious larvae seemed not to be affected. *CfVas* appeared in this peculiar model to regulated the proliferation phase during development and determine the fate of clones as becoming reproductive larvae [26]. Recently, cDNA libraries from *Copidosoma floridanum* embryos allowed to report several candidates to insight occurrence of polyembryony [29].

Symmetry breakage and establishment of axis of polarity in models such as ascidians, drosophila and amphibian have brought to mind a

more complex reality which involves spindle orientation, maternal mRNA distribution and gradient, cytoskeleton rearrangement, oscillatory mechanisms and signaling pathways activations. Proteins interact, sets of genes are activated and establish a complex network that drive cells into proliferation, differentiation or even death. Future territories within the adults are even materialized by changes of pigmentation like in ascidians [30,31] or in amphibian [32], where the egg grey crescent points out the future dorsal part of the embryo. Noticeably, specific areas have been determined in amphibian by Hans Spemann (1869-1941) and Pieter Nieuwkoop (1917-1996), as group of cells with the ability to induce adjacent cells to change for the mesoderme induction and the gastrulation stages, successively being the Nieuwkoop Center and the Spemann Center. Such areas were defined as organizing center [33-36] (Table 1), ruling out polarities through gradient diffusion of morphogens and specific interactions in time and space. Similar organizer were found in birds and mammals [2,3], where induction is also the key process by which a cluster of cells influences the fate of surrounding cells, through induction mechanisms. Morphogens are classically described as molecules generating gradient in space, towards which cells respond in distinct ways, according to their competences or through threshold effects. The notion that cells react according to specific concentration and acquire positional information has been popularized by the French flag model of Wolpert [37]. How the morphogens gradient are generated and integrated, and how morphogens move their way through embryos have been largely addressed both by theoretical and biophysical approaches, for example by using morphogen-GFP, FCS and FRAP (Table 2 and [38-41]). Nevertheless, the coefficient of diffusion detected by FCS and FRAP appeared to be different, according the biophysical approaches used. FRAP appeared to provide more accurate measurements reflecting long-range movements within different experimental situations [42,43], even if rapid morphogens moves can be extrapolated. FCS is more indicated in faster diffusion rates, with lower morphogens concentrations.

Morphogens-GFP can either form gradient through a model of Synthesis-Diffusion-Clearance, or gradient may result from cell lineage transport, like in the case of FGF8. In the simplest cases, morphogens diffuse under monomers or dimers, but lipidation may modulate the movement of some morphogen, as the oligomerization with diffusible molecules [41]. Though extracellular diffusion (extracellular matrix components like heparan sulfate proteoglycans may modulate morphogen spreading, either restricting it or allowing its spreading on long range), and endocytosis (transcytosis) are considered as major modes for morphogens movements, other structures like filipodae have been involved in morphogens signaling [41].

Do mechanical forces also shape cell fate?

For developmental biologists inductive signals are often seen as molecules but they might also proceed through physical forces: cellular mechanosensitivity can be integrated into forces before any molecular signaling pathways. Indeed, most efforts to understand morphogenesis during development were focused on soluble morphogens spatio-temporal gradients inducing dose dependant biochemical response and changes in gene expression [2,3]. Thus, many genes and chemicals regulating tissue formation have been identified as candidates for organizing the tissues. Recent studies however demonstrated that these factors alone are not enough to explain morphogenesis and that mechanical force, generated by cells and tissues, may be involved in an equal proportion to this regulation. If we consider the segmentation period with its accelerated rhythm of cell division and organization of cellular mass with a cavity (i.e. blastocoel within blastocysts in mammals), mechanical forces are at work at different levels: (1) spindle morphogenesis and cytokinesis, (2) modulation of transcription rates and genes expression and (3) adherence between cells. Each of these levels are discussed below. A few mathematical models for segmentation and cleavages have been proposed [44-46]. Many parameters were taken in account, depending of the model: (1) tension of surface and cell shape, (2) viscosity and elasticity within the cells, (3) polarity of the molecules of adherence, (4) physical constraints (i.e. egg shell), (5) distance between centrosomes, (6) attraction forces toward the cortex (*C. elegans*), (7) repulsive force between the centrosomes, (8) chemotaxis and (9) modulation of the actin filament network (stiffness modulation).

Mechanical forces are firstly solicited at the intracellular level during karyokinesis and cytokinesis. The mitotic spindle positioning is regulated by physical interactions between microtubules and actin filaments and determines the symmetry of cell division. In most vertebrates oocytes, asymmetry is requested and the spindle is positioned at the plasma membrane to separate in one side, the gamete, and on the other side, the polar bodies, which are condemned to degenerate. Alteration of this physical forces or the molecules regulating the cytoskeleton architecture and dynamics, could lead to production of abnormally large polar bodies in mice [47-49] or arrest in meiosis I in case where cytoplasm viscosity and yolk presence disable cytokinesis [50,51]. Cytokinesis physically separates daughter cells, via an actin contractile ring, being the main component of the furrow progression. Such constriction is facilitated by the softening of the cell surface. This could be under the control of centralspindlin (Zen4) [52] and might involve RacGTPase [53].

Mechanical forces are also involved at the multicellular level. Indeed, dividing cells in the morula centrally secrete viscous fluid, creating a central cavity inducing the cell aggregation called blastocyst in mammals [54]. The cell-cell adhesion also serves to mechanically couple cells, allowing long range transmission of forces as well as mechanotransduction. Understanding the mechanical regulation of cells thus became a topic of major interest and tools have been developed to describe this new piece of the puzzling picture of embryogenesis [5]. The study of cadherin, one of the most important proteins for dynamic regulation of cell to cell transmission of forces can be used as an example of the potential use of microscopy in mechanobiology also at different scale.

At the cadherin level, single molecule force sensor has been developed by inserting a coiled-coil spring like sequence separating fluorophore FRET pair into the cytoplasmic domain of E-cadherin between the transmembrane domain (enchoring the caderin to the membrane) and the β-catenin binding domain [55]. Thus, when cadherin is under tension, fluorophores are distant and the FRET signal is reduced compare to when the cadherin is relaxed. Measuring FRET images thus provides a map of tension applied to the cadherin in space and time.

At the cellular level, numerous surfaces functionalized with cadherin were developed to measure the cell interactions with it neighbors. For example, cells can be seeded on microneedles coated with cadherin. When the cell applies cadherin mediated traction forces, it will result in deflection of the microneedles. Thus imaging the microneedle subtract and measuring this deflection while knowing the rigidity of the needles provides a dynamic traction force map [56].

At the cell-cell interface, methods like the dual pipette assay are developed to measure adhesion forces between cells. Two cells are attached at the end of pipettes tips, brought into contact then moved away until breaking the junction to measure the separation force. For a view of long range multi-cell force, cell monolayer can be suspended between two mobile roads to measure the force needed for monolayer disruption [57].

Regarding polarity establishment in mammals, several models have been proposed, some of them integrating mechanical forces (cell shape, cell to cell adherence, cell polarity). The inside-out was first proposed by Tarkwosky and Wrobleska [58], in contrast to the pre-patterning model. In the inside-out model, the cellular fate is determined by the cell position within the segmentation mass : internal cells will provide the inner mass cells and thus, the embryo itself while the external cells are devoted to differentiate into trophoblast and embryonic annexes. Arguing in favor of the inside-out model, local changes in mechanical properties of the extracellular matrix were shown regulate the Hippo/YAP pathway involved in the differentiation either on trophoblast and epiblast cells [59]. The cell polarity model [60], involving cadherin distribution, completed the inside-out model. One has to note that undeniably, Gene expression can also be impacted by mechanical forces. Though improperly using the term of self-organization, an integrative model for the establishment of polarity axis and symmetry breaking in mammals has been proposed [61].

Conclusion and Perspectives

In conclusion, to get a full picture of the early developmental steps we have to built in our mind a picture taking into account the following points : (1) the maternal transcripts, inherited by the zygote break symmetry immediately after fertilization (i.e. amphibian) or before fertilization (i.e. drosophila). This first developmental asymmetry referred to the metaphor of molecular gradient or mosaic, where determinants have been polarized and disseminated, pre`uring the future zones of differentiated cells. Such metaphor is not difficult to envision for small molecules that may be shared through gap junctions, or segregated between the dividing daughter cells, but it begins to be tricky to understand when the message is delivered on a longer distance in the later embryo; (2) self-organized events, being major event, occur without being genetically encoded; (3) morphogens signals have to be interpreted, in a manner that has to consider morphogens dynamics itself as a signal [41,62]; (4) Cells are subjected to mechanical forces and tensions, which are translated and integrated into cellular decisions.

There is also an undeniable and unquestionable plasticity in the molecular network underlying the orchestration of early embryogenesis [63-65]. Through molecular network wiring and rewiring, different topologies may results in similar "signatures", novel properties being besides the sum of intrinsic properties of the network components may arise, and be integrated into the same cellular decision. Within these networks, enzymes have graded levels of activities, and more complex dynamics are now seek, like for kinases to understand their role in fundamental process such as cell division [66].

The morphological events of development and embryogenesis might be described and characterized in a sequential manner. Nevertheless, the underlying physical and molecular mechanisms are intimately intricate, and need, now, to be analyzed in an integrated manner, far from the reductionist approaches accomplished so far. We are in the need to integrate decades of experimental observations, with an explosion of new data brought by high throughput strategies. How can this be achieved? Even if genocentrism has agonized, we still need to get a clear picture of the epigenetic process by quantifying and determining (1) the set of organizers involved in the various cells clusters in space and time but also by quantifying and determining (2) the impact and the nature of the embryos environments, which is specific to each species. We are also in a need to use simple theoretical and technical tools to get more intelligible the plasticity of the early events. For example, from the reductionist approaches, self-organization has been observed and modelized at several levels, but we can question its physiological relevancy in cell. Another point is to determine the best way to measure the mechanical forces at work during early embryogenesis and to understand how the arising messages are integrated into cell fates commitments. At the end, we need tools and ways to integrate all these data together, thanks to systems biology development, that will seeds new & intelligible paradigms and metaphors.

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