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Embryology, from vertigo to vertigo^{1,2}

Jean-François Nicolas³

In the 1970s, the problem of embryo development was perceived as potentially insurmountable. Forty years later, molecular genetics elucidated the explanatory principles, and the emerging field of embryology demands a new synthesis of the Theory of Evolution. In this brief historical account of what transpired between these two moments, as I experienced it, I present the following questions: 1) What were the driving forces behind this narrative? 2) Does a new theory of development emerge, and where does it stand in relation to those predating this revolution?

¹ From the vertigo of **observing** the course of development to the vertigo in the face of the **astonishing results of experimental embryology**, to the challenge of explaining it, perceived in the 1970s as perhaps impossible to overcome, and finally to the vertigo in the face of the three answers obtained forty years later, of which two are probably **impossible to understand without the help of machines**.

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In the last century, biology was revolutionized by the emergence of molecular biology, which combined the disciplines of biochemistry (the science of molecules) and genetics (the science of inheritance and variability of Mendelian factors). The origin of this hybrid science can be traced back to early attempts to connect genotype and phenotype (see glossary for these terms) notably through the work of George Wells Beadle, Boris Ephrussi⁴, and Edward L. Tatum and their "one gene, one enzyme" hypothesis (Beadle and Ephrussi, 1936; Beadle and Tatum, 1941). Their method involved deducing the role of genes from the phenotypes of mutants. The achievements of molecular biology were remarkable (Hayes, 1970; Morange, 2020)⁵.

With these methods in the 1970s, François Jacob and Jacques Monod⁶ demonstrated that the products of certain genes, known as *regulatory genes* (see glossary) (Jacob and Monod, 1961; Monod and Jacob, 1961), and *certain proteins*, known as *allosteric* (see glossary) (Monod et al., 1965), perform logical operations capable of explaining complex biological phenomena. These discoveries marked a turning point (Morange, 1987).

Molecular biologists then turned their attention to developmental problems. Sydney Brenner created his genetic model, *C. Elegans*, a worm, F. Jacob chose a mammal, the mouse (Morange, 2000; Morange and Peyrieras, 2002), George Streisinger the zebrafish, while others, favoring the biochemical approach, turned to amphibian models (J. Gerhart, D. Melton, R. De Robertis) or the sea urchin (E. Davidson). However, genetic analysis was impossible with the two latter models.

Before this turning point, "Drosophilist" geneticists had shown interest in development by studying curious mutants (Morgan, 1926), some of which were termed homeotic mutants (see glossary, homeotic transformation). The reason newcomers did not choose *Drosophila* was their belief that its study would not contribute to understanding vertebrate development (see glossary).

Meanwhile, established embryologists, following the remarkable successes of experimental embryology, were limited by the failure to characterize the "inducer" (see paragraph 2) and sought alternative approaches. These attempts largely failed, with the exception of Nicole Le Douarin's work, which, starting in 1969, would become one of the most brilliant chapters in this discipline (Le Douarin and Kalcheim, 2009; Le Douarin, 1969). Conrad H. Waddington, on the other hand, conceived the concepts of canalization and genetic assimilation (see

glossary for both terms) following James M. Baldwin and Ivan I. Schmalhausen (Waddington, 1962) (see also Gilbert, 1991b; Morange, 2009c). These concepts are still under discussion in theories and debates concerning the links between ecology, development, and evolution (Gerhart and Kirschner, 1997; Gilbert, 2021; Huneman et al., 2017; Wagner, 2005; West-Eberhard, 2003).

What exactly were these people trying to answer?

At the dawn of the molecular revolution, three articles appeared that left their mark. An article by R. Britten and E. Davidson (Britten and Davidson, 1969) on a theory of gene regulation, following that of Jacob and Monod, had a clear goal: « *Perhaps in this we are offered an insight into the elegance and the complexity of the regulation system, the understanding of which is now a major objective in the experimental study of gene action in development* » (Davidson, 1968), see also (Morange, 2009b). An article by Lewis Wolpert in which he proposed that cells in a developing system have their position specified, this non-genetic information determining their molecular differentiation (Wolpert, 1969). The goal was clear: to offer a solution to the question of how a cell is instructed to perform its functions based on its location. Finally, articles by Stuart A. Kauffman (Kauffman, 1969a; Kauffman, 1969b), aimed to construct formal models of genetic regulatory networks (see glossary). In 1975, Wolpert and Kauffman, along with Sydney Brenner, attended a meeting also joined by Antonio Garcia-Bellido. Garcia-Bellido presented a theory based on the concepts of clonal compartment (see glossary) and selector genes (see glossary). Note his conclusion: « *Discussion has been focused on the problem of how morphogenetic genes can operate to control developmental pathways... A hierarchy of genes may be involved in this process, and the operational characteristics of 'activator', 'selector' and 'realisator' genes have been described... It is hoped that analysis of cell interaction mechanisms, such as diffusion or cell contact mediated induction, will help to close the gap between the developmental and genetic approaches and lead to an understanding of morphogenesis* » (Garcia-Bellido, 1975). This illustrates the goals of researchers at the time; see also (Love, A.C., (ed), 2015).

In the 1990s, developmental biology claimed to have elucidated the explanatory principles of development and asserted a major role in the Theory of Evolution (Carroll et al., 2001; Gerhart and Kirschner, 1997; Gilbert, 2021; Kirschner and Gerhart, 2005; Newman and Müller, 2003; Raff, 1996; Wagner, 2014; West-Eberhard, 2003), etc., following (Jacob, 1977; Raff and Kaufman, 1983), with, admittedly, contrasting interpretations (Morange, 2011).

⁴ Boris Ephrussi (1901-1979) held the first university chair in Genetics in France in 1946. His pioneering contributions to biology are numerous.

⁵ Michel Morange, Director of the Cavailles Center for the History and Philosophy of Sciences at ENS, is one of the most brilliant

philosophers of the history of biology in general and molecular biology in particular.

⁶ Jacques Monod (1910-1976) and François Jacob (1920-2013), researchers at the Pasteur Institute, are the founding fathers of molecular biology.

What follows is a personal history of what happened between these two moments, as I experienced it (not as a historian or philosopher of science) that I present. The questions are: 1) What were the driving forces of this narrative and how did the different systems interconnect? This is addressed in the section titled: "What has developmental biology found?" 2) What new theory of development follows from this, where does it stand compared to those before this molecular revolution? These questions are explored in the sections titled: "Has the new embryology answered experimental embryology?" and "Elements of a theory of development?". I conclude with a metaphor, "The three languages of the embryo."

1. WHAT HAS DEVELOPMENTAL BIOLOGY FOUND?

From the actors of the 1970s, *Drosophila* geneticists, joined by molecular biologists in the 1980s and mathematical modelers in the 2000s, emerged victorious. The fly, which played a crucial role in establishing modern genetics at the beginning of the 20th century, triumphed once again at the end of that century. The development of *Drosophila* is by far the best understood, and by uncovering its universality, it provided other models with the means to translate its findings.

Let's present the essentials of *Drosophila* development as understood at the end of the 1990s.

1a. *Drosophila* Development Through the Lens of Molecular Genetics

Three key points to know. Firstly, insect development begins within a syncytial structure, where nuclei divide in the egg without cellular membranes. This greatly simplifies molecular exchanges between nuclei. Secondly, transcription factors (or TFs, the products of regulatory genes as per Jacob and Monod, see glossary) bind to specific DNA sequences (called cis-regulatory elements or CREs, see glossary) near genes, either activating or repressing their expression. Thirdly, insects have a segmented body, comprising, from front to back, the head, 3 cervical segments, 3 thoracic segments bearing legs and wings, and 8 abdominal segments.

1aa. Dividing the Embryo into Compartments

After fertilization, two TFs deposited as RNA during *Drosophila* oogenesis at the anterior (Bicoid) and posterior (Oskar) poles of the elongated egg diffuse and activate or repress other TFs, which, in turn, activate or repress additional TFs. This results in transverse sectors within the egg, where different TFs are expressed. These sectors consist of nuclei and then cells. After only 4 or 5 steps, these TFs activate selector genes, which impart identity to their respective sectors. They will control cell activities in these sectors even in the adult fly. These sectors are known as compartments. Three hours after fertilization,

gastrulation (see glossary) has not yet occurred, and the embryo comprises approximately thirty transverse (antero-posterior, AP) compartments and 10,000 cells. Simultaneously, from molecules present in the egg, dorso-ventral (DV) longitudinal compartments are defined by other activation/repression cascades (see glossary).

1ab. What Are These Compartments?

Compartments are defined by cellular boundaries that prevent cells from entering or exiting; the cells in the compartments form the foundational cells for the structures they will give rise to. They all share the same identity conferred by the expression of selector genes. Compartments are both cellular (founder cells) and genetic (selector genes) units of development.

These compartments do not align with the 14 segments of the *Drosophila* body. They define what has been termed parasegments in the larva. Their boundaries fall in the middle of segments, wings, halteres, legs, and other *Drosophila* organs. Selector genes thus determine the identity of half-wings, half-halteres, or other organs. Mutation of the selector gene *Bithorax* changes the identity of parasegment T3a to that of T2a, resulting in a fly with a structure that is half wing and half haltere (Fig. 1). Selector genes do not control individual organs or segments but territories.

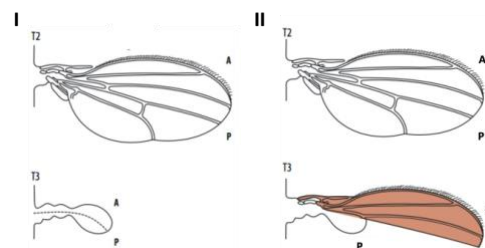


Figure 1: Compartments curiously dissect the fly; selector genes bestow identity upon them. T2, T3: thoracic segments 2, bearing the wing, and 3, bearing the haltere of the fly; A anterior compartment, B posterior compartment of these segments. The *Bithorax* gene specifies the identity of the anterior compartment T3, which constructs the anterior part of the haltere (I, T3). Mutation of this gene changes this identity to that of the anterior compartment T2, which constructs the anterior part of the wing. I) T2 and T3 segments of the normal fly. II) T2 and T3 segments of the mutant fly. It exhibits a baroque thoracic segment T3, half wing and half haltere.

1ac. Subdividing Compartments through Signaling Pathways

Cells within a compartment all share the identity conferred by their selector gene. Their allocation to the structures within the compartment occurs by subdividing it into new territories characterized by new TFs. The mechanism that makes the cells of the compartments different is as follows: cells at compartment boundaries secrete substances received by surface proteins of neighboring cells, activating a specific TF in those cells. This TF, in turn, activates new genes. In this process, the secreted substances are ligands (see glossary) for transmembrane receptors that modify internal proteins of the cells carrying them,

ultimately activating a specific TF, which then activates new genes. These components constitute a signaling pathway (SP, Fig. 2 and see glossary), which transduces, transforms, a received message into a genetic response (message transduction). Specificity in the response results from the combination of the SP's TF and the TFs in the receiving cells. Signaling pathways are the means to introduce heterogeneity into a group of identical cells. This is how the biggest problem (in my opinion) of development is resolved.

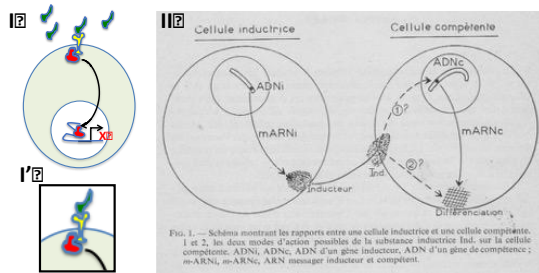


Figure 2: Signaling pathways transform a received message into a genetic response. I, I': The substances secreted by the inducing cell are ligands (in green in I and I') of transmembrane receptors (in yellow in I and I') that modify internal proteins (in red) of the competent cells that carry them and ultimately a specific TF (also in red in I), which then activates new genes X. II: As early as 1967, Étienne Wolff⁷ had imagined that induction could be a mechanism of this nature.]

1ad. An Example

Cells within the territory that will form the wing belong to two compartments, anterior (A) and posterior (P), delimited by a clonal boundary. Cells at the clonal boundary of the posterior compartment express a TF, *Engrailed*, which activates the expression of a signal, *Hh*. *Hh* is secreted and activates the *Hh* signaling pathway (SP) in anterior compartment cells, which then activates the expression of the *Dpp* signal. *Dpp* is secreted and diffuses into both A and P compartments. At high concentration around the boundary, *Dpp* activates the expression of the *Spalt* TF, and at low concentration, it activates the *Omb* TF. Thus, three regions are created -one central (spanning both compartments) and two peripheral. However, the wing territory is also divided into dorsal and ventral compartments. The selector gene *Apterous* (a TF) determines the dorsal identity. It controls the Notch/Delta signaling pathway, which in turn controls the expression of the Wingless signaling pathway at the dorso-ventral boundary. Wingless, like *Dpp*, subdivides compartments D and V. There are five signaling pathway interventions in just this initial phase! This pattern of activating new TFs in new territories through cascades of signaling pathways is universal and recurs in all other *Drosophila* compartments.

1ae. Forming an Extra Wing Through Signaling Pathways

To appreciate the importance of these signals, let's activate *Hh* in cells of the anterior compartment using genetic techniques. An extra wing forms (Fig. 3, II): *Hh* activates *Dpp*, which plays its role as a morphogen (see glossary) (Zecca et al., 1995).

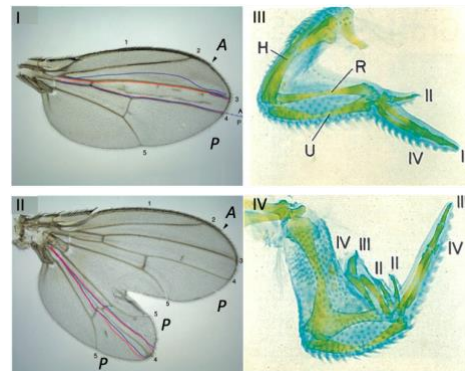


Figure 3: Formation of an extra wing through signaling pathway action. I, Normal wing, A anterior compartment, B posterior compartment. II: Forced expression of the *Hh* signaling pathway ligand in the posterior compartment of the wing activates the development of an extra wing in that compartment. *Hh* activates the expression of *Dpp*, similar to what happens at the A-P clonal boundary. The activation respects the "posterior" genetic identity of the cells, resulting in an extra wing that exhibits a "PP" mirror symmetry. The normal activation, which occurs at the boundary of compartments A and P and propagates into both compartments, forms an A-P wing. (C, D) The same story with homologous activating genes for chicken wing development.

1af. The End of the Story?

Does this cover all the principles of *Drosophila* development? If, as widely accepted, compartments are indeed independent units, then there is nothing more to add. Furthermore, one of the major mechanisms discovered by experimental embryology, induction (see 2a and b and see glossary), has found its molecular translation in *Drosophila*.

Thus, Drosophila development can be summarized as follows: the egg contains determinants (TFs or elements of signaling pathways) that activate short cascades of TFs, ultimately activating selector TFs in territories called compartments, which dissect the embryo into independent cellular and genetic development units. These units are subsequently subdivided, as many times as necessary, until the organism's structures appear, through cell interactions (signaling pathways and others) that give these subdivisions their own identities (unique combinations of TFs).

1ag. A Brief Historical Overview of This Story

From 1915 to 1980: Compartments associated with selector genes. This episode ended in the 1970s (García-Bellido, 1975; García-Bellido et al., 1973; Lawrence and Morata, 1976), but the interpretation of

⁷ Étienne Wolff (1904-1996), an embryologist and professor at the Collège de France until 1974, specialized in teratology and was a mentor to Nicole Le Douarin.

parasegments dates to 1984 (Martinez-Arias and Lawrence, 1985; Struhl, 1984). The foundations were laid by TH Morgan, Bridges, E. Lewis with the *Bitborax* complex, and on the other hand, C. Stern and C. Tokunaga, P. Bryant. Their methods, including clonal analysis, mitotic recombination, gynandromorphs, demonstrated the power of genetics, leading to the establishment of a new experimental embryology described perfectly in (Lawrence, 1992).

The 1980s to 1990s: Unraveling the Genetic and Molecular Basis of Drosophila Development. *The genetic foundation:* Heroes emerged in the form of Nüsslein-Volhard and Wieschaus for saturation mutagenesis (see glossary) of larval segment formation genes. These genes encompass 1) maternal genes (of gap and pair-rule types) responsible for activating selector genes in compartments, and 2) segment polarity genes that structure these compartments (Nüsslein-Volhard and Wieschaus, 1980). The former are termed TFs (Transcription Factors), while the latter are genes of various natures organized into signaling pathways (SD). Through four saturation mutagenesis experiments, the major developmental genes were identified. The most astonishing revelation was their remarkably low number. It would take two decades to fully comprehend their roles and implications for the theory of Evolution.

The molecular foundation was established through molecular biology techniques: cloning and sequencing of genes, genomic and cDNA libraries, hybridization, and more. By the end of 1982, thanks to David Hogness's "chromosome walk," several labs had obtained cDNA sequences of *Antennapedia* (Garber et al., 1983) and *Bitborax* (Bender et al., 1983). Cloning selector genes revealed a conserved sequence, the homeobox (see glossary) (McGinnis et al., 1984b; Scott et al., 1983), suggesting that Hox genes bind to DNA and control the expression of other genes. It also became a tool for cloning other genes in *Drosophila*, such as *Engrailed* and genes that control selector gene activation from 1985 onwards, followed by *Bicoid* in 1989, and so forth. These genes constitute non-linear genetic networks of TFs. The first syntheses began to emerge around 1988 (Ingham, 1988).

Regarding the nature of TFs and CREs (Cis-Regulatory Elements): In the 1980s, molecular biologists discovered CREs in viruses and eukaryotic cells, demonstrating that these elements function at a distance (Banerji et al., 1983; Banerji et al., 1981; Gillies et al., 1983; Moreau et al., 1981).

The 1990s to 2000s: The Discovery of SP (Signaling Pathway). The primary challenge, in my opinion, was to understand the segment polarity genes (*Hh*, *Wingless*, *Gooseberry*, *Armadillo*, *Dishevelled*, *Porcupine*,

Fused, *Patched*, *Naked*, *Dpp*, etc.). It was a convoluted task that engaged numerous laboratories from various backgrounds, but it eventually elucidated the process of compartment subdivision into sub-compartments. These genes were eventually organized into SP, much like *Hh*, *Wingless*, and *Notch*. Solving this problem would not have been possible without saturation mutagenesis followed by gene cloning. For *Wnt*, see (Bejsovec, 2018); for *Hh* and *Dpp*, see (Tabata and Kornberg, 1994; Zecca et al., 1995). Comparing these two articles reveals the difference between genetic and biochemical approaches. To appreciate the challenges encountered in organizing these genes, note that, for example, the position of *Patched* in the *Hh* SP was only recognized in 1996 (Marigo et al., 1996)!

The saturation mutagenesis experiments by Nüsslein-Volhard and Wieschaus from 1980 to 1984 and the discovery of the homeodomain in 1983 in Drosophila were, in my opinion, pivotal moments in all of biology. Subsequent developments unveiled the molecular and cellular mechanisms of development, their universality, and revolutionized our thinking about genes, genomes, and Evolution.

1b: Development in Vertebrates as Seen Through the Lens of Drosophila

By 1984, *to everyone's surprise*, the homeodomain was found in vertebrates (Carrasco et al., 1984; McGinnis et al., 1984a)! A frantic race began. In 1985, the floodgates opened with the cloning of many *Hox* genes in mice. In a few years, vertebrate models, which had been in a slump since the 1940s, were inundated with genes exported from *Drosophila*, along with... its interpretations.

Between 1988 and 1992, it was established that, as in *Drosophila*, vertebrate Hox genes are organized into clusters, express in distinct domains along the embryo's AP axis from an early stage, and that their inactivation results in respecifications. In parallel, the discovery of *MyoD* in mice, the master gene (see glossary) for muscle differentiation (Davis et al., 1987; Sassoon et al., 1989), marked the beginning of understanding the associated genetic network (Buckingham and Rigby, 2014)⁸. In 1993, the second big shock came when vertebrate homologs of *Emx* and *Otx*, the selector genes for anterior compartments in *Drosophila*, were found in vertebrates (Boncinelli et al., 1995). In 1994, the third shock: the master eye gene, *Ey*, in *Drosophila* was discovered in vertebrates as *Pax6* (Quiring et al., 1994). The same logic of dividing the embryo into genetically independent territories of development, using the same selector genes, seemed to be at work in all bilateral animals since their origin!

But there was more. In *Drosophila*, cells in compartments are made distinct by SP activated at their boundaries. In the wing, *Hh* activates *Dpp*, which

⁸ Margaret Buckingham's laboratory at the Pasteur Institute played a leading role, especially in the challenging aspects of "embryos" in mice.

in turn activates new TFs in the A and P compartments, and Notch/Delta and Wingless act similarly in the Dorsal (specified by *Apterous*) and Ventral compartments (see 1ac). In 1993, vertebrate homologs of *Hh* were cloned (references in Ingham, 1994), and it was shown that in the limb bud, Shh activates the *Dpp* homolog (BMP2) posteriorly, while dorsally, the homologs of *Apterous* and *Wingless* (*Lmx1* and *Wnt7a*) act. The homologies extend to the proximo-distal axis with Notch/Delta (Fringe) and the TF Distalless. The structuring of limbs in vertebrates and wings in insects not only utilizes conserved genes but also **conserved mechanisms** and thus **homologs**. These mechanisms consist of the same combination of selector genes and several VDS, with the VDS themselves being multigene combinations. The concept of deep homology (see glossary) stems from this (Held, 2017; Shubin et al., 1997).

Finally, also in 1993, it was shown that Shh activation in the anterior part of the bud mirrors it (Fig. 3, IV) (Riddle et al., 1993)!

As at the end of section 1ae: Is this the end of the story? With induction having once again found its molecular translation, what more is there to add? Especially considering that this example is not unique. The SP-TF sequences involved in D-V partitioning in *Drosophila* and vertebrates are also conserved (Holley et al., 1995) (see 1be). The specification of light-sensing organs in mollusks, insects, or vertebrates by the same TF, *Pax6*, is equally impressive (Halder et al., 1995; Quiring et al., 1994).

Since 1985, Scott F. Gilbert, in his re-editions of "Developmental Biology," reports the revolution he had foreseen. In the 3rd edition in 1991, TFs had made their appearance, and in the 5th edition in 1997, the outlines of SP began to emerge. He noted then: "*This newly empowered science (Developmental biology) identified molecular mechanisms for the two most central processes of developmental biology: differentiation and induction.*" In 1998, "Principles of Development" by Wolpert was published. The first sentence reads: "*Developmental biology... deals with the process by which the genes in the fertilized egg control cell behavior in the embryo and so determine its pattern, its form, and much of its behavior.*"

2. HAS THE NEW EMBRYOLOGY ANSWERED EXPERIMENTAL EMBRYOLOGY?

Embryology was initially descriptive, with Ernst Haeckel's assertion, "ontogeny recapitulates phylogeny," serving as a stimulus for zoologists (Morgan, 1936). It later became experimental, starting in 1881, as attempts were made to understand its mechanisms in sea urchins and amphibians (Gilbert, 1991a; Sander, 1997). Its spectacular results (Driesch, 1891; Harrison, 1918; Hörstadius, 1939b; Spemann, 1901; Spemann and Mangold, 1924) added to the excitement of embryologists and led to the development of a range of concepts. However, chemical embryology, which aimed to synthesize tissue and chemical levels, was a failure (Bautzmann et al., 1932; Holtfreter, 1934; Waddington et al., 1936). The inability to decide between opposing concepts (such as Spemann's organizer versus Child's metabolic gradients) muddied interpretations. The results and concepts of experimental embryology (Gallien, 1958; Sander, 1997)⁹ remain exceptional.

Here, I argue for a thorough analysis of the history of these concepts, which were remarkably presented by Spemann in 1938 (including those from Driesch's *Fundamentalsatz*, 1892, and *Analytische Theorie der organischen Entwicklung*, 1894). Apart from those abandoned, some were directly assimilated by the new embryology, while others remain under discussion.

Among those assimilated are: induction (Spemann's action-reaction system), preformation/mosaic development, epigenesis, potentiality, determination, emancipation, topographic regulation, and essential regulation (see glossary for all these terms).

Among those under discussion are: morphogenetic fields¹⁰, local morphogenetic fields, organizing centers, organizers (see glossary for these two terms), gradients, physiological gradients, metabolic activity gradients, double gradient fields (vegetative and animal). Local morphogenetic fields do not coincide with presumptive or determined rudiments of organs; they extend beyond their boundaries. In Spemann's view, induction in morphogenetic fields becomes a "field action" that involves all physical and chemical elements that can play a role in development. Gradients oppose morphogenetic fields. They embody

⁹ Louis Gallien (1908-1976) held the chair of embryology at the Sorbonne from 1954. His book on experimental embryology provides a clear and comprehensive summary of the subject.

¹⁰ Morphogenetic fields: A concept of experimental embryology borrowed from physics (as was the concept of induction), aimed at explaining regulation and equipotency of its cells (the field is a harmonic-equipotential system: the whole is contained in each part, as per Driesch), induction (in Spemann's case, induction is a "field action"), and determination. The field is seen as *independent* (A. Gurwitsch) or *associated* (P. Weiss) with the material embryo. For Spemann, the field includes not only physical but also chemical factors. Until the 1950s-60s, the notion of fields remained

important among embryologists. Gallien speaks of "the region of the germ or organizational factors whose activity leads to the establishment of defined and specific territories, constituting a morphogenetic field." These organizational factors are related to the intensity of certain metabolic activities and cytoplasmic structural states. Organization in gradients ("gradient fields"), relative strength of fields, limits of organizing power surpassing those defined by organs, and regulation are characteristics of morphogenetic fields.

the idea that embryos have one or more axes linked to gradients of "something" that determine organs (Child, 1936; Hörstadius, 1952; Runnström, 1929). Sometimes, fields and gradients combine! Fields and gradients are higher-level entities than those associated with concepts from the first category. Further analyses would help identify the problems they address. The problem of "wholeness" as in Spemann and Driesch's concept of the "harmonic-equipotential system" is part of it (see 2c).

The analysis of these concepts should include the peculiar usage that was made of them during the period that interests us. After proclaiming that "words like induction, morphogenetic fields, and all the classical terminology are just bla-bla," Ed Lewis (see Ghysen, 2009) and (Galperin, 2000), that "Spemann's organizer set developmental biology back by 50 years" (in Eddy De Robertis - (Niehrs, 2021)), and that "Gradient was a dirty word in the 1960s and 1970s" (Lawrence, 1992), these same concepts, , along with others derived from them (position information, for fields, axis formation for gradients) were used, sometimes without justification, often with changing meanings and recklessly, and sometimes (like position information, for example) to the point of delirium. What's even more astonishing is that they still persist in textbooks and elsewhere!

Central to experimental embryology, induction and regulation, "the most striking phenomena of life" (Spemann, 1938). How does the new embryology interpret them?

2a. Induction

As indicated in 1ad, forced expression of *Hb* activates the development of an additional wing in *Drosophila*, and activation of *Sbb* duplicates the wing in chickens (Fig. 3, II and IV). Signaling pathways serve as agents of induction. Secreted ligands represent the action system, and transcription factors (TFs) of the signaling pathways, combined with TFs of the receiving cells, represent Spemann's reaction system. About ten signaling pathways intervene repeatedly during development. The concept of induction poorly suits this permissive activation mechanism (see glossary).

2b. The Spemann-Mangold Organizer

The most spectacular result of experimental embryology is the formation of a secondary embryo by grafting the dorsal lip of the blastopore (Spemann and Mangold, 1924), interpreted through the concept of the organizer (Spemann, 1938). The unachieved goal of chemical embryology was subsequently to elucidate this phenomenon.

The explanation in developmental biology is as follows: the organization of a second embryo is due to the secretion, by the organizer, into the extracellular space, of antagonists of Bmp and Wnt signaling pathways. By binding to Bmp and Wnt, which are produced by all cells of the embryo, these antagonists neutralize them. This establishes gradients of Bmp and Wnt distribution (low near the organizer, high further

away). Cells that no longer receive Bmp or Wnt signals respond to this absence according to their pre-programmed genetic circuitry. Regarding Bmp, the choices are binary: for ectoderm -Bmp, neural/+Bmp, epidermis; for mesoderm -Bmp, somites/+Bmp, lateral mesoderm. These choices structurally organize the embryo dorsoventrally. As for Wnt, the outcome is an anteroposterior structuring of the neural plate by the underlying mesoderm: -Wnt, corresponds to the anterior brain and anterior midbrain, +Wnt, leads to the midbrain and anterior hindbrain, and ++Wnt, results in the posterior hindbrain and spinal cord.

These elements fit into the framework proposed in 1ae. The organizer creates new territories within homogeneous territories. The cell's response involves the modification of transcription factors, such as Smad1 for Bmp, and beta-catenin/TCF for Wnt, leading to changes in cell phenotype. The action of the organizer is simple, but the *preprogrammed* responses of the cells are highly elaborate. These preprogramming factors include the specification of endoderm and ectoderm by maternal factors (VegT and Foxl1e TF), the induction of mesoderm from ectoderm by endoderm, and the positioning of Spemann's organizer by the superimposition of VegT, Xnr (nodal), and beta-catenin.

The organizer and organizing center are antagonistic secreted molecules of signaling pathways. They are neither organizers nor inductors! In the new embryology, they represent an example of double negative regulation: Bmp *inhibits*, through the Smad1p TF, neural and somitic development pathways, and the antagonists secreted by the organizer *inhibit the inhibitor*, Bmp, thus releasing these pathways (Gerhart, 2015).

We are far from Spemann's assumptions (Spemann, 1938): "*It (his use of metaphors from psychology) was meant to express my conviction that the suitable reaction of a germ fragment, ... in an embryonic « field, » ... is not a common chemical reaction, but that these processes of development, ... are comparable, to those vital process of which we have the most intimate knowledge, viz., the psychical ones.*" p 372. We are closer to what he wrote on page 369: "*If the most diverse kinds of tissues may be inductive, if formations of complicated morphological structure may be called forth by simple chemical substances, then almost the whole complication must lie on the part of the system of reaction ; but in consequence the conception of the organizer no longer applies to these cases. A dead organizer is a contradiction in itself*". It must be inferred that he was close to abandoning this concept (his book "Embryonic Development and Induction" refers not to the organizing center but to induction, of which he says, "their analysis affords a deep insight into the nature of animal development"). Neither embryology textbooks nor even some current actors, for example, (Anderson and Stern, 2016), have fully integrated the consequences of these results yet.

However, this relative simplicity of the "organizer" is, I believe, essential. It makes the embryo, at this stage,

more flexible. Changing the location or timing¹¹ of the signal can occur quite easily and alter the organization of the embryo without affecting its reactivity, which is independent of the signal. The neural plate and somites naturally find their new place in the new structure. In fact, the "shield" of the zebrafish, the AVE and "node" in mice, Hensen's node in chickens, which share properties, several antagonists, and probably the mode of action of Spemann's "organizer", adapt to very different conditions, as seen, for example, in (Bachiller et al., 2000). Perhaps for this reason, the exploitation of double negative regulation is frequent in development. It is a beautiful example of "weak regulatory linkage" that facilitates variation (see glossary), according to John Gerhart and Marc Kirschner.

2c. Regulation, « *the most striking phenomena of life* » (Spemann, 1938)

Regulation is the process by which a system subjected to disturbance reacts to tend towards achieving all normal morphogenetic performances (Gallien, 1958). It is topographical regulation in the case of eggs: each of the meridian halves produces a normal embryo (Driesch, 1891; Morgan, 1895; Wilson, 1893), or two embryos when fused, result in a single giant embryo (Driesch, 1910, see (Sander, 1997), (Mangold and Seidel, 1927). Driesch interprets these facts by postulating an equipotential harmonious system (Driesch, 1891). It is also essential regulation in the case of the sea urchin, where one meridian half and one animal half of the egg produce, through regulation with potential transfer for cells, a normal pluteus (Hörstadius, 1939a). Hörstadius interprets this by postulating two gradient fields, animal and vegetative, which mutually influence each other. There are multiple examples of regulation, from cnidarians to mammals. This process has not received much attention from the new embryologists, except for De Robertis in *Xenopus*, following his work on Spemann's "organizer" (De Robertis and Moriyama, 2016; Reversade and De Robertis, 2005).

But *Drosophila* once again took the lead, this time joined by modelers and computer simulations. These approaches were applied to the network, established through genetics, of extracellular proteins, Dpp, Sog, and Tld, which structure the dorsal region of the *Drosophila* embryo. They helped understand that the network's logic confers robustness (maintains its function, see glossary) against fluctuations. This robustness requires the diffusion of Dpp-Sog complexes, the action of a protease that cleaves Sog (Tld), and the absence of diffusion of uncomplexed ligands. This mechanism, called "transport (of Dpp)-shuttling by (Sog)," concentrates an excess of Dpp in a small region of the embryo where Sog is absent.

Predicted by modeling, it is experimentally confirmed (Eldar et al., 2002).

The genes *Dpp*, *Sog*, et *Tld* are conserved in *Xenopus* (they are *Bmp*, *Chordin* et *Xlr*), where they structure the dorsal region of the embryo (Spemann's "organizer," see 1b and 2b) with the same "transport-shuttling" mechanism. Furthermore, by introducing the Bmp-like ligand *Admp* into the model, which is present in *Xenopus* as well as in most bilaterians, it accounts for a new property of the network, the rescaling of the Bmp gradient. This automatic restoration of the gradient accounts for topographical regulation (Ben-Zvi et al., 2008).

A few remarks: 1) topographical regulations in *Xenopus*, perhaps in all chordates, can be explained by the robustness of the protein interaction network used to structure the embryo's dorsal region. The elements of this property, molecules - identified through genetics - and interactions, transport-shuttling and rescaling - identified through modeling and simulations - are known. It is interesting to note that "rescaling" is due to a particular molecule (*Adpm*). 2) Other embryology experiments that show regulation often test the robustness of a mechanism, many of whose elements are unknown. 3) But if these networks involve elements with conflicting actions, only the explanatory and predictive power of mathematical models will allow understand them; intuition will be ineffective. 4) Robustness, a fundamental concept for biological systems that persist despite natural genetic or environmental variations, applies to developmental processes.

The analysis of regulation cases in sea urchins (Davidson, 1989; Davidson et al., 1998) and "secondary morphogenetic fields" is, to my knowledge, not very advanced. As one can expect as many mechanisms as SP or combinations of SP involved, there is no doubt that modelers will take an interest in them.

From the 2000s onwards, embryologists' recourse to modelers became the norm. Systems biology (Alon, 2006-2020) and synthetic biology (Barkai and Shilo, 2020) became indispensable to them (Morange, 2009a). Embryologists are experiencing a new vertigo: machines that solve problems by testing countless possibilities! One last point I want to note: Driesch and Spemann's regulation, in finding its solution, has disappeared in favor of a mechanism, rescaling. The associated concept, robustness, covers much broader datasets that do not belong solely to embryology. This is how concepts die.

2d. Some Dates Related to the Elucidation of the Nature of the Organizer and Regulation

¹¹ In Evolution, it is called heterotopy or heterochrony.

This required more than 10 years under the unfavorable conditions characterizing *Xenopus* (lack of genetic approaches and far from the exactitude of its reasoning). The story involved several laboratories working with *Xenopus*, combining molecular techniques, cell assays, embryo injections, etc. (Bouwmeester et al., 1996; Cho et al., 1991; Hemmati-Brivanlou et al., 1994; Leyns et al., 1997; Piccolo et al., 1996; Reversade and De Robertis, 2005; Sasai et al., 1994; Wilson and Hemmati-Brivanlou, 1995; Zimmerman et al., 1996). The contribution of *Drosophila* (Eldar et al., 2002; Ferguson and Anderson, 1992) and then computer modeling and simulations (Ben-Zvi et al., 2008) was crucial.

2e. Concepts of the New Embryology?

Molecular genetics offers mechanisms for many concepts in experimental embryology. Therefore, the new embryology no longer sees their usefulness. By breaking down the embryo into simpler elements (see 3 and 4), it has shifted problems to other frameworks, such as networks with their own concepts. Furthermore, the "evolution" aspects that the new embryology addresses with the evolutionary developmental biology ("evo-devo") have caused more conceptual turmoil on the side of evolution than within embryology (Dickins, 2021; Huneman et al., 2017; Love, 2015; Pigliucci et al., 2010; Tobias et al., 2019). Does this partly explain the quasi-absence of explicit concepts in the new embryology? Those that remain: pattern (see glossary) as distinct from form, self-organization, gradient; for the network level: robustness, noise, flexibility, evolvability; for concepts that have been emptied of their meaning: positional information, information, genetic program, developmental genes, see also (Keller, 2004; Morange, 2020). New embryology uses metaphors extensively, like axis formation, formation plan, program, the dreadful "toolbox" in the context of tinkering, to poorly evoke modularity (see glossary), parsimony, and "weak linkage." In my opinion, it would benefit from abandoning these metaphors and redefining its concepts, which inevitably exist, and undoubtedly revisiting the crucial one: compartment, the cellular and genetic unit of development.

3. ELEMENTS OF A THEORY OF DEVELOPMENT

The three "bible" textbooks of developmental biology, "Developmental Biology" (S.F. Gilbert), "Principles of Development" (L. Wolpert), and "Essential Developmental Biology" (J. M. W. Slack), give an idea of the vast amount of

accumulated knowledge. However, strangely, none of them present a theory of development, even a partial one. The fact that developmental biology has not produced a theory is suggested in (Minelli and Pradeu, 2014; Pradeu et al., 2016), although see (Morange, 2014) for a different perspective. What a strange situation for a science without concepts that claims to be without a theory! But is it even conceivable that experimentation in developmental biology does not test theories, is not the consequence of theories? So what is the reality?

When Spemann published his Silliman lectures of 1933, the title was: "Experimentelle Beiträge zu einer **Theorie der Entwicklung**" ("Experimental Contributions to a Theory of Development"). In 1936, the English translation changed it to "Embryonic development and **induction**." Therefore, induction is what he acknowledges as contributing to a theory of development. When Morgan wrote in 1934, in "Embryology and genetics,"¹² *"The common meeting point of embryology and genetics is found in the relation between the genes, and the protoplasm of the cell where the influence of the genes comes to visible expression...It is known that the protoplasm of different parts of the egg is somewhat different...The initial differences in the protoplasmic regions may be supposed to affect the activity of the genes. The genes will then in turn affect the protoplasm which will start a new series of reciprocal reactions. In this way we can picture to ourselves the gradual elaboration and differentiation of the various regions of the embryo."*, everyone saw it as one of the earliest theories of development. When in 1975 Garcia-Bellido discussed his results (see the introduction), isn't that once again proposing a theory of development?

Induction, these "determinants" in the egg, these genetic hierarchies, and the mechanisms of cell interactions have been deciphered. I have recounted "my" history here.

I argue that developments only require three conditions: a primary condition, processes to create diversity within a cluster of cells, and two ancillary conditions (already realized in the unicellular ancestor), regulatory genes, and cell proliferation. Together, they form the basis of an explanatory theory of all developments, whether they start from a fertilized egg or a group of asexual cells, as in blastogenesis, regenerative development, or even neural crest cells (see glossary). This theory identifies cell interactions as the innovation at the origin of developmental emergence, and then their driving force. They are the primary condition because they underlie the embryo's structuring ("patterning") and morphogenesis, with regulatory processes and genes as the executors.

The processes to create diversity among cells are cell-to-cell signals, transduced into genetic signals (SPs are

¹² Translated in French ("Embryologie et génétique") as early as 1936 by Jean Rostand (1894-1977), a distinguished member of the French Academy and an exceptional popularizer, with a pristine style, of genetics, biology, and their societal implications.

part of this). Genetic signals regulate other regulators (FTs and CREs are part of this). Interactions of this kind between different cell groups gradually generate an increasing number of territories (compartments in insects) that structure (pattern) the embryo. Each type of signal corresponds to a type of structuring, and signals can combine. The SP Notch does not produce the same types of structuring as SPs Bmp, Wnt, or FGF, which themselves do not achieve what the Notch-FGF combination accomplishes. This is how the only real problem of development is solved: creating diversity among the cells of a group.

In each territory thus created, regulators set the activity state of the cells, which corresponds to a specific state of activity in their genetic network. The global organization of this genetic network is produced by all regulators (FTs and CREs are part of this). It determines all possible activity states.

By the end of the process, specialized cells of different types (muscles, neurons, keratinocytes, etc.) express different combinations of regulators that control genes characterizing their function (myosins, neurotransmitters, keratins, etc.). Their position in the organism and their morphogenesis result from both all the signals they have received (from their history of cell interactions) and their successive behaviors (from their history of genetic activity states). Morphogenesis gradually emerges from these two histories.

Finally, as an organism is a cluster of cells, cell proliferation accompanies its development.

This theory of development, which is the production of differences among cells in a cluster through messages they exchange and transduce into genetic signals, specifying distinct activity states of their regulatory network, aligns with the simplicity of life: the inexact reproduction of molecular structures performing chemical reactions by borrowing energy and molecules from the external environment (Morange, 2010), and evolution: phenotypic variation, differential adaptation, and heritable adaptation (Lewontin, 1970), if we restrict ourselves "to the pillars that together are responsible for the emergence of all other characteristics" (Morange, 2010).

Having an explicit theory of development has multiple advantages. It allows for the logical ordering and classification (see 4.2) of the multitude of information on the countless topics that have been addressed over the past forty years. It provides a solid foundation for addressing the problem of the emergence of developments 600 million years ago and their evolution. Finally, for the novice, it is the thread to navigate the incredible labyrinths that developmental biology textbooks have become.

This theory, when extended to the evolutionary developmental biology, should be able to propose plausible mechanisms for variation, novelty, and innovation (see glossary for these three terms), major concepts in evolutionary theories.

4. CONCLUSION, THE METAPHOR OF THE THREE LANGUAGES

4.1 In the 20th century, *Drosophila* was the paradise of genetic developmentalists, while vertebrates were their hell.

Drosophila was a miracle. Besides being ideally suited for mutagenesis screens, it had other characteristics that proved incredibly advantageous: 1) a simple genome without the two massive duplications that occurred in vertebrates. Genetic redundancy was low, mutants had clear phenotypes. 2) its syncytial blastoderm: in the absence of membranes, molecules had more direct access to nuclei. This tremendous simplification allowed the deciphering of the non-linear cascades of FTs that lead to selector genes. Other developments involve cellular interactions or invariant lineages. 3) its long germ band, which greatly simplified the gene activation process as it occurred without temporality. Again, an extraordinary simplification. Consider that, as I write, we still do not know how, or precisely in which cells, Hox genes are set up in the mouse (Deschamps and Duboule, 2017; Wymeersch et al., 2021). 4) giant chromosomes, which allowed the cloning of genes like Bithorax, Antennapedia, Engrailed, using Hogness methods. 5) imaginal discs, perfectly isolated units of natural development. 6) the minute mutation (Bridges, 1919), without which defining compartments would have been very difficult (Morata and Ripoll, 1975). 7) wings and eyes: two easily observable organs. The first mutants discovered (Morgan, 1926) included 22 out of 85 affecting the wing, including Notch (VSD), Apterous (selector gene), Fringe (VSD Notch), Vestigial (selector gene), and 15 affecting the eye, including Eyeless (selector gene), Furrowed, Deformed, and White. By 1915, several of the genes involved in development were already isolated. *Drosophilists* had quite an advantage!

4.2 The Three Languages of the Embryo

Between cells: This retrospective shows that before the great molecular revolution, the problems had already been posed. In 1975, García-Bellido suggested cellular interactions as the solution to morphogenesis. We now know that local and long-distance cell-cell interactions structure the embryo, defining an ever-increasing number of territories. The dozen SPs used and reused in animal development constitute the words of the first language, that of cells interacting with each other. In conventional metaphors, we say that with this language, the embryo generates a plan of formation, becomes geometric, etc., meaning that it defines territories.

Between regulators: That genes can interact with each other was recognized by a few. For example, in 1961, before Monod and Jacob, Waddington wrote, after discussing feedback: "I am mentioning these excessively simple models only to show that the interaction of gene activities to form organized systems does not involve any very surprising or

unknown properties, but is something which we have a perfect right to expect" (Waddington, 1961). Similarly, García-Bellido had seen that selector genes were elements of a hierarchy, and Kauffman and his early global gene regulatory networks (Kauffman, 1971). We now recognize the role of genetic hierarchies and network motifs in the vast network formed by FTs (at least 1700 in humans) and other regulators (RNAs). These arrangements give rise to functions (memory, clock, oscillators, etc. (Alon, 2006-2020; Peter, 2020)) that endow territories with particular properties. The second language, with its alphabet as regulators (FTs and CREs), words as network motifs, and text as the genetic regulatory network determining the countless activity states of cells in the territories.

Between molecules: In cells, dozens of genes are activated or repressed at any given moment. Their products, which individually or in interactions constitute their behaviors (migration, adhesion, shape, division, apoptosis, etc.), cause cells to change shape, tissues to move, and rearrange (Jamie, 2013). Gastrulation, neurulation, etc., depend on this (Solnica-Krezel, 2020). These molecules, many of which are structural proteins such as tubulins, cadherins, actins, myosins, etc., are modules and constitute words of this third language.

The embryo simultaneously mixes these three languages, nested within each other, determining tissue, cellular, and molecular dynamics that create organisms in an apparent cacophony, much of which remains to be deciphered!

From one vertigo to another¹³.

¹³ For those interested in a comprehensive and accessible description of development principles from the perspective of the new embryology, I refer you to my work, "La Fabrique des Organismes," available for download in PDF at <https://hal.archives-ouvertes.fr/hal-02890738v2/document>, (version 3, in english, "The Making of Organisms", coming soon); for a more in-depth view, see "Principles of Development" by L. Wolpert, 2019, and for a very in-depth view, "Developmental

Biology" by S.F. Gilbert, 2020. Finally, "Molecular Biology of The Cell" by Bruce Alberts et al., 6th edition, 2014, or 7th edition, 2022, is the unparalleled reference that answers all questions about molecular components, levels of organization, structure, functioning, and properties of isolated cells and in the context of organisms. The chapter on development, like the others, is remarkably clear.

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GLOSSARY¹⁴

A

Activation (Molecular Biology): Induction of gene transcription. See Repression.

Activation, permissive, instructive: An activation (induction, signaling, interaction) is considered permissive when the recipient's response is already constructed but repressed, and activation only releases the repression. It is instructive when the recipient's response is not pre-constructed, and activation provides information to construct the response. Embryonic inductions are permissive interactions. The induction of beta-galactosidase by lactose is also a permissive process.

Allele: One of the forms of a gene; in diploid organisms, each gene has two alleles, which may be identical or different, one on each of the two homologous chromosomes.

Allosterism, Allosteric Proteins: Allosteric proteins have, on their surface, in addition to their active site that binds their substrate, an allosteric site that binds activators or repressors of the active site; it is a site for regulating protein function. These proteins have two possible conformations (caused by the binding or absence of the regulatory ligand) that differ in the degree of activity of the active site.

C

Canalization: The reduction of developmental plasticity (flexibility) that makes an adaptive phenotype* resistant to environmental and genetic perturbations, preventing it from deviating from the phenotypic optimum. Canalization is a consequence of genetic assimilation*.

Central Dogma of Molecular Biology: A dogma stating that the flow of information can move from DNA* to RNA* and from RNA* to proteins*, but not from proteins* to RNA* or DNA*.

Cis-regulatory Elements (-region, -sequence, -module) (CRE): A DNA* sequence near a gene* on which transcriptional regulators* (repressors or activators) can bind to control its transcription* level. The "control regions" of a gene contain many CREs organized into modules. It is called cis-regulation of genes.

Cloning (Molecular Biology): The isolation of a piece of DNA (a gene, a regulatory sequence, etc.) from the rest of the genome; it is accompanied by the amplification, to billions of copies, of that DNA fragment.

Compartment (in Embryology): An independent developmental unit containing all the descendants of a small group of founder cells, the founder cells of this embryonic compartment, and only them. In the compartment, a specific selector gene* (or combination) dictates its identity (behavior, destiny, properties) by regulating the activity of other genes. At its borders, the compartment expresses one or more signaling proteins (SPs*) used to subdivide it.

D

Determination: For experimental embryology: a phenomenon at the end of which the destiny of the rudiments (in morphogenetic fields) is fixed, these rudiments now being capable of self-differentiation. Therefore a determined rudiment (or a cell) when transplanted into a non-neutral environment, such as grafting into other regions of the embryo, develops according to its normal fate; at this stage, it is no longer reprogrammable, and its destiny is fixed (it is the emancipation of the rudiments, the parcellation of P. Weiss's germ); but when a field has acquired its determination, its parts are not determined. Cyto-differentiation or differentiation follows determinations. Concept adopted by the new embryology: an assumed irreversible change in cell state that fixes their destiny. This process is supposed to follow specification, but often the distinction between the two is not made. See also Specification and Differentiation.

Deep Homology: Historical continuity (homology), of regulatory genes and the circuits they form, whether the morphologies they control are homologous or not.

Development: The discipline that studies, in multicellular organisms, the processes of their construction from the egg, metamorphoses, regenerations, and the renewal of organ cells (from so-called stem cells).

Differentiation: A concept common to experimental embryology and the new embryology: the process by which a cell becomes a particular mature cell type (muscle, neuron, keratinocyte, lymphocyte, etc.) after being determined. See also Specification and Determination.

DNA Sequencing, RNA Sequencing, Gene Sequencing, Genomics, etc.: Determination of the nucleotide sequence (a sequence of A, T, G, C, or A, U, G, C) of genes, genomes, DNA, RNA, and protein sequencing is the determination of their peptide sequence (a sequence of amino acids linked by covalent peptide bonds).

Drosophila, Drosophila melanogaster (black-bellied): Also known as the "fruit fly" or "vinegar fly," is an insect (bilaterians, arthropods) of the order Diptera; has been used as a laboratory model since the late 19th century.

E

Embryology: The study of developmental processes. It has successively been descriptive and comparative (19th century), experimental (early 20th century), chemical, molecular (from 1980, Developmental Biology), and then molecular and systemic (early 21st century referred to here as "new embryology").).

Epigenesis: The doctrine that development gradually forms structures in each generation through a series of stages. See Preformation its antonym.

Epigenetics: The study of heritable phenotypic changes that do not involve alterations in DNA sequence but result from various mechanisms, including cytosine methylation, histone acetylation or methylation, changes in DNA compaction, and the destruction of transcripts by small

¹⁴ English Adaptation of part of a Glossary to be Published in the Special Issue "Biology and the Molecular Revolution: Selected Narratives" in 2023 in "Contemporary Research History," CNRS Editions.

RNAs*, among others. These modifications can have environmental causes or be part of the organism's normal development.

Essential Regulation: Example: Half of a meridian plus half of an animal of an egg gives a normal embryo through regulation with an adjustment of cell potentials.

G

Gastrulation: The process by which prospective endoderm and mesoderm cells are displaced from the embryo's surface to its interior.

Gene: A region of DNA* that is transcribed into an RNA* unit carrying the information for a trait corresponding to a protein or that RNA. The gene is flanked by a regulatory region that contains binding sites (CRE*) for transcription factors*.

Gene Expression: The collective operations involved in producing a functional protein or RNA from genes. Activation* by transcription factors*, transcription* (production of an RNA copy), and translation (production of a polypeptide chain) are some of its steps, along with RNA splicing, maturation into messenger RNA, folding of the polypeptide chain into a protein, and activation.

Genetic Assimilation: The process by which a somatic response to an environmental stimulus becomes hereditary. The new phenotype can then be produced in the absence of the environmental stimulus. The success of assimilation depends on the genetic variation available in the population.

Gene Regulatory Networks (GRN): All molecular interactions of genes encoding transcriptional regulators (TFs, RNAs, and others) among themselves as defined by the genome (via CRE and others). Each genome corresponds to a single gene regulatory network, which is invariant, but it can have numerous activity states, each defined by a specific gene expression profile that depends on internal and external factors.

Genetic Assimilation: The process by which a somatic response to an environmental stimulus becomes hereditary. The new phenotype can then be produced in the absence of the environmental stimulus. The success of assimilation depends on the genetic variation available in the population.

Genetics: The study of the foundations of heredity and variations.

Genetic Engineering: A technology for modifying the DNA* of a cell or organism.

Genetic Screen: A technique to identify genes (or all genes in a saturation screen) involved in a given phenotype by screening a large number of mutagenized individuals. Example: searching for all mutants that alter the pattern of bristles on the larva of the fruit fly* (see Mutagenesis).

Genome: The complete sequence (a sequence of the four bases A, T, G, C) of an organism's DNA; it carries hereditary information. The human genome is a sequence of 3.4 billion bases.

Genotype: An individual's genetic constitution. In a narrow sense, it is the allelic combination of one or more loci.

Gradients, see Morphogenetic Fields.

H

Homeodomain (homeobox, homeotic box). A 180-base pair sequence that codes for a DNA-binding domain of transcription factors*, such as homeotic genes and "paired box" proteins like Pax6.

Homeotic Transformation: The replacement of one structure with another, such as the development of a wing in place of a haltere in the *Bithorax* mutant of the fruit fly.

Homology, Deep Homology In evolution, homology is referred to when a resemblance arises from a shared ancestor, and deep homology when non-homologous organs are controlled by homologous genes or regulatory circuits.

Hox Genes: A family of genes encoding transcription factors* that specify the identity of territories (compartments*) in the posterior half of the head and trunk of all animals. Each of these territories is characterized by the expression of a particular combination of Hox genes. They are selector genes*, master genes*.

I

Induction: In embryology: the process by which one group of cells influences the development of another group of cells. See Signaling Pathway*. In Biochemistry, induction refers to the initiation or enhancement of enzyme synthesis by a substance called an inducer. In both cases, induction can be instructive or permissive*.

Innovation: A radical modification of a pre-existing morphological character to which it is homologous. It does not require a change in the overall body plan (see Novelties*).

L

Ligand: A substance that binds to a protein at its binding site, such as ligands of membrane receptors.

M

Master Gene: A gene encoding a transcription factor whose expression is necessary and sufficient for the development of a cell type, tissue, organ, or even a compartment. It is generally expressed continuously and regulates the activity of many other genes. Myo-D, Neuro-D, Pax6, or the Hox genes are examples of master genes.

Modularity, see Module.

Module: One of the semi-independent units of a set. Elements within modules are more strongly linked (joined, attached, constrained) to each other than modules are to each other.

Modularity (of development, genetic networks, genes, their control regions, etc.): It allows changes in one module without interfering with other modules (dissociation), thus enabling cooption and duplications followed by divergence in evolution.

Molecular Biology: The combination of genetics, the study of the transmission and variability of Mendelian characters, and biochemistry, the study of molecules.

Molecular Cloning: A method for amplifying a DNA* fragment inserted in vitro into a bacterial vector capable of multiplying into a large number of copies in bacteria*.

Morphogen: A signaling molecule to which cells respond differently depending on its concentration, thereby generating a pattern. Morphogens specifically bind to receptors in signaling pathways* (SP) present on recipient cells.

Morphogenetic fields: A concept of experimental embryology borrowed from physics (as was the concept of induction), aimed at explaining regulation and equipotence of its cells (the field is a harmonic-equipotential system: the whole is contained in each part, as per Driesch), induction (in Spemann's case, induction is a "field action"), and determination. The field is seen as *independent* (A. Gurwitsch) or *associated* (P. Weiss) with the material embryo. For Spemann, the field includes not only physical but also chemical factors. Until the 1950s-60s, the notion of fields remained important among embryologists. Gallien speaks of "the region of the germ or organizational factors whose activity leads to the establishment of defined and specific territories, constituting a morphogenetic field." These organizational factors are related to the intensity of certain metabolic activities and cytoplasmic structural states. Organization in gradients ("gradient fields"), relative strength of fields, limits of organizing power surpassing those defined by organs, and regulation are characteristics of morphogenetic fields.

Mosaic Development: Development is considered "mosaic" when cells are autonomously determined, without interactions between them, based on the distribution of cytoplasmic determinants located in the egg. See also Epigenesis.

Mutagenesis: The exposure of cells or organisms to an agent that causes changes in their DNA.

N

Neural Crest (in vertebrates): The neural crest is one of the evolutionary innovations underlying vertebrate development. It appears at the boundary between the neural plate and the epidermis. During neurulation*, the cells that make up the neural crest migrate and give rise to various cell types, including bone and cartilage in the face and jaws, neurons and glial cells in nearly the entire peripheral nervous system, melanocytes in the skin and hair, etc.

Neurulation: The process of forming the neural tube (brain and spinal cord) from the ectodermal neural plate.

Novelty The appearance of a morphological characteristic that does not preexist (with no homology to any other characteristic) in common ancestors. It requires a change in the organizational plan (cf. innovation).

O

Ontogenesis: The development (the making) of an organism.

Organizer, see Organizing Center.

Organizing Center: The dorsal lip of the blastopore in amphibians (Spemann-Mangold organizer), Hensen's node in birds, the shield in fish, the AVE in mice; they have the

ability upon transplantation, of "organizing" a supernumerary embryo. In experimental embryology, the organizing center directs the development of the entire embryo. In the new embryology, it loses its status as an organizer, as the instructions are already in the responsive tissues that the organizing center only activates (see Permissive Activation*).

P

Pattern Formation (Patterning): The process by which embryonic cells form spatially ordered tissue arrangements.

Phenotype The set of an individual's characteristics, regardless of the observation scale. Phenotypic variation can be due to genetic and/or non-genetic causes.

Phylogenesis: The evolutionary history of a species, represented in the form of a branched tree of the species' ancestors.

Positional Information: The hypothesis that embryonic cells acquire positional values relative to field boundaries, conceived as a positional information field, specifying the nature of their differentiation. A concept developed by Lewis Wolpert to account for the spatial organization of cells.

Preformation An abandoned theory that suggests adult structures are already present in the egg or sperm See Epigenesis (antonym).

Protein: A polymer of amino acids linked by covalent peptide bonds. Proteins are major constituents of cells.

R

Regulation (Embryology): The ability to develop normally even after ablation, injury, or rearrangements. Essential regulation, for example, occurs when a meridian half plus an animal half of an egg gives, through regulation with potential transfer between cells, a normal embryo. Topographical regulation, for example, in eggs: each of the meridian halves produces a normal embryo, or two embryos when fused give a single giant embryo. See also Robustness.

Regulatory Gene: See Transcription Factor.

Repression (Molecular Biology): Suppression of gene activity.

RNA: Ribonucleic acid; a polymer of ribonucleotides. Messenger RNAs are responsible for translating the genetic message into specific proteins; ribosomal RNAs, transfer RNAs, microRNAs, piRNAs, siRNAs, etc., are other classes of RNA.

Robustness: The maintenance of a function or phenotype despite environmental or genetic changes.

S

Selector Gene: A gene encoding a transcription factor expressed alone or in combination with others in a compartment (territory) of an animal, determining its behavior and properties (destiny, identity) by activating or repressing other transcription factors and self-activating. See Hox Genes and Master Gene.

Signaling Pathways (SP), Cell Signaling, Signal Transduction: A signaling pathway converts an extracellular signal into an intracellular signal that triggers a cell response. The elements of SP include extracellular

signals, membrane receptors, a series of chemical reactions within the cell leading to changes in gene transcription or cell shape and motility.

Specification: A group of cells or a cell is specified if, placed in a neutral environment, it develops according to the destiny it would normally have; however, at this stage, it remains reprogrammable. Also referred to as the specification of a pattern, axis, or position. The distinction between specification and determination is not always made and is not always clear. See also Determination and Differentiation.

Systems Biology: An emanation of molecular biology that focuses on the properties of interaction networks. It brings together molecular biologists, bioinformaticians, physicists, and modelers.

T

Theory of Evolution: Phenotypic variation, differential adaptation, and heritable adaptation are the three pillars of the theory of evolution, responsible for the emergence of all other characteristics. This constantly revised theory has gone through three periods: Darwinism, which emphasizes natural selection, Neo-Darwinism or the "modern synthesis" that incorporates Mendelism, population genetics, and the central dogma of molecular biology, and the "post-modern synthesis" that incorporates epigenetics, self-organization of genetic networks (resulting in the incorporation of genetic assimilation, the Baldwin effect, and elements of neo-Lamarckism), and more broadly, bio-complexity.

Topographical Regulation Examples: Half of a meridian of an egg gives a normal embryo; two fused embryos give a single giant embryo.

Transcription (DNA Transcription): Copying one of the two strands of DNA into a complementary RNA sequence.

Transcription Activation: Induction of gene transcription* (see Repression). Transcription regulation can occur: *In cis:* This is a positional effect due to the insertion of a sequence (see Cis Regulatory Element) near the gene capable of enhancing its transcription (promoter-activator). *In trans:* In this case, a protein factor directly interacts with the gene's transcription machinery (transactivator).

Transcription Factor (TF), Transcriptional Regulator: A protein that binds to a DNA sequence (a cis-regulatory element or CRE) and regulates the transcription (activity, expression) of a gene. See Activation, Repression.

Transcriptional Regulators: TF, but also some RNAs.

V

Vertebrates: Vertebrates are primarily characterized by the possession of an internal skeleton composed of a skull and a vertebral column. Fish, reptiles, birds, and mammals are vertebrates. They are bilaterians (with bilateral symmetry), deuterostomes (with the mouth forming after the anus), and chordates (with a dorsal nerve cord). In comparison, insects, as well as crustaceans or nematodes, which are also bilaterians, are protostomes (with the mouth forming first) and ecdysozoans (with development occurring through molting). Sponges, jellyfish, or ctenophores are non-bilaterian animals.