

# The development of concepts on development

## A dialogue with Antonio García-Bellido

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### Introduction

The Dialogue is a traditional literary form with multiple uses, from the presentation by Plato of the philosophy of Socrates to the ruthless satires of Lukian or the defense of heliocentrism by Galileo. The present Dialogue is less of a fake than most of its literary predecessors. The authors actually dialogued in Rome in July, 1997, and those conversations served the same purpose as this text: to extract the views on the conceptual evolution of Developmental Biology from the sophisticated and specialized brain of Antonio García-Bellido and allow them to nest in brains unfamiliar with his experience. The solvent for this extraction had as its most active ingredient and buffer our common friendship, which celebrates this year its thirtieth anniversary.

The conversations were transferred to notes, from which I wrote a draft and had it corrected by Antonio. I was afraid that, unhappy with my rendering, he would require that I give pseudonyms to the speakers, as they used to do in the Renaissance, and I was quite prepared to call them Simplicio and Critilo, for example. Fortunately, he liked my text more than I expected. In any case, I bear the final responsibility for the defects and errors and he should receive full credit for his views.

ECO.-The subject of our talk, the development of multicellular animals, could be condensed in the middle of the 19th century to

the question of how a single cell produces an integrated body of different kinds of cells. This was made possible by microscopic observations that had produced abrupt changes of paradigm. According to the cellular theory (Theodor Schwann, 1839, and Mathias Jacob Schleiden), all organisms are made of cells. Even before that, the theory of germinal layers (Karl Ernst von Baer, 1827, and previous work by Christian Pander and Caspar Friedrich Wolff) had established the correspondence between tissues of the adult and layers of the embryo. Did it take long to propose a theory backed by experiments?

*AGB.-Active experimentation in embryology became a new science, Developmental Mechanics, in the hands of Wilhelm Roux. He founded the first research institute for this science (1889) in Breslau, now Wroclaw, and the first journal, Archiv für Entwicklungsmechanik der Organismen (1894), which is still being published.*

ECO.-The choice of name seems a tribute to the achievements and beauty of nineteenth-century Mechanics and, at the same time, a statement of faith: Clockwork Biology...

*AGB.-Such was indeed the attitude of Roux and the foundation of his scientific method: he tried to reduce vital phenomena to physical and chemical events with precise hypotheses that he expected to confirm or reject by cutting and pricking embryos and*

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*exposing them to various agents. Influenced by evolutionary thought, the school was very concerned with the diversity of life. Comparative Embryology provided convenient experimental subjects and supported general theories.*

ECO.-Developmental Mechanics was an academic success, with many active research groups, a pilgrimage temple in the zoological laboratory of Naples, founded by Anton Dohrn in 1873, and an American branch after Jacques Loeb and Edmund Wilson moved to the USA around 1890. Many concepts of developmental mechanics have survived a century: determination, causality, interaction, self realization, dependent and autonomous differentiation, determinant factors, realizers. But such a group could hardly remain united in the intellectual turmoils of that period.

*AGB.-Developmental Mechanics soon segregated its own opposition, which preferred the term Developmental Physiology. Hans Driesch, one of the best experimentalists of the group, was shocked by his own observation that cells separated from an early embryo formed a full animal. He concluded that living beings have an inner drive for completion which he called "entelechy", after a term from Aristotle. Driesch was a vitalist because he thought that entelechy could not be reduced to physics and chemistry.*

ECO.-He had a complex personality. He was very attracted by parapsychology. At the arrival of Nazism his leftist sympathies made him retire prematurely from his chair in Leipzig. Anyway, his use of a name like entelechy followed a common practice of the period. Text books were loaded with empty concepts such as "the cell responds to stimuli because irritability is a property of protoplasm", to mention a simple one.

*AGB.-Such terms may be useful if they incite to explore the underlying mechanisms. I have proposed recently an "entelechia model" to explain why the cells that build an organ keep dividing until the final size of the organ is reached.*

ECO.-Your model destroys the original concept, which was a general property of the embryo.

*AGB.-The predominant approach to development for many decades was holistic. Most mechanisms of development were believed to involve the whole organism or complex interactions between many elements. As biochemistry progressed, these elements were viewed as enzymes and hormones, then a general term for diffusible molecules. Cells were viewed as little more than bricks to be layered in the proper position.*

ECO.-Perhaps not bricks in a building, but sand in a beach. The size, density and aggregation properties of sand grains contribute to the shape of a beach, but grains have no inner drive to complete a beach; they are commanded by collective and outside forces, such as water currents, temperature gradients, gravity, waves and wind. These names remind me very much of those of holistic development: gradients, fields, positional information, inductions, interactions. The extreme opposite would be an Academy of Sciences: a shapeless body of strong personalities.

*AGB.-The cells would have very strong personalities if they differed in their hereditary information, as suggested by Roux and by*

*August Weismann in 1883. The genetic information of a cell would not always be passed on complete to its daughters, thus producing genetically different cell lineages. The hypothesis was supported by observations in Ascaris and many other animals that germline cells contain chromatin that is absent from somatic cells, but was weakened when differences in chromosome structure between the cells of an organism turned out to be exceptions, rather than the rule. The hypothesis was killed when molecular analyses confirmed that in general each cell of an organism contains all its genes.*

ECO.-A very egalitarian principle: all cells are born genetically equal. And a good lead for collectivist proposals on development.

*AGB.-They became common. From individual cells little more was asked than the housekeeping functions common to all eukaryotes and obedience to higher instructions to grow and multiply. These instructions could be physically located in the cytoplasm and vary in different cells because of initial heterogeneities in the cytoplasm of the zygote, as proposed by Theodor Boveri.*

ECO.-Whatever the mechanism, there was growing evidence for cell specialization, for example, from ectopic transplants of embryonic parts.

*AGB.-There is a fantastic degree of cell specialization and organization. This was shown very clearly, for example, in the nervous system by Santiago Ramón y Cajal around the turn of the century. Intercellular recognition was proven when dissociated cells from sponges (H.V. Wilson in 1907) or from parts of amphibian embryos (Johannes Holtfreter in 1939) reagggregated spontaneously in the original fashion.*

ECO.-In your hands, *Drosophila* flies turned out to be an excellent material for reagggregation experiments.

*AGB.-Weismann recognized that many larval cells perish during metamorphosis; the major structures of an adult fly, let's say, a wing, derive from small and specific groups of cells in the larva, the so-called imaginal discs. After being dissected from a larva, implantation in the abdomen of an adult fly allowed imaginal-disc cells to multiply, but not to differentiate. Implantation in other larvae led to differentiation. Ernst Hadorn showed that the final structure is predetermined, even if the implant is away from its usual location, and that changes of determination are rare. By dissociating imaginal-disc cells prior to the implant and by mixing cells from genetically-marked organisms, I found precise cell recognition and reassembly. In the case of *Drosophila melanogaster*, technical convenience is coupled with advanced genetic analysis.*

ECO.-In Genetics, holistic thought was replaced very early by quantal-combinatorial explanations. Let me mention a piece of work that seems to have been forgotten. Michel Sageret (1826) observed that melon hybrids present various combinations of parental characters, but usually no intermediate forms. He recognized the individuality and stability of hereditary characters and explained natural diversity through their combinations. He suspected the existence of "a type or template that contains all organs in germ, that sleeps or awakes, develops or not according to the circumstances". The terms type and template (*type, moule* in the

original), taken from printers and smiths, are an extraordinary anticipation of terms we frequently use for genes and DNA and his metaphor is an excellent description of regulated gene expression. Thus, discrete characters and combinatory appeared in Genetics much before Gregor Mendel and his rediscoverers.

*AGB.-Some developmental biologists, such as Hans Spemann, completely disregarded Genetics, but many of the major early contributions to Genetics were made by people trained as embryologists. Thomas H. Morgan was a specialist of invertebrate regeneration, traveled several times to Naples and other places in Europe, and was a friend of Dohrn and Driesch. After his successes in Genetics, he returned to the study of regeneration. Morgan's genetical research can be considered a long and successful digression, surprisingly exempt from developmental connotations. Late in his career he wrote the book Embryology and Genetics (1934), which presents both disciplines separately and states that they were not ripe for convergence, and wouldn't be for a long time.*

*ECO.-A convergence that was already being tried by Waldemar Schleich ("Entwicklungsmechanik und Vererbung bei Tieren", 1927) and Richard B. Goldschmidt ("Physiologische Theorie der Vererbung", 1927; "Physiological Genetics", 1938), although they threw in more clouds than light.*

*AGB.-It was certainly a merit to bring together development and gene action for the first time and to understand the unavoidable overlapping of their explanations. The proposed links were very complicated. Goldschmidt, under the name Phenogenetics, provided global, intuitive, non-reductive descriptions of how genes, endowed with autocatalytic powers, acted through hormones, enzyme products, and growth factors to determine the final phenotype of an organism. For most developmental biologists, particularly those working with Drosophila, the concepts of gene and gene action were diluted in a maelstrom of complex interactions. As a consequence, in the concept of canalization of Conrad H. Waddington (1962), the action of a gene was buffered by the actions of many other genes. Genes could well code for enzymes and structural proteins, but any state of an organism would be defined by enormous numbers of both. At each step, complex interactions of so many agents would define the next step in a cascade process. Experiments of induction and transplantation, particularly in the vertebrates, stressed the role of interactions in development.*

*ECO.-Genetic analysis should have clarified these complexities.*

*AGB.-Only to a limited extent. There were early indications that the phenotype of a cell was determined by its own genes. For example, Alfred Sturtevant (1929) obtained Drosophila gynandromorphs (mosaic flies composed of two cell populations, XX females and X males) from zygotes that were heterozygous for recessive mutations in the X chromosome; in most cases, mutant male cells exhibited the mutant phenotype, showing that they were not influenced by the neighboring female cells. The eye imaginal discs transplanted by Boris Ephrussi and George W. Beadle (1935, 1936) from mutant to wild-type larvae produced mutant eyes. The overwhelming weight of the evidence favored the cellular autonomy of gene expression, but this conclusion was blurred by a few results. For example, mutant pale testes transplanted by E.W. Caspari*

*(1933) from larvae of the moth, Ephestia kuehniella, to wild-type larvae became dark, like those of the wild type. The same lack of autonomy was shown by transplants of imaginal discs of vermilion and cinnabar eye-color mutants of Drosophila. These cases were explained by the absence in the mutant of a diffusible substrate that could be supplied by the wild type. The substrate missing in vermilion was shown by Adolf Butenandt and his coworkers (1940) to be a simple chemical, kynurenine, which is a precursor of eye pigments. One could think that integrated development implies the metabolism of many diffusible chemicals.*

*ECO.-Ephrussi then moved on to Saccharomyces and Beadle to Neurospora. The success of Beadle and others with the Genetics of metabolism must have triggered attempts to reduce development to metabolism, in the line proposed long before by Loeb, for whom development processes were essentially chemical processes.*

*In the early 1940s Donald Poulson found that genes act consecutively in development, much as they were being shown to act in metabolic pathways, but few people saw parallels between development and metabolism. On the contrary, developmental mutants were set apart from metabolic mutants. Hermann J. Muller proposed a classification of mutant alleles: hypomorphs, hypermorphs, amorphs (nullimorphs), depending on their level of activity in relation to the wild type, and antimorphs and neomorphs, if the mutant made something qualitatively different. For many people developmental mutants were incompatible with this classification.*

*ECO.-Muller was perhaps too much of an outsider, although his scientific achievements were impressive and numerous. He emigrated temporarily to the young USSR to set up a laboratory for Drosophila development in revolutionary Moscow ...*

*AGB.-Whatever his personality, his classification was easily understood by enzymologists. Developmental biologists lost interest in genes because they thought that various specific alleles, and not a standard wild-type allele, had been adjusted by selection to interact with various other alleles.*

*ECO.-Drosophila geneticists contributed to the confusion with their complex loci and obscure concept of the gene, not to mention the ridiculous nomenclature. Bacterial and fungal genetics had simpler views of genes and their relationships. When François Jacob and Jacques Monod described the first operon (1961), responsible for an instance of metabolic regulation, one could dream of connected operon circuits as the foundation of development.*

*AGB.-The dream turned into reality when genetic analysis of Drosophila showed that development is ruled by time- and space-dependant expression of regulatory genes. The first instances were homeotic genes, defined by mutants that change the developmental specification of a part of the animal. Some of these mutants, for instance the bithorax series, had been isolated in the 1920s by Calvin B. Bridges in the laboratory of Morgan, but their genetics remained obscure for more than forty years until the work of Edward B. Lewis.*

*ECO.-That was a revival period for Drosophila, when Seymour Benzer titled a review "Drosophila flies again".*

*AGB.-This was an obvious exaggeration: it had been flying all the time. A powerful tool in the discovery of the function of homeotic mutants was the ability to render homozygous, just in a single cell and their clonal descendants, a developmental mutation and an indiscreet cellular marker. It was then enough to look at the morphology of marked cells. Unexpected topographical restrictions were discovered. Cell clones expressing a certain homeotic gene kept together and did not cross certain boundaries. The embryo is divided in territories defined by specific gene activity. The homeotic genes were recognized as "selector genes", whose role is to order teams of "realizator" genes to act.*

*ECO.-These groups of genes seem to correlate with the "structural" and regulatory genes in operons. "Structural" is a poor adjective, since most of the genes it designates code not for structural proteins, but for enzymes. Let me call them "blue-collar" genes. Regulatory genes would then be the "white-collar" ones that give instructions to the others. White-collar jobs tend to be hierarchical everywhere.*

*AGB.-Above selector genes there are "activator" genes that respond to external cues. Activator gene products define the cells that will express selector genes. On the other end, realizator genes are a varied group and include those that determine the shape and size of final structures. This is largely mediated by gene products, located on cellular outer surfaces, that permit recognition and crosstalk between neighboring cells. The final view of an animal is a mosaic of cells that express combinations of different groups of genes. The mosaic changes with time as genes are turned on and off and cells multiply.*

*ECO.-To this point, the conclusions should be limited to *Drosophila*, or the insects, already suspect of being very different from us and the other vertebrates.*

*AGB.-The extension of the analysis to the molecular level and improvements in the genetic manipulation of other animals, including mammals, have shown, not only that developmental genes are highly conserved in all animals, but that they maintain*

*the essentials of their functions. Each gene has a standard wild-type allele; genetic polymorphisms do not play critical roles.*

*ECO.-Animals are then for you much less diverse than they look, since they all share the same basic developmental program. This is unsettling to people trained to classify animals in separate phyla, each with a specific fundamental structure, and to view evolution as a drawn-out process in which groups of animals replaced one another many times.*

*AGB.-Contrary to these views, the Burgess Shale fauna and other treasure troves indicate that all major groups of present day animals appeared within about 50 million years, in the Cambrian geological period, a little over 500 million years ago. The present developmental genes must have been already available, and in fact most of them have homologs in unicellular eukaryotes and even in bacteria.*

*ECO.-The developmental jump and the rapid diversification (assuming that they are not artefacts of fossilization or observation) and the prolonged maintenance seem hard to reconcile with the idea of evolution as a giant random walk, in which each step, taken with considerable statistical freedom, limits the possibilities of the next.*

*AGB.-There may be unsuspected invariants, constraints and rules in the development of multicellularity that limit the possibilities. The result, after all, may be more deterministic than historically contingent.*

*ECO.-In general terms, we are what we are because we couldn't be otherwise!*

*AGB.-In any case, animals resulted not so much from the appearance of new genes as from new combinations of pre-existing ones. Developmental Biology has become quantal and combinatorial. At the same time the old opposition between preformation and epigenesis has been replaced by a synthesis. Preformation is represented by DNA, not by the presence of a tiny adult in every sperm or zygote; epigenesis is represented by the successive cycles of specific gene activation.*