From Epigenesis to Epigenetics

The Case of C. H. Waddington

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ABSTRACT: One continuous thread in this volume is the name of Conrad H.
Waddington (1905–1975), the developmental biologist known as the inven-
tor of the term *epigenetics*. After some biographical notes on his life, this
article explores the meaning of the Waddingtonian equation and the con-
text wherein it was developed. This equation holds that *epigenesis + genet-
ics = epigenetics*, and refers in retrospect to the debate on *epigenesis* versus
preformationism in neoclassical embryology. Whereas Waddington actual-
ized this debate by linking epigenesis to developmental biology and prefor-
mation to genetics, thereby stressing the importance of genetic action in
causal embryology, today's *epigenetics* more and more offers the possibility
to enfeeble biological thinking in terms of genes only, as it expands the
gene-centric view in biology by introducing a flexible and pragmatically
oriented hierarchy of crucial genomic contexts that go beyond the
organism.

KEYWORDS: development; epigenesis; epigenetics; gene-centrism; genomic
context; genotype; phenotype; preformationism; Waddington

CONRAD HAL WADDINGTON (1905–1975)

In unraveling the original meaning of the term *epigenetics* in Waddington's
work, little attention will be paid to biographical elements. Nevertheless,
some noteworthy elements may help to situate this extraordinary figure.

Conrad Hal Waddington was born in Evesham, England, on the eighth of
November 1905, as the son of Hal and Mary Ellen Waddington-Warner. Until
the age of four, he lived on his parent's tea plantation in South India, after
which he went back to England to live with an uncle and aunt on a farm in
Sedgeberrow. From this uncle—a naturalist in heart and soul—Conrad ac-

quired a fascination for collecting fossils. Later, at the beginning of World War I, he moved to his grandmother's house, where he grew fond of an old mid-Victorian book on chemical and physical experiments. This led to his first experiments and a love for curious Latin and Greek names. Any spare time went into the erection of "Con's Museum" in a barn attached to his grandmother's house, where he exhibited his fossil foundings.[a]

These childhood interests remained vivid in his academic career, which started with scholarships in natural science and paleontology and extended over philosophy to poetry and visual art. In the 1930s, Waddington entered the field of embryology, where he concentrated on the study of induction. This brought him in close contact with embryologists like Hans Spemann and the Needhams. From 1947 onwards—the same year he was elected a fellow of the Royal Society of London—Waddington started from scratch to found and lead a genetics department in the Institute in Edinburgh. Quite soon, a degree in animal genetics was started, and by 1957 there was a full honors course in genetics. The department was highly successful and grew to be one of the largest genetics departments in the world. By the end of the 1950s, however, the Institute became more and more compartmentalized, and Waddington spent less time in Edinburgh, mainly because he planned to build an *epigenetics* laboratory. After receiving financial support from different sources, the Epigenetic Research Group—with Waddington as Honorary Director—was formed in 1965. However, the concept of epigenetics did not develop as Waddington had hoped. An important reason was, shortly after the laboratory started to operate, a huge flowering of molecular biology arose from the discovery of DNA and RNA hybridization techniques. Most funds went to this field, which had no immediate relevance to the topic Waddington wanted to investigate, which was embryological development.[1]

Waddington, having suffered from heart trouble in the years before, suffered a fatal heart attack outside his residential house on the 26th of September 1975, two months before his 70th birthday. Leaving a wealth of publications, filled with famous ideas, controversial standpoints, and wise observations, Waddington's legacy is overwhelming and still attracts many people.[2] He is rightly recognized as having coined the term *epigenetics*. To do justice to his many original ideas, it is necessary to investigate exactly where his legacy with regard to epigenetics stands today, and what has been left behind. Or, as Waddington himself said: "the work of the biologists in past centuries laid the foundations on which we have to build, and it is worth having a short glance at it to discover the points of lasting value it contains."[3]

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[a]In 1928, when his parents returned permanently from India, Waddington lived in Cambridge and was already married to a woman named Lascelles, who had strong artistic leanings. The marriage resulted in a son, Jake, who became a professor of physics, and ended in 1936. The same year, Conrad engaged himself in a second marriage with Justin Blanco White, an architect with whom he had two daughters, Caroline and Dusa, and which lasted until his death in 1975.

WADDINGTON'S EPIGENETICS: GENES ARE NECESSARY!

Bringing Genetics into Embryology

An often forgotten aspect of the original concept of epigenetics is that Waddington promoted it while stressing the importance of genetics as an underlying unifying factor in biology. Already in his 1939 student's handbook *Introduction to Modern Genetics*, he stated that the connection between genetics and other branches of biology, such as cytology, embryology, evolutionary theory, and cell biology, is much closer than is often admitted, and that "the boundaries between these subjects deserve less attention than is usually paid to them."[4]

Today, as the media has their mouths full of genes, genetic modification, and the Human Genome Project, this statement seems to be upside-down. In the 1940s, however, genetics was just a beginning science in which *a gene* was assumed to equal a unit of heredity, without being fully materialized.[b]

The gene concept, lacking a physical identity, was used with caution in embryology, because (a) genetic information on the embryological model organisms, like amphibians, was practically nil; (b) genetics had shown that alternative developmental pathways could be followed, despite an identical genome, making the genome not a very decisive factor; (c) embryology concentrated mainly on cytoplasmatic factors outside the realm of the nucleus; and (d) inside the nucleus, the chromosomes as a whole seemed to present themselves as a candidate chemical unit that influences the activity of separate genes.[c]

In the 1960s, Waddington revived the idea that chromosomal structure (presenting itself as a structurally coherent entity on a supra-molecular level) had a functional advantage over a system of separate genes, namely a refined control of activity.[9] Hereby, older work on chromosomal control of gene expression regained interest. Alfred Sturtevant's discovery of position effect

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[b]Although the Swiss biochemist, Friedrich Miescher, discovered the DNA substance in 1869, it was not until 1952—when Alfred Hershey and Martha Chase conducted their famous experiment with labeled bacteriophages—that DNA (and not protein) was accepted as the material basis of heredity.[5] Up until 1962 it was problematic to physically define one gene from another. Waddington suggested the possibility of crossing-over as the best way to distinguish genes. However, "one difficulty with this definition is that certain regions of chromosome are known … in which no crossing-over occurs. Further, … shall we be satisfied if no crossing-over occurs in one thousand individuals, in ten thousand, one million, or how many?"[6] Chromosomal breakage by x-rays or mutagene chemicals were equally dissatisfying in defining a gene, since it was not clear if breakage occurred in between genes or within a gene. In the following years, a more precise biochemical analysis of the DNA double helix followed, as well as better insights on transcription and translation.[7,8] More and more, the gene concept became restricted to a specific DNA sequence, whereas the genome was seen to contain life's blueprint.

[c]In 1939, C. D. Darlington speculated that chromosomal duplication helped during cell division. Although by 1958, because of a lack of experimental evidence, he renounced his hypothesis in favor of the idea that chromosomes are merely the result of spontaneous DNA polymerization.

variegation[d] in *Drosophila* in 1925, as well as Barbara McClintock's study of controlling elements acting on nearby chromosomal parts, promised to be excellent examples. Although the exact processes were not known, chromosomal structure was more and more considered a crucial context for gene expression. The interaction between different genes on these chromosomes came permanently under Waddington's interest.

Although still being theoretical entities with hypothetical characteristics, genes played a crucial role in models on evolution and heredity, as the Modern Synthesis of the 1920s had shown. Waddington now focused on implanting this extended genetics into embryology, a field that was excluded from the Modern Synthesis because it lacked an overall theory of development.[e] According to Waddington, the Synthesis should be build not only on the ultimate slow evolutionary process of natural selection and the less slow genetic process of random mutation, but also on once-in-a-life-time and rapid physiological processes, leading to a renewed appraisal of reproduction and development. By bringing genes into development, not only would embryology gain more clarity than biochemical concepts such as organizer, gradient, competence, and embryonic field could provide,[f] but also genetics would move from its static position[g] and become "the science whose labors are devoted to the elucidation of the phenomena of heredity and variation; in other words to the physiology of Descent."[11] In trying to convince an audience of

[d]Position effect illustrates the inactivation of genes through a relocation of those genes to a chromosomal site that is densely packaged. Waddington held this inactivation as a mutation to an inactive allelomorph, as in certain cases it occurs in germinal tissue and breeds true in the inactivated form. He did not agree with McClintock's hypothesis that cellular differentiation might depend on gene-mutations controlled by some mechanism of this sort, involving an interaction of heterochromatic and euchromatic segments of the chromosomes: "in the examples known at present the mutations occur in a disorderly fashion, giving rise to flecks and spots which have little relation to the main anatomical features of the organism. Moreover, to explain differentiation we should need not only the orderly mutation of one gene, but of the whole complex set of genes active in the tissues concerned."[10]

[e]Embryologists had since long tried to unite developmental insights into one general principle. The different versions of recapitulation theory by Meckel, Von Baer, and others came the closest to succeeding. In general, this theory states that a developing embryo passes through the adult (in Meckel's version) or embryonic (Von Baer's version) stages of lower ancestral lifeforms. Today, recapitulation is seen as mere history because the theory offers no real explanations of developmental processes and is based on analogy.

[f]The concept of *embryonic field* was frequently used in the 1930s–1940s. Its wide definition, that is, any spatial region that initiates the development of a complete structure, created the illusion that it explained different kinds of phenomena. Waddington opted for more restricted terms, like *individuation field* for processes in a region resulting in the formation of a specific structure, and *region of competence* for regions holding the capacity to develop a structure that normally does not form there. He saw these classical embryological terms as operational terms, useful in describing experimental results, but feeble and even deceptive as a guide to the nature of underlying elements that bring about the discovered processes.

[g]The few geneticists that engaged themselves in embryological research in the 1950s merely tried to unravel what kind of protein changes a genetic mutation could lead to, and not how developing substances and tissues were influenced by the cellular genotype or how differential gene activation proceeded.

embryologists that was generally[h] not interested in genetics, Waddington therefore subtly stressed the importance of the genetic component for embryology, that is, he *contextualized* gene activity within the cytoplasm:

> In all these [developmental] reactions the genes are not acting alone, but are co-operating with the living matter outside the nucleus, or cytoplasm….Probably the cytoplasm provides much of the fundamental mechanism by which development is brought about, and the genes act as directing and controlling agents. The cytoplasm, on this view, could be, as we might say, the drilling-machines and lathes with which the animal is made, and the genes would be the particular tools, jigs, and drills which are fitted on to the machines for the actual job on hand. This idea may seem to suggest that the real fundamental thing is the cytoplasm and that the effects of the genes are purely superficial. To some extent this may be true for any one embryo. But one must not forget that the cytoplasm itself has been evolved through a long series of ancestors and has probably been affected by the genes which they contained…. Of course, to insist on pursuing the argument *ad infinitum* leads to a ridiculous question, like asking whether the hen or the egg came first, because finally the gene and the cytoplasm are dependent on each other and neither could exist alone."[12]

Next, Waddington speculated that every gene affects several different processes, and that genes work together to form gene networks. During development, these networks themselves were thought to undergo changes in competence. Here, Waddington did not speak of a theory of genes, but of *a quasi-atomic theory in which collections of genes play the fundamental role*.[13] Where Jacob and Monod's bacterial operon model had shown a nice example of gene regulation, bacterial research demonstrated that genetic processes need not be singular. Around 1966 the synthesis of argenine in *Neurospora*, for example, was known to be controlled by a minimum of seven genes, whereas Waddington described wing development in *Drosophila melanogaster* as affected by more than 40 genes. In addition to this, he stated that there is no simple one-to-one relation between a gene and a phenotypic character, as such a relation only exists between the phenotype and the genotype as a whole. In the case of eye development in *Drosophila*, for example, Waddington considered it incorrect to say that the gene $w+$ corresponds to red eyes, and w to white eyes. Rather, "we should say that, in the usual genotypes met within *Drosophila melanogaster* a substitution of $w+$ for w will change the eyes from white to red. The whole of the genotype other than the particular gene in which we are interested can be referred to as the genotypic milieu or the genetic background."[14] In other words, a second context to be considered when talking about gene action is the genetic background as present in the cell.

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[h]Richard Goldschmidt presents an exception, although he was convinced that genes controlled only the quantity, and not the quality, of the reaction.

Waddington's Equation: Epigenesis + Genetics = Epigenetics

Waddington's view questioned the biological disciplines that were in use, as it made the distinction between developmental mechanisms (or *experimental embryology*) and developmental genetics (or *phaenogenetics*) look artificial. Where the former studied embryonic development after chemical or surgical interference and the latter studied genetic function by genetically induced changes in developing embryos, both equally relied on gene action and developmental processes. This common property, synthesized with genetics, Waddington saw as crucial to the search for causal mechanisms in embryology. This *causal embryology* included three major processes in embryological development: histogenesis (differentiation in time), organogenesis (differentiation in space), and morphogenesis (differentiation in shape), which were renamed *histological differentiation*, *regional differentiation*, and *individuation* later on (FIG. 1).
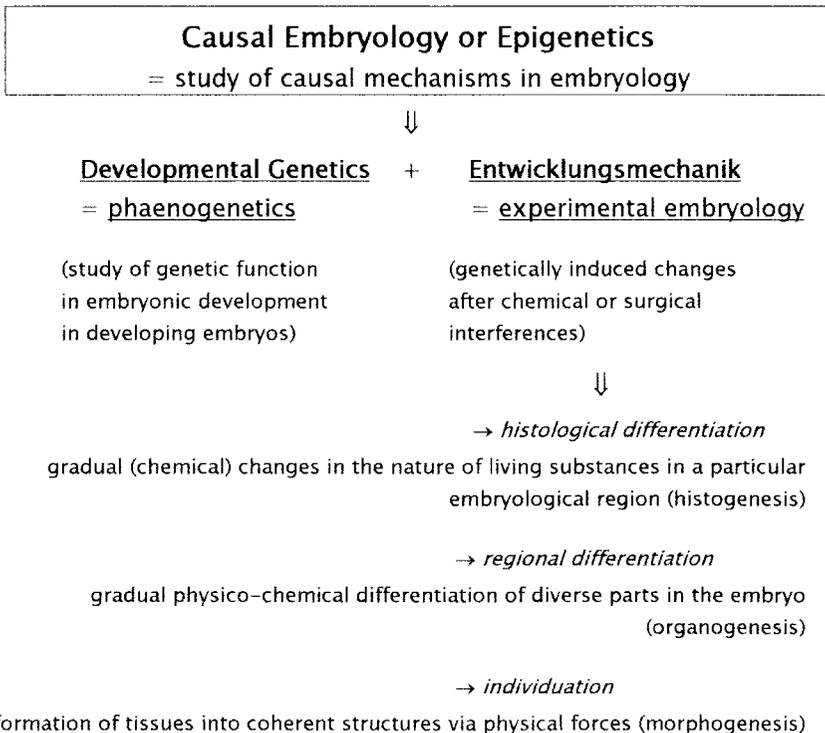

Causal Embryology or Epigenetics
= study of causal mechanisms in embryology

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Developmental Genetics   +   **Entwicklungsmechanik**

= phaenogenetics   **= experimental embryology**

(study of genetic function   (genetically induced changes
in embryonic development   after chemical or surgical
in developing embryos)   interferences)

⇩

→ *histological differentiation*

gradual (chemical) changes in the nature of living substances in a particular
embryological region (histogenesis)

→ *regional differentiation*

gradual physico–chemical differentiation of diverse parts in the embryo
(organogenesis)

→ *individuation*

formation of tissues into coherent structures via physical forces (morphogenesis)

FIGURE 1. Waddington's Synthesis of Causal Embryology in 1956. By 1962, next to histo- and organogenesis, he included the importance of interactions between a hierarchy of more complex organized entities (organelles, cells, tissues, organs) with a more or less definite spatial structure.

Causal embryology also deserved a special name. Waddington disliked names like Wilhelm Roux's *developmental mechanics* or *Entwicklungs-mechanik*, because of their association with machines and the inorganic and because they did not have an explicit link to genetics. Waddington sought inspiration in the 16th–17th century debate on preformationism *versus* epigenesis. He saw these theories as the first to bring the idea of heredity under general acceptance and, despite their "deceptive air of simplicity,"[15] he felt that they did better than pure philosophical approaches. Whereas *preformation*—claiming that all characters of the adult organism are present in the fertilized egg and only needed to unfold or grow—stressed the static aspects of development, *epigenesis* presented the old term for embryological growth and differentiation, thereby focusing on the interaction of the constituents of the zygote.[16]

In Waddington's interpretation, preformation and epigenesis become complementary: preformation is not so much understood as every feature of the adult being present in the fertilized egg; it is rather understood as every feature being *represented* by something in the egg, though this may be quite different from the adult form of the feature. This "something" holds the potential to develop robustly into the actual features of the adult organism. Epigenesis is seen to hold that the fertilized egg contains a small number of elements and that during development these react together to produce the much larger number of adult features that were not represented before. A fertilized egg does more than merely reproduce itself; it produces something new.[i] Putting these theories under a modern spotlight, Waddington links preformation to genetics and epigenesis to classical development, and synthesizes them:

> We know that a fertilized egg contains *some* preformed elements—namely, the genes and a certain number of different regions of cytoplasm—and we know that during development these interact in epigenetic processes to produce final adult characters and features that are not individually represented in the egg. We see, therefore, that both preformation and epigenesis are involved in embryonic development…. In the present stage of biology, the study of the preformed element in the fertilized eggs, taken in hand by the geneticists, has made such enormous progress that nobody is likely to be able to overlook it for long. Embryologists certainly have to accept it as part of the basic groundwork from which they start. Their attention is more immediately concentrated on trying to

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[i]Waddington often describes development as a gradual, self-invigorating process by stressing epigenesist aspects of embryonic cell differentiation. For example, "the differences between fully differentiated cells relate not to one single crucial substance, but to many. The differences…arise gradually and progressively; the tissue of an early neural plate is already recognizably distinct from that of the contemporary epidermis, but both will gradually undergo further change as the former develops into fully functional nervous tissue and the latter into the adult skin….These further changes cannot be considered as a mere uniform continuation of the first stages of differentiation; thus of the early neural plate, some will become eye-cup and some will not….The whole process is not the simple unfolding of a single trend, which has only one time of origin; it must on the contrary be regarded as involving a series of successive steps."[17]

understand the causal processes by which the genes interact with one another and with the cytoplasm of the egg. The focus of their interest is in the processes that we have referred to as epigenesis.[18]

The junction of *epigenesis* and *genetics* resulted in *epigenetics*, a most appropriate neologism for the causal study of development, emphasizing its fundamental dependence on genetics and its interest in processes. However, Robertson reminds us that "some of his [i.e. Waddington's] concepts turn out under use to be rather fuzzy at the edges—perhaps because he was often content with presenting them as analogies and did not develop them into precise theories."[1]

The concept of epigenetics fits this description to some extent. The epigenetic project started in the early 1940s as an ambitious, scientific goal, continuously stressed by Waddington, up to his final work, *The Evolution of an Evolutionist*, which was published post-mortem in 1975. Nevertheless, the project was never concrete, and Waddington was aware of the fuzziness of his attempt to update embryology from a descriptive to an experimental science. He realized that embryology could no longer be wholly satisfied to operate in terms of organizers, fields and the like, which were discovered in the first successful experimental forays. On the other hand, it was still too early to find biochemical approaches that threw light on the scene. Therefore, he was not just content, but rather inspired to deliberately formulate conceptual schemes in general terms, which were useful as "abstract guides to possible directions which our thoughts may take."[19] Above this, Waddington was not looking for precise definitions and exact theories, an idea that is reflected in most of his books, which hold the middle ground between popular literature and exact science. Whitehead totally inoculated him "against the present epidemic intellectual disease, which causes people to argue that the reality of anything is proportional to the precision with which it can be defined in molecular or atomic terms."[20] But, if epigenetics was indeed nothing more than an abstract guideline, a heuristic conceptual tool, to develop a new embryological science, where then does it fit in with Waddington's other work and what further impact did it have?

DEVELOPMENTAL EPIGENETICS: A HOLISTIC ONTOGENY

Seeing Development Epigenetically: Genotype + Epigenotype = Phenotype

At the time Waddington started to develop his ideas on epigenetics, genetics had two things to offer: Mendelian laws of heredity and a refined chromosomal theory, on the one hand, and a bag of growing exceptions, on the other.[j] Waddington recognized the importance of the Mendelian laws, but he real-

ized that they only count for clearly marked features, which do not represent the organism in total. Likewise, he shaded the meaning of the distinction between *phenotype* and *genotype*, respectively "the characters of an adult individual" and "the representatives of those characters which are present in the germ-cells and pass on into the next generation,"[21] by reminding us that this distinction was invented after the basic theory of genetics had been developed. This meant that the terms were to some extent colored by their conception and a more flexible interpretation of these terms was in order. Why not, suggested Waddington, consider the genotype as "the whole genetic system of the zygote considered both as a set of potentialities for developmental reactions and as a set of hereditable units; that is to say, it includes not only the mere sum of the genes, but also their arrangement, as expressed in position effects, translocations, inversions, etc."?[22][k] And as for the phenotype, why not include—next to anatomical and physiological characters—developmental processes and see it as "the whole set of characters of an organism, considered as a developing entity"?[22]

Both terms are normally used to refer to differences, caused by genetic or environmental changes, *between* whole organisms. Waddington argued, however, that when brought together, these terms can also be used adequately for the development of differences *within* a single organism, for example:

> the difference between an eye and a nose…is clearly *neither* genotypic nor phenotypic. It is due…to the different sets of developmental processes which have occurred in the two masses of tissue; and these again can be traced back to local interactions between the various genes of the genotype and the already differentiated regions of the cytoplasm in the egg. One might say that the set of organizers and organizing relations to which a certain piece of tissue will be subject during development make up its *epigenetic constitution* or *epigenotype*; then the appearance of a particular organ is the product of the genotype and the epigenotype, reacting with the external environment." [italics added][23][l]

[j]For example, crossing over, gene-linkage, translocation, duplication, and so forth. These were all based on the study of chromosomes or the study of phenotypic effects after crossing.

[k]Waddington continued: "the question arises as to whether the cytoplasmic characteristics of the zygote are to be included in the genotype, but although they are obviously a very important part of the developmental potentialities of the zygote, it seems advisable not to include them in the genotype: probably it is better to consider them as part of the phenotype determined by the genes of the mother."[22]

[l]The phenotype–genotype distinction is linked to the theory of the germ track, which describes the continuous succession of germline cells through the generations. In Waddington's words: "this line of germ-cells represents …an *immortal* piece of living matter, which may increase in size but need never die. The thing which does die is the individual animal body which the germ-cells make to provide themselves with a temporary house."[24] This idea is unmistakably echoed in Dawkins's *The Selfish Gene*.[25] However, Waddington shades the so-called germ–soma distinction, because the temporary body and the immortal germ cells are not always that rigidly distinct. Germ cells often belong to the body in that they develop in the same way as the other differentiated cells in the body, that is, in response to some organizer. Waddington therefore prefers to speak of the immortality of the cell in general, because all young embryonic cells are potentially immortal.

development as an *epigenetic process*

FROM

genotype + environment = phenotype

TO

genotype + *epigenotype* + environment = particular phenotype

⇓

= epigenetic constitution of tissue/cell

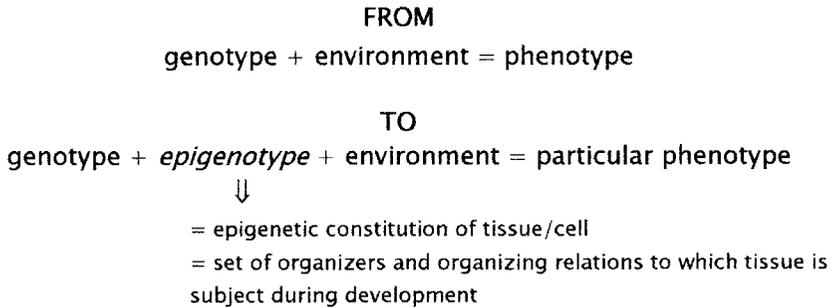= set of organizers and organizing relations to which tissue is subject during development

FIGURE 2. Scheme of Waddington's expansion of the classical model on the phenotype-genotype distinction.

The above citation points out that Waddington considers development to be an *epigenetic process.*[26] In other words, the phenotype—at any instance—is the result of the interrelations among genetic processes, their potentialities and constraints, cytoplasmatic differentiations, and the external environment. Waddington thus further expands the classical genetic vision on the phenotype by including the *epigenotype*, that is, genetic and nongenetic developmental interactions that organize the organic substances and tissues. This expansion is considered necessary, as genetics only deals with the behavior of genes during inheritance and needs to be supplemented by an epigenetic theory, which deals with their behavior in the developmental processes by which the fertilized egg becomes the adult with which most biological investigations are concerned. Epigenetics thus provides the turning point between heredity, evolution, and development (FIG. 2).

Organization, Contexts, and Holism

The expansion of the genotype–phenotype distinction demonstrates the somewhat holistic nature of Waddington's program on causal embryology. The single harmonious living organism under the action of causes brings about the unifying arrangement of innumerable separate processes. Although the organizational principles are still studied via reductionistic means, the developing organism–as the whole being organized—is the major guide for research. However, as *organization* is a rather abstract concept with little explanatory power in itself, guidance rather lies in its exploratory epistemic

value than in its ontological or direct experimental value.[27] Whereas one could consider organization in general, as if all possible modes of deriving parts would be of equal interest, the real value of the idea of organization comes with quantifiability: that is, the degree of organization depends on the degree of specific part–whole dependencies in the context of the organized whole. Organization thus needs to be defined in relation to its context. Through interactions of elements, new relevant contexts or a new level of organization can occur, excluding a total reduction of the hierarchy of organizational levels, because "a new level of organisation cannot be accounted for in terms of the properties of its elementary units as they behave in isolation, but is accounted for if we add to these certain other properties which the units only exhibit when in combination with one another."[28]

Waddington's goals were two-sided in that he wanted not merely "to explain the complex by the simple, but also to discover more about the simple by studying the complex."[28] From this theoretical angle, he held it possible to study the problem of causal embryology experimentally, while remaining holistic in interpreting the results.

Cellular Epigenetic Action Systems

In practice, Waddington studied induction in animal embryology, that is, the development of embryonic cells triggered by and conforming with other elements. Via these studies, Waddington hoped to gain insight into the organizational principles of development that lead to flexibility and stability. The work of Hans Spemann on the presence of internal organizing determinants in embryonic organo- and histogenesis, especially the experience that early embryonic development could recover after cutting away certain regions of cells, stimulated Waddington's views on the existence of a *harmonious equipotential*, *individuation fields*, *developmental fates*, *evocation* and *self-differentiation*. The main idea behind these terms is, time and time again, that a given stimulus can induce a change. This change only occurs because the reacting tissues or cells have the potentiality to make the change. Where before the focus was often on the stimulus, Waddington put the spotlight on potency and on competence[m] or reactivity itself. This idea is fundamental to embryonic cell differentiation and development: cells react to different chemical and organic stimuli in a way that is allowed by their state at that time. Each new reaction is likely to differentiate the cell further and bring it, on the one hand, under more constraints, while on the other, it opens new possibilities or potentialities. Waddington did not agree with Driesch that these cellular potentialities should be called a *developmental soul or non-material entelechie*,

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[m]*Potency* or the ability to act refers formally to what can take place in future events. *Competence* is the concrete, current characterization of the element (tissue, cell,…) making a real reaction to a stimulus possible.

because "a hypothesis like this is in the first place a confession that we do not understand how the harmony of development is brought about, but more than that, it denies that we shall ever find out."[29] On the contrary, Waddington had confidence in the capacity of molecular research on developmental pathways and gene expression to find answers.

With induction as part of a developing epigenetic system, Waddington brought some general principles together to account for the role of genes in it: the development of an organ or a complex substance takes place in a series of steps following an *epigenetic path* or *chreod* (from the Greek *chre*, necessity, and *hodos*, trajectory).[30] Each step is defined by instructions in the genotype that interact to produce a system that moves along a stabilized time trajectory. Genetic action thus forms a major influence, making development the result of all gene-influenced tendencies. The diverse paths in organismic development are protected or canalized[n] by threshold reactions and by the interrelations between elements and processes of the living system, providing stability. This canalization is based on the genetic capacity to buffer developmental pathways against mutational or environmental perturbations.[31] The system is, so to speak, "to some extent 'self-righting,' like a well-designed automobile which has a tendency to straighten itself out after being put into a slight curve."[32] This tendency of development is seen in many contexts and at many levels of organization. It was, in fact, much more widely known and better recognized in embryological connections than in the field of genetics. Waddington long held the intuition that the stability resulting from canalization arises in part as the result of natural selection, and in part as the inevitable consequence of a system in which very many genes are interacting with one another.[o] Nevertheless, epigenetic crises[30] or instability can arise, meaning that during development a small change in the normal conditions can have a great impact on stadia later on in the process.

The idea of canalization is metaphorically illustrated by Waddington's *epigenetic landscape*—a landscape of valleys separated from one another by hills diverging down an inclined plane. This landscape represents the tendency of cells to pass from an immature stage to an adult and specified condition. In other words: during development, a population of homogeneous cells differentiate into the diverse cell types of the organism.[34] The idea of canalization represents those paths within a competent cell that allow certain cell fates

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[n]With regard to the theme of developmental stability in epigenetic space, Waddington coined the term of *homeorhesis* (from the Greek *rheo*, to flow) in analogy to homeostasis. Where homeostasis is the general term for the maintenance of any stable value during a certain period, homeorhesis denotes the maintenance of certain changes in value during a certain period. If a homeorhesic system is disturbed, it is likely to fall back on track: not, however, in the place where the disturbance took place, but in the place where the system normally would be without the disturbance. Homeorhesis thus includes a time factor.

[o]In this context, Waddington refers to a young graduate student, Stuart Kauffman, analyzing complex interacting systems as Boolean networks with self-organizing characteristics, a theme Kauffman continues to promote up to this very day.[33]

to be achieved more readily than others. If the walls are very steep, the equilibrium state of the track is very high, and it is hard for the cells in the valley to escape from this pathway or from their *developmental cell faith*. Low walls indicate the likelihood of cells changing from one pathway to another. Because of the presence of these walls, the developmental pathways are canalized: if the walls are high enough, even a huge disturbance will not be able to bring development out of its normal track, and determined and stable development will proceed.

What controls the steepness of the walls, and thus the entire epigenetic landscape, is not just genes and their products, but gene–gene interactions and gene–environment interactions, an idea Waddington already stated in 1939 even though it was only in 1956 that a genetic landscape was visualized as underlying the epigenetic landscape. In this scheme, Waddington's genetic holism becomes clear anew: although the contribution of certain genes can be zero in a pathway, all genes are seen to be coupled in a genetic network, and the total of this network is connected to the epigenetic landscape. This approach differs firmly from Beadle and Tatum's atomistic and static *one gene—one enzyme* hypothesis of the 1950s.

Waddington inserted other new labels: a *gene–protein system*, being the set of biochemical processes leading from a gene[p] to its protein level; and a *gene action system*, being the set of all biochemical processes leading from a gene to a phenotypic character. In general, a gene action system makes up the epigenetic action system of the cell,[35] in which the many feedback loops should be stressed. The importance of these feedback relations lies in the fact that they admit a flexible view on gene action. Although genes obviously have sufficient stability to be reliably transmitted through many generations, we need not feel bounded to regard genes as nothing more than isolated sequences of DNA, modifiable in no other way than by rare events of mutation. The door to further enlarge the genomic context is hereby opened (Fig. 3).

Waddington himself kept his focus within cellular development and concentrated on the cytoplasmatic context of the genome. He described the cell as a double-cyclic system in which (i) genes make gene products, which elaborate the final cytoplasmatic elements, while (ii) the cytoplasmatic elements feed back to the genetic level to regulate gene-activity.[36,37] The message is clear: genes not only regulate, but they also are regulated by nongenetic factors—relative concentrations of substances, and so forth. Thus, a gene itself

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[p]Regarding genetic influences, Richard Goldschmidt promoted linear reaction pathways (in which a cascade of gene action unfolds). Waddington included branching pathways (in which, after expression of gene A, a bifurcation comes into existence which enables gene B or C to be triggered, leading to phenotypic differences), analogous to embryological bifurcation pathways in which an external factor (or evocator) can push a competent cell line into a new track. As for the physical interaction between entities, Waddington adhered to a dialectial materialism—the transition of quantity into quality—in which entities in contact modify one another. Through these modification genes can have secondary effects.
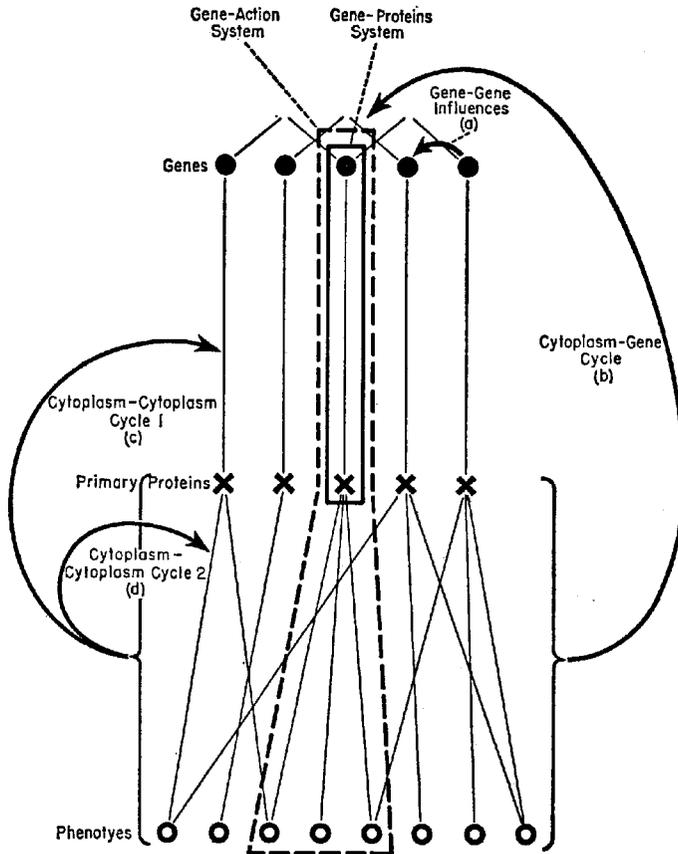
FIGURE 3. Waddington's original scheme of an epigenetic action system of a cell: the feedback reactions from **(a)** gene to gene, **(b)** cytoplasm to gene, **(c)** cytoplasm to gene-protein system, and **(d)** cytoplasm to the primary-protein-to-phenotype processes illustrate the gene contexts that Waddington focused on. (Reprinted from *New Patterns in Genetics and Development* by Conrad Hal Waddington © 1962, Columbia University Press; used with permission.)

could not be seen as a unit of *developmental activity* since it underwent so many influences, as the discovery of position effect variegation had shown:

> Gene-activity is not to be attributed to circumscribed particles, which could be considered as separate 'beads along the chromosome thread,' but … the basic elements are short stretches of chromosome which are not sharply bounded off against each other, but rather shade into or overlap one another. A change in the order of the chromosome thread will in that case alter the character of the fundamental reactions carried out by it.[38]

WADDINGTON'S LOSS AND LEGACY

Embryonic Cell Differentiation

A true epigenetic theory had to give a clue about what caused cell differentiation. In the 1960s, the mutational theory held that during cell differentiation only those genes responsible for the differentiation were activated. However, the facts never supported this view. Neither was it appropriate to regard differentiation as brought about by irreversible mutations in genes, because these had a slow rate and appeared to be random, while development proceeded as a well-organized process. Also, studies of coelenterates had shown that after cell division new differentiation patterns could appear, despite a similar genetic makeup. In addition to this, the idea of plasmagenes [q] with long-lasting genetic continuity of character, seemed inconsistent with the facts.

Waddington himself speculated in the direction of cytoplasmic non-homogenity, the physical location of the nucleus in a specific cytoplasmic region, differential protein synthesis due to small changes in chemical gradients or to genetic mutations, and the existence of autocatalysis. To account for the interconnectivity between individual cellular epigenetic systems, Waddington did think of expanding his theory to an intercellular, organismic, and environmental level. But because much information on intercellular morphogenesis was lacking, his approach could not continue beyond a vague theoretical plane with some attention to the connectivity between cells of the same line (which were seen as a network of different gene-action systems constituting a developmental region). Also, it looked as if cell–cell interactions were not that abundant, as muscle cells and nerve cells could lie next to each other without any sign of mutual interference with protein synthesis. Therefore, the main accent of Waddington's epigenetics remains on the interconnectivity and the existence of continuous feedback relations within a cell and its direct progeny.

Here, Waddington briefly collaborated with Brian Goodwin, then working as a Ph.D. student on the (in)stability of states. Waddington was inspired by Volterra's successful mathematical approach on population dynamics and had strong expectations for Goodwin's mathematical approach. The goal was to develop an *epigenetic thermodynamics* as a statistical tool for open biological systems and to provide a mathematical foundation for making embryol-

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[q]Plasmagenes, also called *cytogenes*, *blastogenes*, and *provirusses,* were thought to be cytoplasmic elements with a stable nature similar to genes in the nucleus. They were seen as determinants of cytoplasmatic inheritance. The proof for the existence of plasmagenes relied on multiple backcrosses of the F1 generation with the parental male: it was believed that if the presence of parental female chromosomes slowly diminished in each generation, and if a characteristic of the parental female persisted through the generations, it could only be inherited via the cytoplasma of the fertilized egg.[39]

ogy quantifiable via terms like *epigenetic temperature* and *epigenetic kinetic energy* in order to come to a general theory of development and evolution. Waddington's comment after the first trials is reserved, as the approach dealt with factors (such as fluctuations of the concentration of particular protein species) which one could not yet measure, but also hopeful, as it presented a novel pattern of thought.[40] Although the years to come provided more insight in quantifying biological matter and processes (electron microscopy alone made insight into metabolic activity much easier), Goodwin's mathematical theory did not achieve its proposed goals, and it was hardly ever touched on by Waddington in later publications. Instead, Waddington leaned more and more toward the fashionable molecular biology as a tool to account for non-genetic components that may be effective at intermediate levels of complexity. A classical genetic approach would miss out on these components:

> If, for instance, in a particular developmental system involved in pattern formation an important part is played by factors such as the permeability or tension of a cell surface, or the resistance to bending of an epithelium, *we should not, by genetic analysis, be brought to realize this*. We should instead find that very many genetic factors were *active*, but we should *not* be able to discover that many of them are *operative* because they *affect such factors as cell membranes or elastic properties*. The genetic analysis of pattern, therefore, reveals to us something about their *complexity in terms of ultimate units*, and also something about the *degree of integration of these ultimate units* into stabilized creodes, but it is *much less informative about the intermediate steps between the genes and the final patterns.…* The fact that a certain gene substitution in *Drosophila* causes the appearance of a four-segmented leg in place of a five-segmented one should be regarded as facing us with a *problem* and not as providing us with an *explanation.* [italics added][41]

The above citation reflects the intertanglement of genetics and development, which became even clearer with the emergence of bacterial genetics in which the genotype–phenotype distinction is less evident and cytoplasmic inheritance is much more obvious. The expansion to eukaryotic and multicellular genetics made the study of developmental cellular changes during embryogenesis necessary. Genetics, in a developmental context, should not only study genes or a genetic code. Rather, (and mind that this is written in 1962):

> We shall want to know, in the first place, *how*, *when*, and *why* a given DNA cistron comes to make a messenger RNA, and secondly how this RNA gets to the microsome. It is in connection with these questions that the cells of higher organisms exhibit phenomena which may yet turn out to be essential components of the whole process of genetic determination [i.e., the specification of an amino acid sequence by a DNA sequence]. One of these phenomena is the almost universal occurrence in higher organisms of feedback relations between cytoplasm and genes, such that the nature of the cytoplasm determines the intensity of the syntheses controlled by the various genes in the nucleus.[42]

Control of gene expression was of particular interest to the new causal embryology. This interest promised to be helpful to other biological branches as well. Immunology, for instance, would prosper from the insight into why and when the different genes for the production of hemoglobin are active.[r]

Waddington's Evo–Devo Program

Waddington did incorporate evolutionary aspects into his epigenetic thinking. For example, he believed that in gene–cytoplasm regulatory interactions a key can be found as to why a species has only a limited amount of distinctive developmental pathways. Also, he tried to figure out how natural selection can influence the degree of developmental canalization, as canalization is highly dependant on genes. In contrast to population genetics, he postulated the idea that natural selection does not work directly on gene pools, nor merely on adult organisms, but on the characteristics of organisms during their lifetime development. In contrast to neo-Darwinists, he believed that natural selection of chance mutations could not account for many natural adaptations and that evolutionary theory would miss out on many biological aspects if it did not incorporate a developmental theory in which "the genotype of evolving organisms can respond to the environment in a more co-ordinated fashion."[43] This led to his speculative model on *genetic assimilation*, exemplified in the famous case of the callosities of the ostrich[s] and experimentally supported by an experiment on the size of the anal papillae of *Drosophila.* In this experiment, when environmental conditions (the food *Drosophila* feeds on) are normal, the fruitfly's papillae do not deviate in size. However, once the conditions change to abnormal (e.g., by feeding the fruitflies food with a

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[r] Human embryonic hemoglobin consist of two α-chains and a γ-chain, instead of two α-chains and a β-chain like in adults.

[s] Where callosities are usually caused by an environmental stimulus (pressure and friction) on an adult organism, ostrich embryos develop callosities on the exact spot where they are needed in adult life *without* the presence of such a stimulus. Genetic assimilation theoretically explains how such a trait—which only has an adaptive function after birth—can be selected for. Fundamental is the idea that although a physiological or developmental adaptation to an environmental stimulus is not heritable as such, the capacity to do so is, because it is under genetic control. As natural selection works indirectly on any genetic control, it will advantage those organisms that show the capacity to develop a trait (*in casu* callosities) sooner than others. As long as the pathway that develops the trait before its adaptive functional time does no harm to the organism, selection is in no position to work an early development of the trait away. Moreover, the development of a such trait can become canalized, once it comes under the control of a mutant genetic variation or a new treshold. For example, where skin cells have the competence to develop callosities after induction by friction, they can be induced by a new factor, like an embryonic gene product. Being controled by a heritable factor, the induction becomes fixated and manifests itself apart from the environmental stimulus without loosing much of the normal effect that the original stimulus triggered. That it is not implausible for an external environmental stimulus to be taken over by an internal genetic factor is counterintuitive to neo-Darwinism thinking. As Arthur Koestler sees it: "it is sheer nonsense to say that evolution is 'nothing but' random mutation plus natural selection. That means to confuse the simple trigger with the infinitely complex mechanism on which it acts."[44]

high salt concentration), a stress reaction occurs, leading to larger papillae. By performing constant positive selection per each new generation for those flies that readily showed large papillae under stress, Waddington succeeded in creating a *Drosophila* stock that also exhibited the larger papillae under normal conditions. The new stock was far better adapted to high salt concentrations in food than the original stock, leading to the conclusion that selection can ameliorate the rate of adaptability and genetically assimilate an environmental reaction by selecting the most appropriate genetic lines.[45] Contrary to the Baldwin effect,[t] one does not have to await an accidental mutation to get an adaptive response. In other words, natural selection does not work on atomistic elements (i.e. separate genes) that randomly change without regard for their context, but on complex, developing phenotypes and on the epigenetic processes that build these phenotypes in interaction with the environment.

Stressing the developing phenotype in evolutionary theory expands the classical focus on the transmission of genetic information with a second focus on gene regulation or instructions on how to use the genetic information. To bring evolution and development to full synthesis, however, a developmental theory is needed. Therefore, Waddington's epigenetics mainly situates itself on the developmental plane, as a model to link the genotype and the phenotype during development in a specific environmental context.

Today's Molecular Epigenetics: Turning away from Gene-centrism

Although most of Waddington's terms never gained popularity, *epigenetics* seems to have caught on. Despite the fact that in the 1960s the concept was barely used in developmental genetics, even though at that time research concentrated on the cytoplasmic context of the genome, the term became abundantly used in the scientific literature from the 1990s onwards. The concept has evolved in time, however. Waddington's epigenetics originated in an embryological era where the gene concept had no strict definition and could be interpreted as broadly as the concept was abstract. As "unit of heredity," it could mean practically anything that fell under the denominator of being heritable. This made the idea of *demarcating gene contexts* less obvious be-

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[t]The *Baldwin effect* was coined at the end of the 19th century by the Americans Baldwin and Fairfield Osborn and picked up again in 1952 by G.G. Simpson. The original idea of Baldwin has not survived, it merely exists through the interpretations of others. Following Alister Hardy, the Baldwin effect means that under stress certain habits are developed by an active organism. These habits spread initially through imitation. But sooner or later, a genetic mutation can appear that makes the genetic heritability of the habit possible. Here, function comes first, structure later, an idea basic to Lamarckism. Following Huxley and Mayr, it means that under environmental stress, organisms can—thanks to physiological adaptations—survive long enough to passively await the appearance of a random genetic mutation that provides the appropriate phenotypic modification to resist this stress. Here, Waddington claims, the basic idea of genetic assimilation is forgotten: that is, physiological adaptations are already under genetic control. Merely a switch is necessary to make this control heritable; one does not have to await a totally new random genetic mutation.

cause what today would be considered as a specific context, then could fall in large part under the heading of the gene concept. But Waddington did define a gene as a DNA sequence, making epigenetics necessary, because it denoted those interactions of genes with their environment that bring the phenotype into being. Waddington mainly approached the problem from within experimental embryology in order to analyze the developmental processes of a fertilized egg. The genomic contexts he studied therefore restricted themselves mainly to intracellular components and networks.

Today, in the molecular age, not only the gene concept, but also the entire phenotype is often reduced to one class of molecules—DNA. Despite its continued success, this extreme genecentrism is refuted more and more by the image of complex molecular networks found in new research and is pressing biologists to shade their thinking. Here, epigenetics can be literally interpreted as epi-genetics, that is, going beyond the genes, an interpretation still fitting Waddington's approach. A more popular current definition sees epigenetics as the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence, but are important for the understanding of the developmental processes and phenotypic traits of the organism.[46,47] Whether this definition correctly covers the cargo or not,[48,49] it does reveal Waddington's legacy in several links. First, there is the renewed interest in the existence and generation of nonrandom, and even non-DNA, variation in biology. Although Waddington focused more on stability and the process of canalization as constraining the possible variation caused by the genome and the environment, he did orient his epigenetic theory toward themes such as genetic assimilation, which is not about the preservation of changes in development, but about their origination. He was also interested in how canalizing constraints exhibit their evolutionary role in guiding the direction of possible changes in evolution. Second, it indicates that not all (heritable) information leading to the phenotype is inscribed in the DNA sequences as such, but that regulation and the developmental context of these sequences should be taken into account—a theme central to Waddington's epigenetics. Third, there is a strong link between genetics and molecular biology, the science Waddington saw as most promising for studying epigenetics. Whereas most experiments in modern epigenetics still study the epigenetic regulation of one specific gene (in contrast to Waddington's focus on gene networks), today, new techniques such as microarrays and chip technology allow the possibility of studying larger gene networks at the same time.

On the other hand, one positive change with regard to the original definition is that today epigenetics is not restricted to the study of embryonic stages of an organism. Embryological topics like parental imprinting (e.g., inactivation of the X-chromosome in female mammals) still fall under the heading of epigenetics, but the list certainly does not end there. Also, a large fraction of epigenetic research takes place in plant molecular biology. Research on trans-

genic gene silencing is especially popular. Waddington would not have been eager to study plants, because he saw them as rather passive organisms, lacking mobility and interaction. Today, epigenetic research on RNA interference and the like show us that plants should not be considered less complex or interesting to study than animals as research subjects. Second, where Waddington mainly investigated the intracellular context of genes and theorized vaguely on larger contexts, today, more and more research covers an expanded hierarchy of crucial genomic contexts: next to the intracellular level, the intercellular level is abundantly studied in terms of cell–cell communication systems (e.g., system-acquired silencing shows how a changed genetic state in one cell can be transported to other cells via signal communication), research on the organismic context is showing expanded forms of heredity, as the genotype–phenotype distinction is not as isolated as once thought, and also the ecological context of a developing organism is increasingly receiving the attention it deserves. This leads not only to a more complex perception of biochemical organization, but also to a new image of the genome, that of *genome plasticity*. Where before there was gene-centrism, epigenetics gradually expands the range of molecular processes influencing the genome, thereby decentralizing the sovereign role of the genome.[50]

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