The Epigenotype: a dynamic network-view of development

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During the late 1930s and the early 1940s, a particularly productive period in his scientific life, Conrad Hal Waddington (1905-1975) started to construct a new synthesis between genetics, embryology and evolution. In the four years between 1939 and 1943, before he became involved in military activity during the Second World War, he published two substantial books and several seminal papers, all of which were explicitly geared towards constructing of an integrated view of biology. 'The Epigenotype',[1] published in 1942 in the semi-popular science journal *Endeavour*, is one of these papers. In it, Waddington presented and developed some of the ideas that he had already discussed in his books, and also defined, albeit informally, a new domain of research, *epigenetics* – the study of the causal mechanisms intervening between the genotype and the phenotype.

Today, epigenetics is a very broad field of study, covering many aspects of biology, including morphogenesis, cell heredity, transgenerational epigenetic inheritance, and the evo-devo approach to evolution that Waddington investigated though his genetic assimilation experiments. In this commentary, we briefly discuss one particular aspect of Waddington's epigenetic approach, the network-oriented view that he put forward in the 1942 *Endeavour* paper,[1] and the way in which this network view, Waddington's epigenotype, is conceived today.

The epigenotype as a network: the many-many relations between genes and characters

In his 1939 book, *An Introduction to Modern Genetics*,[2] Waddington had already introduced the term epigenotype, highlighting its developmental, interactive aspects:

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> 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.*" (p. 156, our italics)[2]

Both in the 1939 genetics textbook and in his subsequent book, *Organisers and Genes*,[3] Waddington makes it very clear that the 'organizing relations' are a dynamic network of interactions occurring during embryogenesis, and that the study of this network, the *epigenotype*, is the subject matter of the new integrative discipline, *epigenetics*. In the *Endeavour* paper, he defines the epigenotype more generally than he did in his 1939 book, and in terms more familiar to geneticists: he describes it as the whole complex of developmental processes lying between and connecting the genotype and the phenotype (p.19).[1] He also defines the term *epigenetics* as the study of the causal mechanisms through which genes bring about phenotypic effects (p. 18).[1] 'The Epigenotype' has a strong and optimistic genetic orientation: Waddington has no doubt that a sophisticated genetic approach, which analyses the effects of the genes, can bridge the gap between the genotype and the developing phenotype. He presented the study of wing development in *Drosophila* as a concrete example of such an approach, and claimed at the end of the paper that 'the analysis of the effects of genes has now progressed far enough to become merged with experimental embryology' (p. 20).[1]

The basis of this optimism was the realization that 'the concatenations of processes linked together in a network',[1] which is the epigenotype, is amenable to genetic analysis. However, the kind of analysis that is required is a sophisticated one, which recognizes that a phenotypic trait is the result of many-to-many relations between genes and characters. On the one hand, one has to consider pleiotropic effects, such as those that had been studied and analyzed by geneticists like Hans Grüneberg, who had shown that one gene can have many effects. For example, as well as affecting fur colour, the 'grey-lethal' gene in the mouse leads to a failure in bone absorption, which has effects on the structure of the skeleton and teeth, and through these influences feeding behaviour. On the other hand, it has to be recognised that many different genes affect a single trait. As

Waddington himself showed, during the 48 hours after puparium formation the development of the wing of the fruit fly *Drosophila melanogaster* is affected by at least 15 genes.

Moreover, because both genetic mutations and embryological manipulations can bring about the same phenotype, studying the epigenotypic network of interactions can be approached in different ways. This was stressed by Richard Goldschmidt, who coined the term *phenocopy* to describe environmentally-induced phenotypes that mimic genetic mutations[4]. Phenocopies were seen by Waddington as clues to the network-nature of the epigenotype. First, since as he puts it, 'there are no developmental events which they [genes] do not regulate and guide' (p. 20)[1], gene mutations can (sometimes) lead to changes in the network of processes, and eventually to a visible change in the phenotype. Second, since the very same altered phenotype can be brought about by environmental changes (e.g. experimental manipulations of embryogenesis) that directly affect the network of interactions itself, the epigenotype must be a responsive (plastic) *developmental system.*[5] Analysis in terms of single genes can therefore tell us little about development, even when a single gene mutation has an effect on the behaviour of the network. The effect of the mutation, like that of an environmental perturbation, is not autonomous, but related to the cybernetic properties of the network. It is through the network view, Waddington suggested, that we can understand the dynamics of phenotypic development, which he depicted in his metaphor of the epigenetic landscape.[3] The epigenetic landscape (a term not mentioned in the short *Endeavour* paper) has some interesting characteristics, which are underlain by epigenotype dynamics: it exhibits canalization, manifests critical periods when particularly big changes can be induced, and shows developmental bifurcations that lead to sharply distinguished alternative tissues. The epigenetic landscape changes during evolution, through processes of genetic assimilation. Evolution, for Waddington, was always the evolution of developmental systems – of dynamic epigenetic landscapes and epigenotypes.[1,2,3,5]

In the *Endeavour* paper Waddington only hints at these issues and presents them as open problems, although he does offer some fairly well-worked discussions of all of them in

other publications from this period.[2,3,5,6] But what Waddington called in the paper 'epigenetic analysis' – the combined genetic and embryological deconstruction and reconstruction of development, which he so confidently believed was going to bridge the gap between genetics and experimental embryology, did not materialize as quickly as he anticipated. It took almost another fifty years for the network approach to be incorporated into the centre of modern developmental and evolutionary biology.[7,8]

The modern epigenetic-network approach

The epigenomic network approach advocated by Waddington was complex and somewhat abstract, attempting to provide a general framework for thinking about the process of embryogenesis. A much simpler type of regulatory gene network was discovered and characterized by Jacob and Monod in the 1960s, and began to dominate thinking about gene regulation. Jacob and Monod's ground-breaking study of the lac operon uncovered two basic building blocks of gene regulatory networks: cis-regulatory genetic elements that reside in the chromosomal neighbourhood of genes, and trans-acting factors (gene products, or externally-introduced factors such as sugars) that bind to the cis-acting elements, leading to the regulation of one or more adjacent genes. Elucidating the network of regulatory interactions of genes, gene products, and external cues has become a major research program, and the bacterial lac operon illustrates some general aspects of regulatory networks, that are also manifested by networks found in higher organisms. In particular, it shows how regulatory networks can integrate different types of information leading to a transcriptional decision.[9] This, we now know, involves interactions between proteins, changes in protein phosphorylation, modification of histones (in eukaryotes), and alterations in DNA methylation, all of which reflect the state of the cell as it responds to its developmental environment. Waddington realized the importance of Jacob and Monod's model and its relevance to his own network thinking, but he was also aware that this simple model would need to be greatly modified in order to work in eukaryotic cells, with their 'proteinaceous chromosomes' and their huge and coordinated gene networks.[10,11] He was aware that the *organization* of the genetic elements themselves (which includes the chromatin) is crucial for understanding gene regulation, and was therefore happy with Davidson and Britten's 1969 model of gene

regulation in eukaryotes, which came closer to his ideas, although he emphasized that additional components and structural considerations needed to be added to it to account for various aspects of embryogenesis, such as competence.[10]

The study of genetic and developmental networks is thriving today, and much effort is devoted to establishing the interactions involved in developmental regulation, for example, in the sea urchin embryo.[12] This research is primarily concerned with cis-regulatory elements and their role in the development of the embryo. It shows, for example, how cell states are stabilized, even when the specification inputs are transitory; how the interactions between cells belonging to one tissue can smooth out local differences; and how processes such as these are involved in cell differentiation. Network interactions include regulatory cues between the genes belonging to the network as well as the regulatory effects of external cues. The source of such external cues may be the transcriptional state that the cell inherited through dedicated cell heredity mechanisms, or inputs acquired from adjacent cells.

Recent research on developmental gene regulatory networks has led to the identification of recurrent 'motifs', such as various kinds of feedback loops, which serve as building blocks of more complex networks.[13] More complex circuits, which are used for particular purposes in embryological development but whose abstract network topologies appear many times, have also been identified.[14] In each particular case, different genes are involved, but the circuit performs the same logical function, and is utilized for the same kind of developmental purpose. These repeatable sub-circuits point to a modular architecture for developmental gene regulatory networks, where modules are responsible for particular developmental functions. It is possible that network sub-circuits appeared earlier in evolution than the complex networks found in modern organisms of which they are components.[14] Although the evolution of regulatory networks was central to Waddington's view of evolution, and he saw evolutionary adaptation in terms of changes in the regulatory architecture of the epigenotype, the lack of detailed knowledge about network architecture in different species precluded, during his lifetime, a comparative

phylogenetic approach based on network organization. We have no doubt that he would have been deeply interested in this line of research.

He would have been no less interested in the discoveries of the more general properties of gene networks, and their relevance to evolutionary change. Network modularity can be studied by examining the properties of the topology of various regulatory networks. For example, Fraser[15] (reviewed in ref 16) partitioned proteins with many connections (hubs) in protein-protein interaction networks into “party hubs”, which interact with multiple partners within a network module, and “date hubs”, which interact with partners in different modules. The former evolve slowly, while the evolutionary rate of the latter is comparable to that of proteins with no partners. Koonin and Wolf made the plausible suggestion that network modules are evolutionarily conserved, while inter-module hubs are a source of innovation and variation during evolution.[16] This focus on network building-blocks reinforces the framework presented by West-Eberhard,[17] who extended Waddington’s approach, emphasizing the importance of modularity as a mechanism of plasticity.

Just as Waddington realized 70 years ago, the network perspective helps to explain the robustness of organisms to genetic perturbations; in other words, it sheds light on the genetic aspect of developmental canalization. For example, by using gene-deletion mutants, it has been shown that only 18.7% of the protein-coding genes in yeast are essential for growth in full glucose medium.[18] The picture that emerges when more than one gene is deleted is, however, much more interesting.[19] It turns out that when two genes, each of which can be deleted with no phenotypic effect, are both deleted, the result can be lethality. Even though each gene on its own may be functionally redundant, both are still active in most conditions. They are therefore exposed to natural selection, so do not accumulate detrimental mutations, and contribute to the robustness of the pathways in which they are involved. This type of robustness may help explain cases in which mutations in multiple genes are required for a phenotypic effect to be manifested, as Waddington argued theoretically from the 1930s onwards, and was able to demonstrate empirically in his assimilation experiments (reviewed in refs 11 and 20). It also partially

explains the limited success of genome-wide association studies in uncovering single genes that are implicated in human diseases.[21]

The complexity of the situation is revealed by recent observations showing that gene pairs that provide backup for one another have different expression patterns in most growth conditions, and that the transcription level of one member of the pair can increase in response to mutations that inactivate its partner. Models that analyze this type of compensatory behaviour show that it filters out environmental or developmental noise from the transcriptional pathway. It has been suggested that the regulatory precision that this provides, rather than compensation for gene loss, may explain the functional redundancy of genetic pathways.[22] This kind of canalization is exactly what Waddington predicted: the network architecture allows for redundancy, partial functional overlap, and dynamic compensation for environmental noise.[20]

As these recent studies on genetic and developmental networks show, Waddington's epigenotype perspective, which was based on the assumption that the relations between genes and characters are many-to-many, is now commonplace. In addition, the regulatory dynamics found in networks and their recurring architectural properties have raised new questions about the evolution of animal genomes, and opened up new explanatory approaches to robustness and the evolution of complex traits.

Epigenetic today

In recent years, epigenetics has certainly become a growth area. However, as has been pointed out many times, over the years the meaning of "epigenetics" has undergone some changes from the rather general notion suggested by Waddington in his *Endeavour* paper, which was the study of the causal developmental mechanisms linking the genotype and the phenotype. It has become a more specific term: epigenetics is defined today as the study of the mechanisms that lead to *persistent* developmental changes in gene activities and effects, but do not involve altered DNA base sequences. An important component of epigenetics is *epigenetic inheritance*, the transmission of phenotypic variations that do not stem from differences in DNA base sequence from one generation of cells or

organisms to the next. (For historical overviews of epigenetics and epigenetic inheritance, see refs 23-28).

Waddington was, of course, well aware of the persistence of developmental phenotypes, and explained them in terms of the re-construction of the network dynamics, but his work did not focus on cell heredity, a line of research that at that time was pursued mainly by microbiologists and cell biologists.[25,29,30] However, as we pointed out earlier, he sensed that the proteinaceous nature of the eukaryotic chromosomes makes a difference to the way gene expression is regulated, and sought chromosomal mechanisms that could help to explain the phenomenon of developmental competence at the genomic level.[10,11] As we know today, epigenetic marks such as DNA methylation and histone modifications, and their associated reconstruction mechanisms, are some of the factors and processes that underlie such competence. Cellular epigenetic inheritance, which is a process he did not anticipate, nevertheless fits beautifully into his general view of the epigenotype with its myriad of stabilizing mechanisms. It is interesting to speculate about what he would have made of transgenerational epigenetic inheritance: it could readily have been accommodated within his evolutionary framework as a stage in the process of genetic assimilation, and as an aspect of active developmental re-construction. It would have been another epigenomic factor in the great development-heredity-evolution entangled web.

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References

[1] Waddington CH. The epigenotype. *Endeavour* 1942;**1**:18–20.

[2] Waddington CH. *An Introduction to Modern Genetics*. New York: Macmillan; 1939.

[3] Waddington CH. *Organisers and Genes*. Cambridge: Cambridge University Press; 1940.

[4] Goldschmidt R. *The Material Basis of Evolution*. New Haven: Yale University Press; 1940.

[5] Waddington CH. The evolution of developmental systems. *Nature* 1941;**147**:108–110.

[6] Waddington CH. Canalization of development and the inheritance of acquired characters. *Nature* 1942;**50**:563–565.

[7] Gilbert SF. Diachronic biology meets evo-devo: C. H. Waddington's approach to evolutionary developmental biology. *Amer Zool* 2000;**40**:729–737.

[8] Morange M. What history tells us XVII. Conrad Waddington and the nature of life. *J Biosci* 2009;**34**:95–198.

[9] Shapiro JA. Genome organization and reorganization in evolution: formatting for computation and function. *Ann N Y Acad Sci* 2002;**981**:111–134.

[10] Waddington CH. Gene regulation in higher cells. *Science* 1970;**166**:639–640.

[11] Waddington CH. *The Evolution of an Evolutionist*. Edinburgh: Edinburgh University Press; 1977.

[12] Davidson EH. *The Regulatory Genome: Gene Regulatory Networks In Development And Evolution*. Amsterdam:Elsevier/Academic Press; 2006.

[13] Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U. Network motifs: simple building blocks of complex networks. *Science* 2002;**298**:824–827.

[14] Davidson EH. Network design principles from the sea urchin embryo. *Curr Opin Genetics Dev* 2009;**6**:535–540.

[15] Fraser HB. Modularity and evolutionary constraint on proteins. *Nat Genet* 2005;**37**: 351–352.

[16] Koonin EV, Wolf YI. Evolutionary systems biology: Links between gene evolution and function. *Curr Opin Biotechnol* 2006;**17**:481–487.

[17] West-Eberhard M-J. *Developmental Plasticity and Evolution*. New York: Oxford University Press; 2003.

[18] Giaever G, Chu AM, Ni L et al. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 2002; **418**:387–391.

[19] Tong AHY, Guillaume L, Gary DB et al. Global mapping of the yeast genetic interaction network. *Science* 2004;**303**:808–813.

[20] Waddington CH. *The Strategy of the Genes*. London: George Allen and Unwin; 1957.

[21] Hartwell L. Robust interactions. *Science* 2004; **303**:774–775.

[22] Kafri R, Melissa L, Pilpel Y. The regulatory utilization of genetic redundancy through responsive backup circuits. *Proc Natl Acad Sci USA.* 2006;**103**:11653–11658.

[23] Wu CT, Morris JR. Genes, genetics, and epigenetics: A correspondence. *Science* 2001;**293**:1103–1105.

[24] Jablonka E, Lamb, MJ. The changing concept of epigenetics. *Ann N Y Acad Sci* 2002;**981**:82–96.

[25] Jablonka E, Lamb MJ. Transgenerational epigenetic inheritance. In: *Evolution: The Extended Synthesis*, ed. M Pigliucci, and GB Müller, pp. 137–174. Cambridge, MA: MIT Press; 2010.

[26] Holliday R. Epigenetics comes of age in the twenty first century. *J Genet* 2002;**81**:1–4.

[27] Holliday R. Epigenetics: a historical overview. *Epigenetics* 2006;**1**:76–80.

[28] Haig D. The (dual) origin of epigenetics. *Cold Spring Harb Symp Quant Biol* 2004;**69**:67–70.

[29] Nanney DL. Epigenetic control systems. *Proc Natl Acad Sci USA.* 1958;**44**:712–717.

[30] Nanney DL. Microbiology, developmental genetics and evolution. *Am Nat* 1960;**94**:167–179.