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Epigenesis and Epigenetics

The concepts of epigenesis and epigenetics have deep historical roots that reach into early philosophical explanations of the organismal world. At the same time, a host of different usages exist in present day science. In using the term "epigenetic," developmental biologists wish to emphasize the context-dependence of developmental processes, geneticists refer to mechanisms of gene regulation that do not require changes of DNA sequence, evolutionary biologists imply non-DNA-based mechanisms of inheritance, and population geneticists evoke phenotypic variation in response to environmental conditions.

These usages seem vastly dissimilar. Although they address different phenomena pertaining to different fields of traditional research, these phenomena and their epigenetic components are related through the continuity of processes that link the succession of ontogenies in evolution. Here we provide an outline for a coherent discipline of epigenetic research. We begin by a brief summary of the historical background (for more detailed accounts, see Maienschein, 1986; Richards, 1992; Jahn, 2000), and then define and present examples of the four prevailing areas of epigenetic research. Finally we discuss the meaning of the epigenetic approach for evolutionary theory and evolutionary developmental biology.

An Explanatory Concept of Development

The historical origin of the epigenesis concept is not related to genetics but is rooted in a debate started by Aristotle about the nature of embryonic development. Reacting to what could be called proto-preformationist views held by the Hippocratic school, Aristotle clearly distinguished between the two possibilities of embryonic organs arising from pre-existing parts or from the formation of new parts, and decided for the latter. From here onward the history of developmental biology can be traced as alternations between these two explanatory concepts.

Contentious debate began in the seventeenth century regarding epigenesis-preformation. In *Exercitationes de Generatione Animalium* (1651, p. 121), William Harvey (1578–1657) first used "epigenesis" to argue that development proceeds as incremental formation of new entities out of nonstructured germ material. Based on the first microscopical observations of Leeuwenhook (1632–1723) and Malphigi (1628–1694), the early preformationists countered that the complete organism exists already in miniaturized form in the egg (ovist view) or sperm (spermist view) and that embryonic development only consists of the elaboration of the preexistent form. Albrecht von Haller (1708–1777) concluded categorically that there is no epigenesis, and he used the term "evolution" (unfolding) to characterize the preformationist concept of embryological development, as elaborated by Swammerdam (1637–1680) and later by Bonnet (1720–1793).

These and other authors favored the view that God had created germs containing miniature organisms, which in turn contained germs that contained miniature organisms, and so forth, with the effect that "the entire human race already existed in the loins of our first parents, Adam and Eve" (Swammerdam, 1685, p. 46). Bonnet also believed that the world had undergone several catastrophes in which the adult organisms had disappeared but their germs survived. These germs would then have developed into new forms after each catastrophe. Here Bonnet connects individual development with that of species renewal and is thus chiefly responsible for the beginning shift of the term "evolution" toward signifying the unfolding of progressively more perfect forms of "organized beings." The embryological notion of unfolding had important consequences for Darwin's understanding of species transformation and the conceptualization of evolutionary theory (Richards, 1992).

In renewed opposition against the rising preformationist explanations, Caspar Friedrich Wolff (1734–1794) developed a theory of epigenesis in *Theorie von der Generation* (1764). He noted, for example, that the heart and circulatory system of the chick form gradually from an undifferentiated mass, and considered the preformationist view that the organs were already formed, but too small to see, a fable. However, because epigenesis assumed the absence of preformed structures, it required an organizing force that produced differentiation out of uniformity, which led to vitalist notions, first expressed by Blumenbach (1752–1840) and subsequently endorsed by many empirical embryologists, such as Karl Ernst von Baer (1792–1876) and Hans Driesch (1876–

1941). In contrast, the main attraction of preformationism was that it made unnecessary the search for additional causes of the transformation from undifferentiated to differentiated form; only more simple processes, such as growth, needed to be explained.

In the nineteenth century, under the new auspices of a well-developed cell theory and a rising understanding of the factors of heredity and species evolution, the epigenesis-preformation debate famously went through another round when August Weismann's (1834–1914) views collided with those of Oscar Hertwig (1849–1922). Weismann had originally been sympathetic to the epigenetic point of view. However, his ideas about inheritance and differentiation forced him to become "convinced that epigenetic development is an *impossibility*" (Weismann, 1893, p. xiv). Weismann envisioned that differentiation during development was regulated by "determinants" already present in the germ plasm or, more precisely, in the chromosomes that had just been detected. These determinants he thought represented the basic units of heredity, constituting a preformed entity in each new generation. His preformationism saw ontogeny as a process in which the inherited determinants became distributed in such a way that each cell receives the correct combination of determinants to make it into a specific cell type.

Oscar Hertwig criticized Weismann in *Präformation oder Epigenese* (1894). Hertwig, whose work pointed to the nucleus as the locus of heredity, argued that by allocating the causal factors that regulate development to "determinants" that could not be directly observed, Weismann had abandoned the scientific method. Despite this criticism, Hertwig agreed with Weismann that the germ plasm was already highly organized, but he emphasized that embryonic development was influenced by external factors, even in its early stages. Hertwig pointed out that complex processes, such as gastrulation, in which many cells must cooperate, are difficult to explain using Weismann's determinants only. He also argued that the way in which embryos respond plastically to changing environmental conditions and experimental manipulations posed substantial problems for Weismann's view.

Around the middle of the twentieth century, with genetic theory in full swing, "epigenetic" began to shift its meaning, mostly through the work and writings of Conrad Hal Waddington (1900–1975). Combining aspects of epigenesis and genetics, Waddington suggested the term *epigenetics* as an English language equivalent to *Entwicklungsmechanik,* instead of the unwieldy direct translation "developmental mechanics"

(Waddington, 1956b, p. 10). Thus, for Waddington, epigenetics generally signified the causal analysis of development and, in particular, all interactions of genes with their environment that bring the phenotype into being. Here we find the roots of the semantic shift from *-genesis* to *-genetic,* which caused much confusion in latter-day interpretations of epigenetics.

At the same time Waddington emphasized the evolutionary importance of the *epigenotype* as a historically acquired, species-specific network of developmental interactions that has further consequences for the evolvability of a phylogenetic lineage. He coined a number of concepts to address these developmental and evolutionary mechanisms, such as the "epigenetic landscape," "chreods," and "assimilation," all related to the new, causal epigenetics he advocated. Most authors in the second half of the twentieth century refer to Waddington's concepts when they speak of epigenetics, and the prevailing usage in developmental texts still evokes the context-dependency of developmental processes (e.g., Løvtrup, 1974; Hall, 1998). Less prominently, and quite independently from the developmental interpretations—although equally rooted in Waddington's views—"epigenetic" started to be used in population genetics to signify environmentally induced phenotypic variation.

The shift of meaning introduced by Waddington strengthened toward the end of the twentieth century by an increasing association of the term epigenetics with molecular mechanisms of selective gene regulation and non-DNA-based forms of mitotic and meiotic inheritance. Although a certain notion of developmental context has remained, the prevailing emphasis now is on the regulatory mechanisms of gene activity, and new definitions of epigenetics are framed in the vocabulary of genetics (e.g., Holliday, 1987, 1994; Russo et al., 1996; Chadwick and Cardew, 1998; Urnov and Wolffe, 2001). This has effectively removed the problem of embryonic form generation from the discussion of epigenetics, although the core questions of the epigenesis-preformation debate started in the seventeenth century resurface in the postgenomic era. They are cast in different terms: Is all information to build a body contained in DNA sequences? In other words, can we predict what an unknown organism would look like if its complete genome were known? And is information contained in the genome deterministically exhaustive, or does information arise through the processes of developmental interaction in which many other, nonprogrammed factors participate?

Not only does the epigenesis-preformation debate continue in new

form, but different usages of epigenetics coexist at the beginning of the twenty-first century. Textbooks of developmental biology do not refer to gene silencing, paramutation, or non-DNA-based inheritance when they address epigenetic factors, and neither do population genetic texts. And molecular geneticists do not refer to reaction norms, environmental factors, or context-dependent morphogenesis when they mention epigenetics. Therefore, a closer look at the current usages and their interrelations is necessary.

Connotations of the Term Epigenetic

Four connotations of *epigenetic* can be distinguished in modern usage. Although the scientific domains to which they apply overlap, the four usages are characterized separately before their interrelationships are re-established in the final section.

Epigenetic development (epigenesis). In developmental biology epigenetic still refers primarily to epigenesis, i.e., the individual generation of embryonic form through a series of causal interactions. It emphasizes the fact that development does not consist merely of the reading out of software-like genetic programs but also depends on a species-specific and context-dependent set of regulatory exchanges, also with factors not encoded in DNA. Epigenetic factors are "all conditional, non-programmed factors that act on the materials of the zygote and its derivatives, including those specified by the genes that are necessary to generate three-dimensional biological form" (Müller and Newman, 2002). Such factors comprise all elements and processes active in the molding of tissues at various times in development. They can be internal, belonging to the embryonic system itself, or external, belonging to the environment.

Internal factors of epigenesis reside in (1) the maternal materials and non-DNA-based templates (e.g., cell membrane) passed on from the parent organisms; (2) the generic physical and self-organizational properties of cells and tissue masses; (3) epigenetic gene regulation; (4) the dynamics of interactions among and between cells, cell populations, and tissues; (5) the spatial, geometric, and biomechanical conditions of expanding cell masses; (6) the material properties of intra- and extracellular cell products; (7) activity of the tissues and of the whole embryo; and (8) the intra- and extracellular presence of foreign materials or parasitic organisms. External factors reside primarily in the physicochemical

conditions of the environment in which development takes place, such as temperature, humidity, gravity, light, radiation, mechanical influences, and the chemical composition of the surroundings and nutrients. External factors can also consist of the activity and products of other organisms.

The epigenetic domain of development represents the transformational interface between molecular and macroscopic levels of organismal organization. Epigenetic factors can be specific or unspecific, permissive or instructive, and are converted by the developmental system into a specific morphological outcome. Much in the same way as the phenotype is composed of structural units, and the genotype of units of gene expression, the epigenotype can be conceived as consisting of units of developmental integration.

Epigenetic gene regulation and mitotic propagation (epigenetics). In molecular and developmental genetics, epigenetic refers primarily to modifications of gene activity that are not based on alterations of DNA sequence and that provide mitotically propagated changes in gene function. Epigenetic repression is responsible for genomic imprinting and related phenomena of allelic exclusion. Its molecular mechanisms include cytosine methylation, histone hypoacetylation, and RNA silencing, but sometimes mRNA processing and other forms of posttranscriptional modification are also termed epigenetic.

DNA methylation, primarily of cytosine, is the best-understood mechanism of gene silencing. Methylation at regulatory regions, especially within the promotor, prevents the binding of regulatory factors at these sites. There is a strong positive correlation between the extent of methylation and the degree of silencing. Already suggested in the mid-1970s, these mechanisms were experimentally vindicated in the early 1990s (reviewed in Urnov and Wolffe, 2001). DNA methylation is essential for the normal control of gene expression in development. Significantly, methylation patterns are mitotically propagated, thus maintaining differential functional states of the genome in cell lineages, also called *imprinting*. Unfortunately, this process is often subsumed under the term "epigenetic inheritance," blurring the distinction between development and inheritance.

The most striking example of imprinting is X chromosome inactivation in female placental mammals. Of the two X chromosomes, one is inactivated and heterochromatized in each cell, forming the so-called Barr body. This inactivation requires a gene on X called *XIST*. *XIST*-

RNA accumulates along the chromosome containing the active *XIST* gene and proceeds to inactivate (almost) all of the other genes on that chromosome (Lyon, 1995).

More common than the suppression of an entire chromosome is the inactivation of single genes or of small clusters of genes. When methylation goes wrong, the effects on the developing organism can be dramatic. In humans, a number of genetic diseases are caused by loss of imprinting, probably triggered by defective methylation (Chadwick and Cardew, 1998). Loss of imprinting is also an important factor in tumor formation (Jones and Laird, 1999), possibly because the effect of a mutation on an imprinted allele will not be rescued by the other, nonmutated allele. The study of epigenetic gene regulation has become an important field of biomedicine including cancer and stem cell research, somatic gene therapy, transgenic technologies, cloning, teratogenesis, and so forth.

Epigenetic Inheritance in Sexual Reproduction

In evolutionary biology, epigenetic inheritance refers to the transmission of epigenetic states from one generation to the next, via the germ line, without a change in DNA sequence. This is viewed as a second inheritance system, based on the same mechanisms as the passing on of gene deactivation patterns in cell lineage propagation. Although differential methylation states are generally erased during sexual reproduction through reprogramming in germ cell and pre-implantation development (Reik et al., 2001), certain epigenetic marks seem to be able to escape erasure.

Parental imprinting is the best known phenomenon of this kind. It confers to the offspring an asymmetry between the activation states of parental genes. Only one allele is normally active, either from the father's or from the mother's pronucleus; the other allele is permanently silenced. In general, the silent allele is hypermethylated, and the active allele is often hypomethylated. Among vertebrates, parental imprinting is known to occur only in mammals, such as in mules and hinnies, in which it can make an important difference whether a gene comes from the mother or the father. In the early 1980s it was first shown that male and female genomes cannot substitute for each other (Surani et al., 1984). The exchange of the pronuclei of mouse eggs produced zygotes with two male or two female haploid genomes, but both failed to develop normally. These effects are now explained by the absence of differently imprinted genes.

Paramutation is another case in which epigentic inheritance is implicated. It is a type of gene silencing that involves, in a heterozygous condition, the inactivation of one allele by a mutated allele, and this condition is retained after the alleles have segregated in the offspring. The mechanism is possibly related to cosuppression, involving homologous pairing of genes with direct effects on transcription. Such meiotic inheritance of epigenetic states was first described in plants (Brink, 1960), but was recently also observed in fission yeast, insects, and mammals.

Epigenetic inheritance reflects the functional history of a gene and thus raises the controversial issue of the transmission of individually acquired, functional states from one generation to the next. Because it is known that methylation and other forms of epigenetic chromatin marking can depend on environmental influences, epigenetic inheritance has been argued to represent a kind of Lamarkian mechanism in evolution (Jablonka and Lamb, 1995).

Epigenetic variation. In population genetics, the variation of a phenotypic trait caused by environmental or behavioral factors is sometimes called "epigenetic variation." Epigenetic variation takes place within the range of phenotypic plasticity for a given trait, depending on its genetic reaction norm (Suzuki et al., 1986). A reaction norm is defined at the population level, representing the complete range of phenotypes that a genotype can express in interactions with the environment. Epigenetic variation thus reflects the influence of the environment on individual development and represents one of the modes through which the same genotype can give rise to different phenotypes (Gilbert, 2001).

Although reaction norms are usually defined in genetic terms alone, the factors of epigenesis, epigenetic gene regulation, and epigenetic inheritance are equally important for epigenetic variation. Individuals that are genetically identical (i.e., sufficiently similar that the small genetic differences play no role) can differ epigenetically and therefore differ in their phenotypes. Hence epigenetic variation is a term that encompasses the phenotypic consequences of the epigenetic factors discussed above.

Epigenesis and Epigenetics: A Common Agenda

The results of genome sequencing projects indicate that organismal evolution has proceeded not so much by an increase in the number of genes

but rather through regulatory modification. Part of this modification is epigenetic. Epigenetic modulation acts at different levels of the evolutionary process, which can be distinguished as the generative level, the integrative level, and the inheritance level. The different types of epigenetic mechanisms interact in a contingent, lineage-specific way and have different roles at each of the levels of evolution. Brief characterizations follow.

The generative level of evolution is the level from which variation and innovation take their origin. Whereas neo-Darwinian theory has focused on variation, the mechanisms of phenotypic innovation are much less explored (Müller and Wagner, 1991; Müller and Newman, 2002). It has been argued that epigenetic factors governing the behaviors of cells and tissues had an important influence on morphological evolution, both at the early origination of multicellularity and metazoan body plans, and during later innovations in advanced organisms. These generic forms would have been based on the physical processes characteristic of condensed, chemically active materials, such as cells and cell masses, and on the conditional, inductive interactions that became established in evolved developmental systems (Newman and Müller, 2000). This concept—that epigenetic mechanisms are the generative agents of morphological innovation—has been suggested to contribute to explanations of complex phenomena in evolution, such as the origins of novelty and homology and rapid morphological change.

The evolution of epigenetic gene regulation might have played an important role both during these generative events and also at the integrative level of evolution, at which generative changes become consolidated and made routine by molecular control mechanisms. Epigenetic regulation probably arose from cellular defense mechanisms against viruses and parasitic DNA and was later recruited for differential gene regulation (Matzke et al., 1999). One important evolutionary aspect is that these mechanisms might have contributed significantly to genome evolution, via the silencing of duplicated gene loci, and thus might have facilitated the evolution of developmental differentiation and determination processes. On the other hand, these epigenetic regulation mechanisms might have assisted in the developmental and genetic integration of successful innovations that arose from the epigenesis-type mechanisms listed earlier. This picture is supported by the finding that gene-silencing mechanisms seem to have gained their major influence only in higher

plants and vertebrates, whereas in other organisms, including most invertebrates, methylation seems to be a relatively unimportant event. In vertebrate evolution, a moderate increase of DNA content was accompanied by a substantial increase of methylation (Bird, 1995), indicating new roles for gene silencing.

At the inheritance level, the transgenerational transmission of methylation patterns and other epigenetic states represents one of the most challenging aspects. It infers that a second, autonomous, non-DNA-based inheritance system is at work, and it includes the possibility that epigenetic states acquired in one generation can be transmitted to the next (Jablonka and Lamb, 1995). Environmental stimuli may thus have a more direct influence on adaptive modification than is generally assumed under the neo-Darwinian model. This type of epigenetic inheritance is likely to have its greatest impact on the evolution of organisms that lack a distinct segregation of soma and germ line, such as fungi and plants. It could also assist in reproductive isolation of higher plants and animals and could indirectly affect DNA base changes by influencing the frequency of mutations at certain loci. Furthermore, there is the clear possibility that epigenetic states become fixed by random mutation and natural selection (Maynard Smith, 1990). This transition from an epigenetic to a genetic state corresponds to the process of "genetic assimilation" proposed by Waddington (1953a).

In each traditional research area—molecular genetics, development, heredity, evolution—"epigenetic" refers to a different issue. The molecular mechanisms of gene regulation, the embryonic generation of form, the transmission of information from one generation to the next, and environment-induced variation do not represent the same biological problem. However, these issues are linked through an integrative, evolutionary perspective. In this view, the epigenetic agenda emerges as the science focusing on the role of the nonprogrammed, regulatory, and modulating factors in biological processes. Whereas evolution was traditionally studied either from a genetic or from a phenotypic perspective, the common epigenetic agenda represents a new level of analysis, focusing on the causal interactions between genes, phenotypes, and the environment. This epigenetic agenda will be central for the formulation of integrative models in evolutionary developmental biology.