PERSPECTIVES

Epigenetics comes of age in the twentyfirst century

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Origins of epigenetics

In the nineteenth century most leading biologists believed that development and heredity were one and the same problem. Even as late as 1896, the first edition of E. B. Wilson’s famous book had the title *The cell in development and inheritance*, even though, in the absence of the recognition of Mendel, almost nothing was known about the mechanism of heredity. It is extremely likely that Mendel’s work was ignored for 30 years precisely because heredity was linked to development (Sandler and Sandler 1985). When his work was rediscovered at the beginning of the twentieth century, the discipline of genetics was launched. Mendel’s genius was to unravel the laws of inheritance without knowing anything about the process of development, and most geneticists followed this path thereafter. In this context, it is interesting to note that T. H. Morgan, the leader of the famous school of *Drosophila* geneticists, started his career as an embryologist, and his last book was on embryology and genetics (Morgan 1934). However, he did not include *Drosophila* development as a major topic of his research. For the most part, the embryologists and developmental biologists immersed themselves in their own studies, and did not think about the way genes might control the unfolding of the developmental programme. J. B. S. Haldane was one of the few geneticists who was interested in the way genes controlled metabolism, following the earlier work of Garrod (1909) on inborn errors of metabolism. In the 1940s, Beadle and Tatum formulated their famous concept of one gene one enzyme, which proved to be largely correct.

There were two leading geneticists who also studied embryology, and who fully realized that sooner or later the two disciplines would coalesce. One was Ernst Hadorn, who studied development in *Drosophila*, and the other was C. H. Waddington, who first introduced the term epigenetics (Waddington 1939). In general terms, this was the study of the way genes controlled and directed the developmental programme. It was derived from the early dispute between the ‘preformationists’ and those who believed in epigenesis. The former asserted that the sperm and fertilized egg already contained a preformed organism with all major features represented. In contrast, epigenesis was the process whereby more and more complex structures emerged during development. Obviously, this view is correct, so the term epigenetics had validity from the outset.

DNA methylation

In development there are many branching processes, whereby a given cell gives rise to two independent cell types. Such a process can be regarded as a developmental switch in which one daughter gains a new phenotype, or potential phenotype, whereas the other retains its original phenotype. This is a stem cell situation, but in other cases both daughter cells may have phenotypes different from each other and also different from the parental cell. There is also the stability of differentiated cells, so that lymphocytes, fibroblasts and keratinocytes, and so on, retain a stable karyotype through many divisions. These cells contain the same chromosomes, the same genes, and the same DNA sequences, but their patterns of gene expression are different. They can be said to have different epigenotypes.

The concept of switching of gene activities, and the somatic inheritance of a given set of active and inactive genes, provided the basis for a new epigenetic mechanism, namely DNA methylation. 5-Methylcytosine (5-mC) is produced by an enzyme, DNA methyltransferase, which was known to add a methyl group to the carbon at the 5 position of the pyrimidine ring soon after replication. In mammalian DNA 2–5% of cytosines are methylated, but not at random because CpG doublets are the major site of methylation, and these doublets are depleted in relation to the cytosine and guanine contents. Holliday and Pugh (1975) and Riggs (1975) independently proposed that 5-mC may have an important role in controlling gene expression during development in higher organisms. Riggs was particularly interested in X chromosome inactivation in female mammals, where early in development one X chromosome is permanently inacti-

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vated in every cell, whilst the other remains active. In eutherian mammals this requires a random switch and subsequent maintenance of the active and inactive states. Holliday and Pugh discussed these fundamental features in the wider context of development. Both publications proposed that the hemimethylated DNA after replication is a substrate for a maintenance methylase, which does not act on unmethylated DNA. Neither of these early papers referred to Waddington’s term epigenetics. The connection between DNA methylation and epigenetics was made explicit in 1987 when I published a paper in Science titled ‘The inheritance of epigenetic defects’ (Holliday 1987). This was concerned in part with a long-standing dispute in somatic cell genetics, where it had been argued on the one hand that inherited variation in mammalian cells was not, or need not be, due to gene mutations, and on the other hand that mammalian cells were essentially similar to microbial systems and mutations could be induced and studied in much the same way. As is often the case in such controversies, both viewpoints turned out to be correct, because it had become apparent that gene inactivation could not only be due to mutation, but also to DNA methylation. This was demonstrated by the use of the potent demethylating agent 5-azacytidine (or 5-azadeoxycytidine) which reactivated at high frequency genes silenced by DNA methylation. The term epimutation had been used earlier to describe heritable changes in phenotype which are not due to any alteration in DNA sequence (Holliday 1984). Also, it had been proposed that heritable changes in DNA methylation might be important in carcinogenesis (Holliday 1979). The 1987 paper in Science elaborated on the possible role of epigenetic defects or epimutations in cancer and ageing, and also discussed evidence that they might be transmitted from one generation to the next, a topic that was explored in much more detail by Jablonka and Lamb (1995).

A major advance in the study of epigenetics was the discovery of genomic imprinting in mammalian gametes. It became clear that the paternal and maternal gametes have different information which is superimposed on the chromosomal DNA of one parent and not the other. The two genomes complement each other to produce a normal zygote, whereas zygotes arising from two paternal nuclei or two maternal nuclei produce abnormal embryos. Many studies have been done that link imprinting to differences in DNA methylation in male and female gametes. It is not known in detail how long imprinting persists in the developing organism, but it is certain that in the formation of gametes for the next generation the normal pattern of imprinting is reimposed.

Much information about DNA methylation and the epigenetic control of gene activity is now available in plants (Martienssen and Colot 2001). Also, in the last few years there has been an explosion of interest in the inactivation and methylation of tumour suppressor genes during tumour progression. In the years since 1995 there have been over 1400 publications on this topic. Another very important advance comes from studies that relate DNA methylation to changes in chromatin proteins, and particularly the acetylation and deacetylation of histones (Rountree et al. 2001).

In many of these studies the term epigenetics has been used, but it is notable that in the area of mammalian development a key role for DNA methylation has not yet been revealed. There are, nevertheless, many reasons for believing that DNA methylation does play a crucial role in unravelling the developmental programme, although that may not be the common view amongst developmental biologists. One reason for this was the frequent assertion that Drosophila DNA contained no 5-mC, but this belief has recently been shown to be mistaken (Gowher et al. 2000; Lyko et al. 2000).

**Other epigenetic mechanisms, and the definition of epigenetics**

DNA methylation is a major epigenetic mechanism, but it is not the only one. For example, the changes in DNA that occur in the assembly of immunoglobulin genes in cell lineages of the immune system should be regarded as an epigenetic mechanism, which in this case depends on the creation of new DNA sequences. In my view, the alternative splicing of gene transcripts that occurs in different cell types is also an epigenetic mechanism, because it results in the formation of particular polypeptide chains and proteins appropriate for a specialized cell. There are regulatory proteins that stably repress or induce particular genes without any involvement of DNA methylation, but it is not usually clear whether these gene activities, or inactivities, are stably inherited through mitosis.

Whether or not a particular process or mechanism should be regarded as epigenetic ultimately depends on the definition of epigenetics, which was the subject of a series of letters in Science (Wu and Morris 2001). It is difficult to find a single definition, and in 1994 I suggested two, namely:

(i) The study of the changes in gene expression that occur in organisms with differentiated cells, and the mitotic inheritance of given patterns of gene expression.

(ii) Nuclear inheritance that is not based on differences in DNA sequence.

In contrast, Wu and Morris suggest a single definition:

The study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence.
I consider this to be far too narrow a definition because it does not encompass changes in somatic cells that should surely be regarded as epigenetic. It does not include an example I have just mentioned, the rearrangement of DNA sequences to form antibody genes. It excludes all changes in phenotype induced by a morphogen, hormone or growth factor that is not followed by cell division. The induction of vitellogenin by the hormone oestradiol is a case in point, where it has been shown that the induction is preceded by specific demethylation of DNA at the oestradiol response element (Saluz et al. 1986). Surely this is an epigenetic change where no cell division is involved? In fact, Wu and Morris’s definition excludes all changes in post-mitotic cells, including neurons. I believe it is unjustifiable to exclude the processing of information in non-dividing cells of the brain, including the imprinting of parental images in young animals and many other examples of learning. Indeed, I have suggested that long-term memory could be based on an epigenetic switch involving DNA methylation (Holliday 1999). This is an invalid use of the term epigenetic according to Wu and Morris.

On the matter of definitions, epimutation has already been mentioned, but we also need the term epimutagens (agents that induce epimutations) and therefore epimutationogenesis. An epimutator is a cell strain in which epimutations occur at unusually high frequency, one of which has already been discovered (Holliday and Ho 1998). The term epigenotype is particularly useful, because it covers the situation common to all higher organisms: their somatic cells, with rare exceptions, have the same genotype, but they have different epigenotypes. That is, they have different patterns of gene expression that give rise to the phenotypes of all the specialized cells in the organism.

Conclusions

What is certain is that the rules governing epigenetic processes are completely different from those governing classical genetics. The geneticists of the twentieth century confirmed Mendel’s results and successfully uncovered the basic mechanism of heredity in a wide variety of eukaryotes, and all the variations that can be imposed on that mechanism. It is a fairly safe prediction that the twenty-first century will reveal many epigenetic mechanisms, and the way they govern or influence the developmental programmes of animals and plants. The relative importance of the epigenetic transmission of information from one generation should also become apparent, as well as its possible role in evolution. Will it merely comprise a set of rather unusual examples of non-Mendelian inheritance? Or will it open new possibilities about the way environmental influences might alter the phenotypes of subsequent offspring, as Jablonka and Lamb (1995) have argued? There are many cases of human inheritance that are at present not understood, and these may be imprecisely labelled as examples of ‘multifactorial inheritance with incomplete penetrance’. Could these ill-understood types of inheritance be due to epigenetic information superimposed on the normal genomic DNA? Finally, it must be said that the sequencing of the human genome can only be the beginning of a very long set of further studies. The term epigenomics has already come into use, and this will encompass the inclusion of the fifth base 5-mC in future studies of DNA sequences (Beck et al. 1999). Not only that, because it will be necessary to examine the DNA of many cell types. We can expect some tracts of DNA to have unchanged patterns of DNA, for example where transposable elements have been silenced by DNA methylation, but others will have sequence specificity depending on cell type. To unravel all these differences will still be an enormous undertaking, even with many improvements in the best techniques that are currently available.

References


