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What history tells us XXXII. The long and tortuous history of epigenetic marks

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1. Introduction

Epigenetics is a very active field of biological research. The introduction of the word epigenetics in the 1940s and the first work on epigenetics are currently attributed to Conrad Waddington (see, for instance, Goldberg *et al*. 2007). However, this historical presentation is problematic. Most of the work performed today in epigenetics consists of the characterization of epigenetic marks – histone modifications (and replacement by minor forms of histones) and DNA methylation. Description of these marks and speculations on their physiological role were initiated at the beginning of the 1960s for histones and in the middle of the 1970s for DNA methylation, more than twenty years after the contribution of Waddington.

In addition, nearly twenty more years were required not only for the recognition of the importance of these epigenetic marks in the control of gene expression during differentiation and development, but also for a link to be established between these two types of modifications.

The history of epigenetic marks has not been extensively studied (an exception being Olins and Olins 2003). This is a pity because the characterization of the obstacles that prevented an early acknowledgment of the importance of epigenetic modifications is not without interest in understanding the origin of the fuzziness that pervades so many descriptions of epigenetics.

2. Histone modifications and their role in the control of gene expression during differentiation and development

The hypothesis that histones inhibit the activity of genes was proposed at the beginning of the 1950s, and the first modifications of histones – acetylation and methylation – were described, respectively, in 1961 and 1964 (summarized in Allfrey *et al*. 1964). In 1962, Ru-Chih Huang and James Bonner demonstrated *in vitro* the inhibitory effect of histones on DNA transcription (Huang and Bonner 1962). Vincent Allfrey and Alfred Mirsky obtained a similar result by adding histones to isolated nuclei (Allfrey *et al*. 1963). By using the same experimental system, they showed one year later that histone acetylation alleviates this inhibitory effect (Allfrey *et al*. 1964) without abolishing the interaction between histones and DNA. In a brief note published in *Science*, Allfrey and Mirsky concluded that these findings 'suggest the possibility that relatively minor modifications of histone structure, taking place on the intact protein molecule, offer a means of switching RNA synthesis on or off at different loci along the chromosome' (Allfrey and Mirsky 1964).

Although this statement would be applauded by contemporary biologists, it was not considered with enthusiasm by biologists at the time it was proposed. In 1968, Eric Davidson, who had been the student of Mirsky and had published many articles with him, discussed this hypothesis in his famous book *Gene activity in early development* (Davidson 1968). In the last chapter, where he proposes 'some hypotheses regarding the nature of genomic regulation in differentiated cells,' he not only refuses to use the operon model to explain the control of gene expression during development in higher organisms, but also rejects the hypothesis that histone modifications might be responsible for this control. He considered that control by repressors would not be stable enough and would require too many genes (those coding for repressors). An additional experimental argument in rejecting the use of the operon model was that the existence of operons had not been confirmed in eukaryotes. For

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Davidson, the principles of genetic regulation operating in eukaryotes were different from those acting in prokaryotes. They consisted of a general, non-specific inhibition of gene expression by histones, combined with positive and selective regulation by activators that Davidson identified with RNAs. These ideas were developed in the famous 'Britten-Davidson' model that he proposed the following year (Britten and Davidson 1969). This conviction that the action of histones was a global, non-specific inhibitory effect was shared by most specialists. From 1973, the discovery of nucleosomes (Olins and Olins 1974) and their progressive structural characterization (Kornberg 1974; Richmond *et al*. 1984) focused attention on the organization of the DNA fibre more than on the control of transcription. These first structural studies did not reveal an obvious effect of histone modifications on the overall structure of the nucleosome. Strong experimental arguments showing the role of these modifications in transcription had to wait till the 1990s and the characterization of the proteins that recognized these marks, and acted on the transcription machinery.

3. DNA methylation and its role in the control of gene expression during differentiation and development

Methylation of DNA (and RNA) in prokaryotes was discovered in the 1950s. Its potential role in eukaryotes was discussed as early as 1964: it might protect DNA or participate in the process of differentiation (Srinivasan and Borek 1964). Scarano proposed that DNA methylation might induce mutations that were required for the progression of the developmental program (Scarano *et al* 1967). However, this model, in which DNA is modified during development, was not compatible with the cloning experiments performed by John Gurdon (Gurdon 1962).

In the mid-1970s, conditions were favourable for the development of these early speculations. X chromosome inactivation in mammals (Lyon 1968), and more generally the formation of heterochromatin (Brown 1966) had received in the previous years a lot of attention, in relation to the development of new staining techniques. More importantly, the modification/restriction system operating in bacteria had been extensively described (Arber and Linn 1969), and restriction enzymes had been purified and used in the first genetic engineering experiments (Jackson *et al*. 1972). Meanwhile, developmental biologists were still searching for a mechanism explaining the control of gene activity during differentiation and development. In 1975, two articles proposed that DNA methylation at the 5 position of cytosines in CpG sequences was responsible for the control of gene expression during differentiation and development (Holliday and Pugh 1975; Riggs 1975). There were no more experimental arguments in favour of this hypothesis than there had been ten years earlier, when it was proposed for the first time. But the recent visibility of prokaryotic restriction enzymes made such a hypothesis more plausible. The similarities between the methylases controlling development and the modification enzymes of prokaryotes were obvious for Holliday and Pugh: both types of enzymes recognized specific sequences of DNA. The stability of DNA modifications resulted from the existence of two different enzymes, the first responsible for the initial modification of one DNA strand, and the second adding a methyl group to the second strand, and repeating this process at each cell division. Subtle mechanisms were proposed to explain how the system could function as a clock, counting the number of cell divisions during development or during life. This meant that the same mechanisms could also be involved in the ageing process.

The article by Arthur Riggs drew a similar parallel with the modification/restriction system operating in prokaryotes. The major part of the article argued that such a mechanism was an explanation of X chromosome inactivation, but its author proposed that it was a particular case of general mechanisms involved in the control of differentiation.

The same year, Ruth Sager pushed the comparison with the modification/restriction system even further, suggesting that DNA methylation might be responsible not only for gene and chromosome inactivation but also for the loss of DNA that occurs during development in some eukaryotes (Sager and Kitchin 1975). In a similar way, Holliday and Pugh proposed that cell death by apoptosis, a recently discovered phenomenon involved in normal development and accompanied by DNA degradation, might result from the same methylation process (Holliday and Pugh 1975).

The narrow parallel established with the modification/restriction system required a sequence specificity for the methylases operating during development that later observations did not confirm. The precise description of the methylated residues in DNA sequences, as well as the effects of a drug – 5aza-cytidine – in preventing DNA methylation and activating gene expression, supported the existence of an antagonistic relation between DNA methylation and gene expression. But the precise nature of this relation remained elusive (Felsenfeld and McGhee 1982; Doerfler 1983). In addition, the discovery in 1981 that DNA methylation favours the transition from the B structure of DNA to the recently discovered Z structure did not help clarify the role of DNA methylation (Behe and Felsenfeld 1981; to appreciate the excessive hopes generated by the discovery of Z DNA, see Morange 2007). During late 1980s, the demonstration of the involvement of DNA methylation in genomic imprinting was an additional argument in favour of a role for DNA methylation in gene regulation, at least in mammals (Reik *et al*. 1987; Li *et al*. 1993).

Faced with these difficulties, Holliday in his later publications emphasized the role of DNA methylation in ageing

and intergenerational inheritance while silently renouncing his earlier models. Additionally, he provided a new, extended definition of epigenetics (Holliday 1987; 1994). He is in part responsible for the plurality of meanings that the word has progressively acquired.

4. Some lessons for current epigenetics

What is most striking in this early history of epigenetic marks is the total absence of communication between researchers working on histone modification and those studying DNA methylation. The two groups of researchers were completely separate. This is redolent of the well-known opposition between molecular biologists, more interested by DNA, and biochemists studying proteins. It found its emblematic origin in the opposition of Mirsky to the demonstration by Oswald Avery that the transforming factor in *Pneumococcus* was DNA. This absence of communication prevailed despite the fact that the study of the two phenomena had a common origin in the doubts concerning the possible extension of the repressor model of gene regulation described in bacteria to the explanation of development in higher organisms.

Both phenomena suffered similarly from a lack of specificity. The problem was immediately obvious in the case of histones, but was progressively attenuated by the discovery of the astounding diversity of histone modifications. In contrast, DNA modification, which had been hypothesized to be highly sequence-specific, progressively lost this characteristic. Eric Selker was one of the first in 1990 to acknowledge that modification in prokaryotes was not a good model for understanding the characteristics of DNA methylation in eukaryotes (Selker 1990). In the case of methylation, an additional difficulty was the apparent absence of this phenomenon in the two organisms that were the preferred models of developmental biologists, *Drosophila* and nematodes. These difficulties recurrently led to questioning of the meaning of observations made on DNA methylation and histone modifications: were these phenomena the cause or the consequence of changes in gene expression?

One could imagine that all these problems disappeared when the two lines of research converged at the end of the 1990s with the description of the mechanisms linking histone modification to DNA methylation. But such was not the case. The characterization of these modifications as ‘causes’ or ‘consequences’ is still debated (Stadler *et al*. 2011; Thurman *et al*. 2012). The difficulty of determining in which physiological processes epigenetic marks are the most important also remains. Their lability, which is optimal for the adaptation of gene expression to a changing environment, is poorly compatible with an alleged role in transgenerational inheritance.

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