Series
DOI 10.1007/s12038-009-0098-z

What history tells us XIX. The notion of the episome

Michel Morange
Centre Cavaillès, Ecole normale supérieure, 29 rue d'Ulm, 75230 Paris Cedex 05, France
(Fax, 33-144-323941; Email, morange@biologie.ens.fr)

1. Introduction

Modern textbooks of biology and molecular biology describe episomes only briefly or not at all. Plasmids receive much more attention. The notion of episome was first introduced in a publication of the French Academy of Science by François Jacob and Elie Wollman in 1958 (Jacob and Wollman 1958): episomes "may or may not be present in a cell, and when present they may be either autonomous or integrated" (in the chromosome) (Jacob and Wollman 1958). Episomes were more precisely described in publications that followed (Jacob *et al.* 1960), as well as in the last chapter of a book on the sexuality of bacteria first published in French in 1959 (Wollman and Jacob 1959a), and translated into (American) English two years later (Wollman and Jacob 1961). The emergence of this new concept was the result of the work pursued in the previous five years on lysogeny and bacterial conjugation by the Pasteur group (Brock 1990).

Ten years later, in 1968, in his introduction to a Ciba meeting on episomes and plasmids, William Hayes proposed abandoning the word episome in favour of that of plasmid, and discussions followed (Hayes 1969; Grote 2008). In fact, the word plasmid was already in common use, and this trend continued to the present.

Words have an important role in scientific knowledge. According to the French chemist Lavoisier "Words must generate the idea, and the idea must represent the fact; and as words conserve and transmit ideas, the result is that language cannot be improved without improving science, or science improved without improving language, and that however well established the facts, however exact the ideas generated by these facts, the facts will still convey false impressions if we lack the right words to describe them" (quoted in Jacob *et al.* 1953). There is a permanent movement of words in and out of science, as they enter discreetly or centre stage, and disappear progressively or abruptly (Hayes 1969). The replacement of one word by another is the result of a tacit consensus, or more exceptionally of a public decision of the scientific community. A good example of the latter was the replacement in 1953 of the expression "enzymatic adaptation" by "enzymatic induction", in the context of the Cold War, and of the opposition of geneticists to Lysenkoism (Cohn *et al.* 1953): enzymatic adaptation was not similar to evolutionary adaptation, and the possible role of substrates in enzymatic adaptation ought not to be considered as a support to a neo-Lamarckian model of evolution, in which the environment directly modifies organisms. Another case was this renunciation of the word "episome" – with the exception of a few well-defined cases – adopted during a meeting which, paradoxically, might have appeared as a celebration of the importance of episomes and plasmids (Wolstenholme and O'Connor 1969)!

I will successively consider the conditions in which this notion of episome emerged, the reasons for its introduction by Jacob and Wollman, as well as the conditions in which its use was "officially" restricted.

2. The emergence of the notion of episome

I will be brief, because this part of the story is well known. The phenomenon of lysogeny, the possibility that a bacteriophage will remain silent within a bacterium for numerous generations, had been denied by many microbiologists, including those of the American Phage group (Wollman 1992), until André Lwoff in 1950 provided an experimental way to induce the development of bacteriophages in lysogenic bacteria (Morange 2005b). Ester and Joshua Lederberg (Lederberg and Lederberg 1953) and Wollman (1953) rapidly showed that the capacity

Keywords. Cancer; chromosome; conjugation; development; differentiation; lysogeny; plasmid

of bacterial cells to produce bacteriophages after induction was closely linked to the galactose locus. A better description of the genetic exchanges between bacteria resulted from Hayes' description of two types of bacteria – donor (F+) and acceptors (F-). It was rapidly discovered that during conjugation the capacity to produce bacteriophages, the lysogenic character, was never transmitted from a donor to a recipient bacterium. If F+ and F- bacteria are likened respectively to males and females, this means that lysogeny is uniquely transmitted through maternal inheritance. These studies remained limited by the low frequency of recombination. This obstacle was overcome by the parallel and independent isolation by Hayes and Luca Cavalli of strains of bacteria that transfer their characters with a high frequency (Hfr for high frequency of recombination). Their immediate use by Jacob and Wollman confirmed that the inactive state of the bacteriophage, the prophage, was closely associated with the galactose locus. They showed that the transfer of the prophage to a recipient non-lysogenic bacterium immediately led to the induction of the prophage, and to the lysis of the recipient bacterium (Wollman and Jacob 1954). The use of a Waring blender to interrupt conjugation at precise times (Wollman and Jacob 1955), a strategy previously used by William Hershey and Martha Chase to separate bacteria and bacteriophages, allowed precise temporal mapping of the genes on the chromosome of the donor bacterium. During conjugation, the chromosome is progressively, and in most cases only partially, transferred from the donor to the recipient bacterium.

Returning to the difference between Hfr and F+ strains, Jacob and Wollman demonstrated that the low frequency of genetic transfer in the F+ population was due to the existence in this population of rare Hfr variants, resulting from the attachment of the F factor to the bacterial chromosome (Jacob and Wollman 1956). The "pure" F+ bacteria only transmitted the fertility F factor, converting the recipient female bacteria into males. It was later shown that in Hfr strains the F factor was attached to the bacterial chromosome at what appeared at that time to be its extremity, and for this reason rarely transmitted to the recipient bacteria. The characterization of different Hfr strains, showing a circular permutation of the characters which were transmitted, obliged Wollman and Jacob to abandon a linear description of the bacterial chromosome for a circular one (Jacob and Wollman 1957b).

Like the bacteriophage λ, the F factor was therefore able to replicate autonomously in the cytoplasm, or to associate with the bacterial chromosome. A similar behaviour was attributed to the colicinogenic factors involved in the production of toxins (colicins) by strains of bacteria against other strains, which had been extensively studied by the Pasteur group in previous years (Alfoldi *et al.* 1958).

It was the existence of this ensemble of genetic factors, with apparently very different functions, but behaving similarly, that pressed Wollman and Jacob to introduce the word episome to designate them, and their remarkable properties.

3. Episomes: more than a word, a functional category

André Lwoff paid great attention to the use of the "right" word, and created at the Pasteur Institute a committee on nomenclature (Jacob *et al.* 1953; Morange 2005b). André Lwoff was quite successful in his enterprise since he introduced many scientific words, like capsid, which are now part of the common scientific language. Among these newly created words, "episome" was particularly important: it describes a group of genetic factors likely to have important biological functions and which were considered in the last chapter of the book on bacterial sexuality (Wollman and Jacob 1959).

It is important to acknowledge that, in contrast to the study of virulent bacteriophages used as a model to study a property common to all organisms – reproduction – the existence of lysogeny was not anticipated, and was an orphan phenomenon waiting to be the model for other biological phenomena (Peyriéras and Morange 2002). Three such phenomena were considered by Wollman and Jacob: maternal forms of inheritance in other organisms such as *Drosophila*, the genesis of cancer, and the control of differentiation and development.

The possible relation between lysogeny and cancer had been already considered by Eugène and Elisabeth Wollman, the parents of Elie Wollman, and by André Lwoff (Lwoff 1953). By hypothesizing that episomes cause cancer, it was possible to reconcile the two antagonistic views of cancer that were dominant at that time: the viral and mutational theories, the possibility that cancer was either infectious or hereditary (Wollman and Jacob 1959b). Simple mechanistic models were proposed to explain how episomes could induce the cellular proliferation characteristic of cancer, by attaching to the genetic material of their hosts: they imposed their rapid rhythm of replication on the genetic material to which they were attached; or, by their attachment and detachment from the chromosomes, episomes might modulate the expression of cellular membrane proteins, allowing cancer cells to escape the body's defences.

Simultaneously, at the beginning of the 1960s, Howard Temin proposed that retroviruses transform cells by integrating their genetic material into the host's chromosomes. The fact that the genetic material of the virus was RNA obliged Temin to hypothesize the existence of a conversion of RNA into double-stranded DNA, which was at odds with the central dogma popularized by Jim Watson (Morange 2008), and required ten years of experimental efforts to be confirmed. Notwithstanding these difficulties, the hypothesis that cancer was due to the insertion of viral

DNA or activation of endogenous viruses, became widely accepted. Lysogeny, and the concept of episomes, were guides for most researchers working on cancer during these years. The discovery of the oncogenes at the end of the 1970s placed the perturbation of normal cellular functions at the heart of cancer, without totally rejecting the role of viruses in this perturbation. But the biological phenomena which seemed the most likely to benefit from the description of episomes were differentiation and development. The results of Barbara McClintock on the controlling elements of maize supported this role of mobile genetic elements in cell differentiation (McClintock 1956). The early experiments on nuclear transfer in amphibians by Thomas King and Robert Briggs suggested that the nuclei of differentiated cells were different from those of early embryonic cells (King and Briggs 1956). Differentiation and development might be seen as the result of the attachment to (or detachment from) chromosomes of an ensemble of different episomes.

Whereas the episome concept remained influential in cancer research for two decades, only two years were required for its abandonment as a model for differentiation and development. In the American edition of the book on the sexuality of bacteria, the role of episomes in the control of cell differentiation was more cautiously presented (Wollman and Jacob 1961). The title of the paragraph "*Les épisomes et la différenciation cellulaire*" (Episomes and cellular differentiation) had been replaced by "Episomes and cellular regulation". The reason is simple: the work done in common during these two years by Jacob and Monod had generated a radically different model of gene regulation, the operon model, which was rapidly adapted to explain development of organisms (Morange 2005a). Differentiation and development are the results of the action of regulatory genes, without any structural modification of the genome. The successful transfer of the nucleus of a differentiated cell into an egg, and the production of viable adult organisms by John Gurdon in 1962 was the last blow to this hypothesis (Gurdon 1962).

The assigning of specific roles to episomes was probably responsible for a mistaken interpretation of the relations between the episomes and the bacterial chromosome which persisted throughout these years. Instead of considering that the episomes were fully inserted in the bacterial genome, Jacob and Wollman favoured the existence of sites of attachment of the episomes on the bacterial chromosome (Jacob and Wollman 1957a; for a clear illustration of this, see Jacob and Wollman 1961). Episomes were not additional genetic information: they were something different.

4. A restricted use of the notion of episome

Despite the failure of its extension to the realm of higher organisms, the concept of episome might have remained a useful concept in microorganisms. Such was not the opinion of Hayes, who proposed dropping the word episome in favour of plasmid, a notion introduced by Lederberg in 1952 (Lederberg 1952) which had remained in use in parallel with that of episomes during these years (Hayes 1969). Hayes compared the word operon with that of cistron introduced by Seymour Benzer in his microdissection of the gene. Both words had been useful in pinpointing some remarkable functional characteristics of the objects under study, but their use had become a source of confusion. The main argument of Hayes was that the clear distinction between stable genetic elements and elements able to shift from an autonomous state to a state in which there were associated with the bacterial chromosome had been blurred by recent observations, which showed a sort of continuum between fully autonomous genetic factors and genetic elements stably integrated into the genetic material of the host. In addition, the same genetic factor may behave as an episome in a bacterial species, and as a factor fully integrated in the chromosome in another species. The category of episomes was not a well-defined or closed one.

Mathias Grote has recently interpreted the partial abandonment of the word episome as the conjunction of different transformations: the development of new techniques, which made the isolation of independent DNA molecules, called plasmids, a standard process; the capacity of plasmids to transfer drug resistance from one bacterium to another, giving these structures a high visibility; and the progressive use of plasmids for what would some years later become the tools of genetic engineering (Grote 2008). There was a de-emphasis on the role of these genetic factors in normal cells, and a parallel emphasis on their importance in medical and technological developments. The more neutral word "plasmid" was better adapted to these new roles.

But the main reason for restricting the use of the notion of episome was certainly the failure to associate it with precise functions. Imagine that the role of episomes in the differentiation of higher organisms had been demonstrated. Their role in microorganisms might have been interpreted as a participation in the simple forms of differentiation exhibited by these organisms. It is not by chance that sporulation was also studied by Pierre Schaeffer and François Jacob in the Pasteur group during this period: spores may be considered as differentiated forms of bacterial cells (Schaeffer *et al.* 1959).

This failure to define a function probably explains why Elie Wollman, present at the Ciba meeting, did not defend the notion of episome, but considered that the whole discussion was not "of great importance" (Wolstenholme and O'Connor 1969). When Alan Campbell argued that science needs both operational and cognitive definitions, and that the definition of plasmids was only operational, whereas that of episomes was cognitive, he failed to convince his colleagues precisely because, in the absence of a general function, the cognitive value of the notion of episome was far from obvious (Wolstenholme and O'Connor 1969).

The notion of episome was not fully abandoned, but its use was limited to a few systems, such as the bacteriophage λ and the F factor. Another category of genetic factors, mobile elements, progressively found their place: they can move from one chromosome to another, but unlike episomes they are never autonomous (Lederberg 1998)

The story of episomes also shows the attachment of many biologists to functions, whose existence is justified by the action of natural selection. This Panglossian view of evolution ignores the stochastic nature of variations and the invisibility of many of them to natural selection, and that selection can also act at levels of organization lower than organisms, in particular at the level of replicators. In a second step, these variations may or may not be endowed with functions. But the functions were not their raison d'être.

Acknowledgements

I am indebted to David Marsh for critical reading of the manuscript, and to Mathias Grote who sent me his article, and attracted my attention to the Ciba meeting.

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*e*Publication: 30 November 2009