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What history tells us XXXI. The replicon model: Between molecular biology and molecular cell biology

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1. Introduction

The replicon model was proposed 50 years ago by François Jacob, Sydney Brenner and François Cuzin to explain the regulation of DNA replication in bacteria (Jacob *et al.* 1963). The 50th anniversary of this event was recently commemorated at the Pasteur Institute in Paris. In this model, a protein encoded by an initiator gene binds to a specific sequence of DNA called a replicator to initiate the replication of DNA. The replicator is attached to the bacterial membrane. After replication has been completed, the two chromosomes are separated by the growth of the bacterial membrane.

It is quite remarkable that the replicon model continuously stimulated work on genome and chromosome replication for 50 years! Today, more than 400 articles related to the replicon are published every year. Despite the addition to the initial model of 'molecular complexities', the main characteristics of the 1963 model remain astonishingly true!

My aim is to return to the emergence of the replicon model, to position it in its historical background. I will show that the model was deeply anchored in the previous work of the Pasteurian group. This did not prevent the creative mind of Brenner from playing its full role in the elaboration of the model. But the model was also a first and significant step from molecular biology to molecular cell biology, as well as a decisive moment in the scientific career of Jacob.

2. A model emerging from previous work at the Pasteur Institute

The brief summary of the 1963 publication that I presented before does not faithfully reflect its content. Most of the communication was devoted to a description of the mechanism of bacterial conjugation, and the way the replicon model explained it. In previous years, Elie Wollman and Jacob had characterized the main steps of bacterial conjugation, a process by which bacteria are able to exchange genetic information: a male bacterium (Hfr) with a sexual (F) factor inserted into its chromosome transfers a partial copy of it to a recipient female bacterium. Two hypotheses were put forward by Wollman and Jacob: the replication of the bacterial chromosome during conjugation was under the control of the F factor, and the coordination between replication and the transfer of the chromosome was achieved by the attachment of the F factor to the cell membrane. One of the main items of the replicon model – the attachment of DNA to the membrane – therefore found its origin in the mechanistic requirements of the conjugation process.

The capacity of some genetic elements to self-replicate was also a major issue in the hypothesis of the episomes that Wollman and Jacob had proposed 4 years earlier (Wollman and Jacob 1959). Episomes were genetic elements able to self-replicate, or to integrate into the bacterial genome and to replicate under its control. The F factor, temperate bacteriophages and

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colicinogenic factors were three families of episomes. In a previous issue of this series, I have discussed the huge expectations that were placed upon episomes (Morange 2009): they might be involved in the control of cell differentiation and development in higher organisms. Such predictions were not confirmed, and at the end of the 1960s the word 'plasmid' was more and more used to designate self-replicating genetic elements. But, before that, the importance of episomes had been for Jacob a strong stimulus towards the elaboration of the replicon model.

It is also obvious that the replicon model was a sequel of the operon model. The initiator gene is equivalent to a regulatory gene, and the replicator similar to the operator. Nevertheless, there is a small, but highly significant difference between the two models: the initiator acts as an activator, and not as a repressor.

The discovery, some years before, that induction of bacteriophages and of the enzymes involved in lactose metabolism was an inhibition of a repression was a big surprise for Jacques Monod and Jacob. It led Monod to believe that activation by a double inhibition was a general principle of gene regulation, and he resisted the results provided by his collaborators and many others showing the existence of a positive regulation of transcription in microorganisms (Schwartz 2003).

The replicon model is a wonderful example of tinkering ('bricolage'). The notion of tinkering was reintroduced (after Darwin) in 1977 by Jacob to designate the way evolution proceeds (Jacob 1977). This metaphor became immensely popular. It is less known that in his late historical and philosophical writings, Jacob also used this metaphor to describe the work of scientists, playing with their models, recombining their pieces to create new models (Jacob 1982)

3. The replicon: A step towards integrative biology

In 1969, at a conference at Cold Spring Harbor, Antoinette Ryter, Yukinori Hirota and Jacob introduced their lecture with the following sentence: 'While many aspects of the bacterial cell have been uncovered in the last two decades, we still know very little about the general system which integrates cellular controls, the regulation of DNA replication, the formation of bacterial membrane, and the process of cellular division with its equipartition of the DNA copies' (Ryter *et al.* 1969). This sentence might be considered as emblematic of modern systems biology. More directly, it points to the transformation in biology that took place at the end of the 1960s and beginning of the 1970s: the emphasis was more and more put on the integration of molecular functions within the cells. The publication, 18 years after the famous *Molecular Biology of the Gene*, of *Molecular Biology of the Cell* was a good sign of this displacement.

The elaboration of the replicon model was obviously a first step in this direction. As mentioned above, the hypothesis of a link between DNA (the replicator) and the cell membrane is an important part of the replicon model. But it was not immediately conceived. Before the conference at Cold Spring Harbor, Jacob and Brenner published a short article describing the model in the proceedings of the French Academy of Sciences (Jacob and Brenner 1963). In this note, the word 'membrane' is not used, and is replaced by the word 'surface'. The introduction of the word membrane was a step towards the characterization of the precise cellular structures involved in DNA replication. To this aim, two strategies were used: isolation of mutants affected in their capacity to replicate DNA, and characterization by electron microscopy of the interaction between replicons and membranes. Structures derived from the cell membrane, called 'mesosomes', seen as early as 1953 (Chapman and Hillier 1953) and precisely described in 1960 (Fitz-James 1960) after the development of a new protocol for the observation of bacteria by electron microscopy (Ryter and Kellenberger 1958), were suggested to be the site of interaction between DNA and bacterial membranes. Ten articles were written by Jacob in collaboration with Ryter to give a precise description of this interaction (see, for instance, Ryter and Jacob 1963 and 1964). In the mid-1970s, a consensus was finally reached by researchers that mesosomes were artefacts resulting from the techniques used for the fixation and staining of the preparations (Rasmussen 1993).

In these years, the study of cell membranes was problematic (Morange 2013). Different models of membranes coexisted, and the Singer-Nicolson model of the fluid mosaic membrane was only published in 1972 (Singer and Nicolson 1972). Well-adapted techniques for the study of membranes were crucially lacking. Immunolabelling took on an increasingly large role in the following years, and greatly contributed to the growing importance of membranes in molecular cell biology. It is interesting that the 1963 article of Jacob, Brenner and Cuzin quotes one of the first experiments done using immunofluorescence to study the structure of bacterial cell membranes (and walls) (Cole and Hahn 1962).

The 1970s were the 'golden years' for membranes as the 1960s had been for nucleic acids: not solely because of the emergence of a new model for cell membranes, and of well-adapted techniques for their study, but mainly because numerous important functions were progressively attributed to membranes. Membranes are essential in cell-to-cell communication through the abundance of receptors at their surface. They also play a major role in the synthesis, maturation, and transport of proteins to the different cell compartments. The complexity of vesicular trafficking within cells was fully acknowledged.

The fast growth of developmental biology at the end of the 1960s and beginning of the 1970s emphasized the importance of cell-to-cell contacts and intercellular signal exchanges in cell differentiation and development, in particular at the early steps of these processes.

4. Back to Jacob

The failure of the work on the interaction between DNA and membranes should not mask the fact that Jacob's work on the replicon bore all the characteristics of his method, already obvious in his previous work on lysogeny and conjugation: start with an abstract model and progressively replace the hypothetical entities by precise structures and mechanisms.

Although unfruitful, this contact of Jacob with membranes was the beginning of a new story for him. In 1970, he decided to abandon the study of bacteria and bacteriophages for the study of mouse embryogenesis (Morange 2000). His strategy was to use cells called embryonal carcinoma cells, derived from tumours named teratocarcinomas, which had properties similar to those of the presently extensively studied embryonic stem cells. With his collaborators, he prepared antibodies against the membrane proteins of these cells. He showed that some membrane proteins were expressed at a very early stage of embryogenesis. Addition of antibodies prepared against them prevented the compaction of the embryo, a step that precedes the formation of blastocysts and the first cellular differentiation. The so-called F9-antigen was later shown to be a member of the cadherin family of adhesion proteins.

Therefore, the replicon model was not only a first step from molecular biology to molecular cell biology in general, it was also a springboard for Jacob towards the second part of his scientific career.

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