What history tells us XVI. A third pillar for molecular biology: Molecular embryology

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1. Introduction

It is generally acknowledged that the rise of molecular biology was a result of the encounter of two different research traditions: the structural chemistry and biochemistry tradition, with the progressive description of macromolecular structures, and the informational vision rooted in genetics (Morange 1998). The development of molecular biology, however, is more complex than this convergence suggests, and other disciplines such as microbiology, cell biology and embryology took an active part in the development of its concepts and of its experimental systems.

Observations made in embryology supported the rise of the new molecular vision. The embryological approach of Boris Ephrussi and George Beadle – transplantation of imaginal disks in *Drosophila* – was at the origin of the one gene–one enzyme relation. Observations made on the variations in nucleic acid and protein turnover during development by Thomas Caspersson, Jean Brachet, Alfred Mirsky and many other embryologists helped to position the different macromolecules on the chart of informational transfer which was substituted for the genotype–phenotype relation.

I would like to argue that molecular embryology was more than a simple contributor to the development of the molecular paradigm. It was a full partner, a third pillar of molecular biology, with a different conception of molecular organization, and different experimental systems. I will demonstrate this by analysing the characteristics of the Britten-Davidson model of gene regulation proposed forty years ago.

2. Two models of gene regulation emerging from two different traditions

One cannot help being struck by the contrast between the two molecular models which were proposed in the 1960s to explain gene regulation during development. The first was the operon model advanced in 1961 by François Jacob and Jacques Monod (Jacob and Monod 1961) and its extension to explain the control of differentiation and development in higher organisms (Monod and Jacob 1961). The other was the Britten-Davidson model of 1969 (Britten and Davidson 1969). The second model was presented by its authors as very different from the first. Even if this statement was aimed to focus attention on the newly proposed model, it rightly emphasized the existence of major differences between them.

These two models can be compared from the point of view of their consistency with present knowledge. The operon model rightly underlined the role of transcription factors in the control of gene expression. The discovery in 1984 that the homeotic genes that regulate the fate of the different segments in *Drosophila melanogaster* encode transcription factors was a beautiful confirmation of this hypothesis. But the operon model gave too much importance to the grouping of genes in operons, and to repression – the negative side of regulation. The Britten-Davidson model emphasized the role of activator RNAs. It can be seen as an anticipation of the present importance of gene activation in eukaryotes, and also of the role of controlling RNAs (Morange 2008b). But it is obvious that its neglect of the role of protein transcription factors was a major mistake, and Eric Davidson himself puts these transcription factors at the heart of his present systemic description of development (Davidson 2006). The different examples of pleiotropic mutations affecting development, interpreted by Britten and Davidson as affecting the production of activator RNAs, such as the *Notch*, *bithorax* and *T* mutations, were later shown to be mutations in genes encoding transcription factors and receptors. In addition, the hierarchical organization of genes postulated by the Britten-Davidson model, and the battery of names proposed to

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designate them – sensor, producer, integrator, receptor genes – have completely disappeared; in fact this vocabulary was never used.

Therefore, at first glance, it would appear that the operon model was better able than the Britten-Davidson model to explain the more and more precise descriptions of molecular mechanisms that rapidly emerged from the development of the new engineering tools in the mid-1970s.

More significant are the utterly different relations of the two models to experiments. The operon model was firmly based on exquisitely designed experiments done in bacteria. Its use in explaining gene regulation during embryogenesis was a "logical extension", not the interpretation of any molecular data from embryology. On the other hand, the Britten-Davidson model was highly speculative, but emerged from the accumulation of recent data done on early embryo development, which had been carefully presented the year before by Davidson in his influential book *Gene activity in early development* (Davidson 1968). This model was proposed to guide experiments: a role for a model traditional in physics, but unusual in molecular biology (Keller 2002), and a role that models obviously have today in systems and synthetic biology.

The evolutionary implications of their model were obvious for Britten and Davidson, whereas the evolutionary consequences of the operon model were minimally mentioned by Jacob and Monod in a cryptic publication (to the Pontifical Academy of Sciences!) (Jacob and Monod 1962). The Britten-Davidson model not only aimed to explain the regulation of gene expression during embryogenesis, but also how simple alterations of these mechanisms were likely to generate important evolutionary transformations. For Britten and Davidson, the "evolvability" – a word not used at that time – of organisms was contained in the organization of their genome (Britten and Davidson 1971).

Another way to compare the models is simply to consider them as emerging from different research traditions. The operon model was the product of a genetic and microbiological tradition, whereas the Britten-Davidson model was the product of molecular embryology, especially of its dramatic expansion at the beginning of the 1960s.

3. The molecular embryology tradition

The search for biochemical and later molecular mechanisms of embryology was ancient. It had its roots in the work of cytologists at the end of the 19th century, in the major *opus* of E B Wilson at the beginning of the 20th century, as well as in the efforts to characterize the nature of the organizer in the 1930s. It expanded rapidly at the beginning of the 1960s: cytochemical observations and the effects of inhibitory drugs were complemented and replaced by extraction of labelled or unlabelled RNAs and polysomes, and a determination of the nature of RNAs by hybridization experiments. A combination of these different technologies rapidly provided a harvest of new results, and a comprehensive and dynamic picture of gene expression during early embryogenesis. It was shown that early development was often controlled by maternal RNAs (Crippa *et al* 1967) prestored in the oocyte (Bachvarova *et al* 1965) in the form of cytoplasmic particles (Spirin and Nemer 1965). The activation of the embryonic genome takes place at different stages depending upon the organism – at the blastula stage in amphibians (Davidson *et al* 1968) – and the informational RNAs generated at this step are only used at later steps of development and differentiation.

These results amply confirmed "the variable gene activity theory of cell differentiation" and "the cytoplasmic localization of morphogenetic potential" (Davidson 1968); the initial phase of genome activation depended on the prelocalization in the oocyte of cytoplasmic factors affecting gene activation. This model was supported by the experiments of John Gurdon on nuclear transfer in amphibians (Gurdon and Brown 1965). It was also supported by the results of the cell fusion experiments initiated by Boris Ephrussi (Morange 2008a) and developed by Henry Harris. The experiments of micromanipulation on early embryos done by Eric Davidson (Davidson *et al* 1965), and direct *in vitro* experiments (Thompson and McCarthy 1968), were also in agreement with this model.

Most of these new data were collected on a limited number of experimental systems, mainly amphibians and sea urchin embryos. The role of hormones in the control of gene transcription and cell differentiation, both in animals and in plants, served as a model (Davidson 1968). What characterizes this "molecular biology of embryogenesis" is the dominant place of the informational vision. It goes so far as to systematically replace the expression "messenger RNAs" by "informational RNAs", a habit that nevertheless was soon abandoned. The Central Dogma of molecular biology is fully accepted. But there are obvious oppositions and differences with the other molecular traditions. The expression of genes involved in embryogenesis has to be precisely organized, and this organization is inscribed in one way or another in the genome. The structural organization of the genome was an important component of the Britten-Davidson model whereas, apart from the grouping of genes in an operon, it did not play any role in the Monod-Jacob model. Even if it was admitted that signals, such as hormones, did play a role in the control of gene expression during early embryogenesis, development was mainly "programmed" in the genome. This fundamental difference between the two models explains why Jacob found it easy in the early 1970s to turn his attention to the major role of membrane proteins (Morange 2000), whereas Davidson maintained his interest in the structural organization of the genome until the beginning of the 1980s (Davidson and Britten 1979; Davidson and Posakony 1982). Still today, the models proposed by Davidson conserve traces of these early interests: the precise structural organization of the *cis*-regulatory sequences is highly important for the control of gene expression, and cell-to-cell interactions have a minor role in the global process of embryogenesis.

The fact that in his 1968 book Davidson paid no attention to the complex models of differentiation and development elaborated by Conrad Waddington, who was deeply influenced by the work of geneticists (Waddington 1966; Gilbert 1996) is additional proof that the molecular visions emerging from embryology and genetics were different. Waddington praised the Britten-Davidson hypothesis and suggested that it was the first speculation on molecular mechanisms controlling embryogenesis that made sense (Waddington 1970). By referring in the discussion of the Britten-Davidson model to his own early models, which had been also ignored by Monod and Jacob, Waddington reminded people of his own contributions to an explanation of the molecular control of differentiation and development. The influential tradition of molecular embryology probably explains why the Britten-Davidson model, so different from present models, is still considered by many as an important step in our understanding of development at the molecular level. The recent phase of evolutionary developmental biology ("Evo-Devo") has its roots in this molecular tradition as much as in the early work of developmental geneticists such as Ed Lewis. The fact that the direction of evolution is linked with the mechanisms of development, and *vice-versa*, the requirement for a more global vision of development, and an emphasis on “novelty” are characteristics of Evo-Devo already present in the Britten-Davidson articles.

4. The specific context of the elaboration of the Britten-Davidson model

The Britten-Davidson model was inscribed in a well-defined tradition of research. The necessity to produce a model was clearly the consequence of the accumulation of molecular descriptions of early development made in previous years. But the specific form of the model was the result of a series of puzzling observations made in the months preceding its construction: the discovery that the genome contained interspersed repetitive sequences (Britten and Kohne 1968) differentially expressed during development (Denis 1966), and that a large fraction of the non-ribosomal RNA pool was produced in the nucleus but not exported to the cytoplasm (Church and McCarthy 1967; Shearer and McCarthy 1967). These two results were the basis on which the model was elaborated.

The contribution of many forms of knowledge to the elaboration of a new theory, some well stabilized during decades, and others that had suddenly sprung up in the previous months, is reminiscent of the model proposed by the French historian Fernand Braudel in his famous book on the Mediterranean Sea (Braudel 1949): any historical event can be considered as resulting from the superposition of different historical trends, each with its own pace of evolution. Braudel's model throws light on scientific discoveries in general, and on the elaboration of the Britten-Davidson model in particular.

For Davidson, the model he elaborated with Britten was at odds with the ideas prevalent in the laboratory in which he had been trained, ideas that emphasized the role of histones and histone modifications (Allfrey *et al* 1964) in the control of gene expression. Without denying the value of the experiments done *in vivo* and *in vitro* showing the importance of chromatin (Paul and Gilmour 1968), Davidson did not think that chromatin modifications were specific enough to account for gene regulation during differentiation and development. He saw these modifications as the consequence of earlier events, such as the production of activator RNAs. Both in terms of time and significance, the relative importance of chromatin modifications and specific control of gene expression by microRNAs and transcription factors is still debated today.

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