Series

What history tells us XII. Boris Ephrussi's continuing efforts to create a "genetics of differentiation"

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1. Introduction

Students in biology know that the work of Boris Ephrussi and George Beadle on the genetic control of eye pigmentation in *Drosophila* was the first step towards the establishment, a few years later, of the "one gene-one enzyme" relationship by George Beadle and Edward Tatum through their work on fungus *Neurospora*. After the Second World War, Ephrussi's discovery of the *petite* mutations that blocked respiration in yeast, and the demonstration of the cytoplasmic inheritance of these mutations, were decisive steps in the development of mitochondrial genetics in the 1960s. Less well known is the equally important role that he played in the development of cell hybridization and somatic cell genetics at the beginning of the 1960s. This development took place simultaneously with the establishment of cell lines derived from teratocarcinomas. Ephrussi's demonstration of the potential of these cell lines for the study of differentiation was exploited later by François Jacob and amply confirmed since by numerous studies of embryonic stem cells (Morange 2006).

The plurality of the projects pursued by Ephrussi during his life has been considered as a dispersion of efforts and as an indication of the difficulties encountered by those who, like him and Waddington, tried to reconcile embryology and genetics. Ephrussi famously said of Thomas Hunt Morgan's 1934 book *Embryology and Genetics* that it was the sum of knowledge accumulated in genetics and embryology, not the expected linking together of these two disciplines. My aim in this article is not to sketch a complete scientific biography of Ephrussi – excellent contributions have already been made in this direction (Roman 1980; Sapp 1987; Burian *et al* 1988, 1991; Burian and Gayon 1990, 1999) –, but to outline characteristics of his early contributions as well as to emphasise the importance of the later ones, which so far have not received the attention they deserve (with the exception of Weiss 1992; Zallen and Burian 1992). Focussing on his last studies is also a way to underline the profound unity of Boris Ephrussi's work: some of the questions he raised are still at the core of biologists' investigations.

2. The "early years" (1901–1960)

After his studies in Russia, and a year and a half spent in Bessarabia living "after the manner of Tolstoy", Ephrussi completed his formal education in France, where he had arrived in 1920. He was deeply influenced by two outstanding scientific personalities: Louis Rapkine, who developed a biochemical approach to early embryogenesis, and Emmanuel Fauré-Fremiet, a pioneer in cell culture at the Institut de Biologie Physico-chimique in Paris. Using cell culture, Ephrussi tried to determine the place and mechanism of action of the mouse brachyury mutation (Ephrussi 1933, 1935; Burian *et al* 1985). Of the collaborative work done with Beadle later, the most remarkable feature was the wonderful coupling of a technique from embryology – organ transplantation, in this case transplantation of imaginal disks – and the conceptual models of genetics. Their goal was to characterise the role of genes in development. Ephrussi selected non-autonomous mutations affecting the pigmentation of the eyes, because, as effects caused in one cell by a mutation in another, they would be ideal for illuminating the inductive effects characterised by embryologists. The interpretation favoured by Ephrussi was that genes operated through the action of hormones.

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This early work was remarkable for Ephrussi's efforts to unify the conceptual models of embryology and genetics, and was very far from the general relation between genes and enzymes that Beadle and Edward Tatum would establish some years later.

The work on *petite* mutations appears as a brilliant parenthesis in the career of Ephrussi. To consider it as a contribution to the study of differentiation might be criticised in the same way as Ephrussi criticised the too rapid, unfounded, application of genetic regulatory models derived from bacteria to the study of differentiation and development. One can probably not understand this interlude in the work of Ephrussi independently of the success achieved by Beadle and Tatum and the rise of the fashionable domain of cytoplasmic inheritance and plasmagenes in the late forties. Most of these new discoveries had been made on simple experimental systems, yeasts, *Neurospora*, although they had huge consequences for understanding the functions of all organisms. What is most striking about the work of Ephrussi is the way he was able to link the demonstrated existence of a cytoplasmic form of inheritance with the existence of a control exerted by the nucleus: there was a complex crosstalk between nuclear and cytoplasmic mutations which was revealed by careful genetic studies. His goal was not to create a new science of cytoplasmic inheritance alongside genetics, but to extend genetics to incorporate what he considered well established facts of cytoplasmic inheritance.

3. The central role of Ephrussi in the rise of cell hybridisation

The trigger was the experiment designed by Georges Barski, Serge Sorieul and Francine Cornefert at the Institut Gustave Roussy at Villejuif near Paris to test for genetic transfer between two mouse cell lines. What was observed was the formation of hybrid cells containing the chromosome complements of both parental cells (Barski *et al* 1960). Ephrussi immediately saw the interest of this observation. In collaboration with Serge Sorieul who, as a cytogeneticist, played a crucial role in the initial observation, he confirmed the result with cells of different genetic origin (Sorieul and Ephrussi 1961). The following year, these two authors observed that the hybrid cells exhibited a progressive reduction in the number of chromosomes (Ephrussi and Sorieul 1962). This was the last publication of Serge Sorieul, who died at the age of 32.

It is astonishing how rapidly the potential of the system was exploited, and how technical improvements were made through the work of Ephrussi, Henry Harris, John Littlefield, Mary Weiss, Howard Green and a few others. Soon interspecific hybrids between human and mouse cells (Harris and Watkins 1965) and rat and mouse (Ephrussi and Weiss 1965) were obtained. The possibility to hybridise differentiated and non-differentiated cells led to the discovery of the phenomenon of extinction **(**the disappearance of cell type-specific gene expression that can occur in hybrids between cells of different phenotypes**)** and to the distinction introduced by Davidson, Ephrussi and Yamamoto between *luxury* and fundamental characters (Davidson *et al* 1966; Ephrussi and Weiss 1969). Ephrussi called the latter *household*: today we would say *housekeeping*. The hybridisation of malignant and non-malignant cells (Scaletta and Ephrussi 1965) allowed determining the dominant or recessive character of the transformed phenotype. The characterisation of hybrids, first limited to an estimate of the total number of chromosomes and the recognition of particular chromosomes with highly characteristic forms, became more and more precise with the simultaneous development of new staining techniques for chromosomes. The preparation of hybrids was also initially limited by the selective growth advantage they had to possess. The use of mutant parental cells and of selective media allowed the isolation of hybrids whatever their growth characteristics (Littlefield 1964). The rate of hybridization was increased by the use of inactivated viruses (such as the Sendai virus) by Henry Harris (Harris and Watkins 1965) and, ten years later, of polyethylene glycol (Pontecorvo 1975; Pontecorvo *et al* 1977).

Even more remarkable was the rapidity with which potential applications were developed. The stochastic loss of chromosomes in hybrids permitted the association of a particular characteristic – enzymatic activity, presence of an antigen on the cell surface – with the presence of a particular chromosome – and allowed the assignement of genes to specific chromosomes (Weiss and Green 1967). The translocation of fragments of chromosomes, as well as the disruption of chromosomes by irradiation, allowed researchers to go one step further: to map precisely the genes on the chromosomes. Finally, the possibility of obtaining proliferating hybrid cell lines expressing specific antibodies was conceived very early, and the preparation of hybridomas by Robert Köhler and Cesar Milstein in 1975 (Köhler and Milstein 1975) was the culmination of a huge number of attempts (Cotton 1994; Harris 1995, 142–145).

4. Boris Ephrussi and teratocarcinoma cell lines

This is the last important scientific contribution of Ephrussi, not only directly through the results he obtained, but also, indirectly, through the role he played in the adoption of this system by the laboratory of François Jacob. Stevens and Pierce had already shown the multipotentiality of embryonal carcinoma cells and the maintenance of these potentialities by *in vivo* culture (Morange 2006). Ephrussi extended these observations to clones isolated from teratocarcinomas and maintained in long-term cultures (Finch and Ephrussi

1967; Kahan and Ephrussi 1970). Most of all, he hybridised these cells with permanent cell lines and demonstrated the extinction of multipotentiality by hybridization (Finch and Ephrussi 1967).

5. The place of cell hybridisation and somatic cell genetics in the recent history of biology

We have already described the immediate applications of the cell hybridisation technique. The phenomenon of cell fusion was not unfamiliar to biologists – examples of cell fusion as well as of the facilitating role of viruses had long been known, for more than a century in the case of the former (Harris 1995, 123–124). Therefore, only a highly specific scientific context explains why work on hybrid cells advanced as quickly as it did in the 1960s: progress in cell cultures and karyotypic determination (Caspersson *et al* 1970); the question of differentiation and development progressively occupying the forefront of research, with the proposal of the first molecular model of gene regu-lation; and the increasing ambitions of biologists to (in a sense) 'master' life and to overcome existing barriers such as that between species (Landecker 2007). The possibility of obtaining hybrid cells between mouse and human, or potato and tomato (Melchers *et al* 1978), was more upsetting than the limited chimerism generated ten years later by the use of recombinant DNA technology (Thomas 1974).

Despite these possibilities, cell hybridisation did not wholly fulfil its promise (Gordon 1994). Consider, for instance, the origin of cancer. The initial observations that hybrids resembled the most malignant parent cell were rapidly contradicted by the results of Harris and George Klein demonstrating that malignancy was initially suppressed, but could reappear with the loss of certain chromosomes (Harris *et al* 1969; Harris and Klein 1969). These observations seemed to pave the way for the isolation of genes repressing transformation. Even if these early observations can be explained retrospectively in terms of the action of tumour suppressor genes, it is obvious that they did not play an active role in the development of the new molecular vision of cancer in the 1980s.

The same is true for the control of gene expression during differentiation and development. The observation of the extinction of differentiated characteristics (Davidson *et al* 1966), as well as that of the reappearance of these extinguished functions by loss of chromosomes (Klebe and Ruddle 1970; Weiss and Chaplain 1971), seemed to confirm the model of gene regulation by repression based on the operon hypothesis, and opened the way to the localization and characterization of the repressors. Despite the intensity of the efforts deployed by Ephrussi, Weiss and others, the variability of the observations did not lead to any major breakthrough. In fact, our present knowledge of the mechanisms controlling gene expression during differentiation and development originated in the patient characterisation of the mechanisms of transcription in eukaryotes made possible by the development of molecular technologies. It is likely that epigenetic changes – DNA and chromatin modifications – partially explain the complex observations made on hybrid cells, and the difficulty of establishing general rules. These early studies on hybrid cells revealed the extraordinary plasticity of cells, a characteristic of organisms and their components which is highly fashionable today.

6. Boris Ephrussi and the search for an "extended genetics"

Neither a French tradition, nor his permanent determination to reconcile genetics and embryology, sufficiently explains the richness and diversity of the scientific work of Ephrussi. Important scientific events clearly played a role for somebody aiming to be a "front runner" in science (Burian *et al* 1985). The adoption of yeast as a model system was the consequence of the success achieved by Beadle and Tatum with *Neurospora*. The rapidity with which Ephrussi developed his work on cell hybridisation was in part a result of the necessity, as he felt, to "turn the page" after the shock of the award of the Nobel Prize to Beadle and Tatum in 1958. His efforts to characterise mechanisms involved in cell differentiation were an answer to the overly simple and fashionable models derived from the study of bacteria, as well as a sign of fidelity to the conceptual distinctions introduced in experimental embryology in the 1930s such as the distinction between determination and differentiation (Ephrussi 1972).

But these historical ups and downs are not sufficient to explain the scientific choices of Ephrussi. His first deep conviction was the absolute necessity to develop simple and experimental systems to study fundamental biological questions. *Ex vivo* cell culture was favoured in his first studies as well as in his last accomplishments; the latter, in fact, being made possible by his personal mastery of the new cell culture methodologies developed in Renato Dulbecco's laboratory (Ephrussi and Temin 1960). The problems of differentiation and development were not likely to be solved "on paper or on bacteria".

The second permanent struggle of Ephrussi was a struggle for an extended conception of genetics. An article by Ephrussi written in 1962, but only published 17 years later (Ephrussi 1979), emphasises the motivations that underpinned his work during forty years. The most important of these was his conviction of the dominant place of genetics, a "definitive theory", the science which by its concepts had supported the work of biologists throughout

the 20th century. Its abstract characteristics make genetics comparable to physics. But his second conviction was that geneticists were wrong when they pretended that genetics, in its present form, had reached its objectives. The price paid for supporting such a belief was an arbitrary limitation of the objectives of genetics, with, in particular, the giving up of any attempt to explain differentiation and development. There is a genetic paradox in the process of differentiation: all differentiated cells have the same genes, and the observed inheritance of differentiation state is an inheritance of cytoplasmic variations. But this inheritance is made possible by the nuclear genes.

Throughout his career Ephrussi sought to found a "genetics of differentiation". He felt that cell hybridisation was the ideal experimental system for this purpose. It has turned out that it was not, and biologists in some way are still searching for it.

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