

Hi Josh,

I will append to this note a text intended for the Perspectives series in Genetics on the start of interspecific somatic cell hybridization from / centered on Ephrussi. If Jim Crow likes it and we don't find any major problems with it, it will probably be in the Sept. issue. IF you have time in the next little while (we probably have to have the revision in in a bit over two weeks) and have comments or suggestions, we'd be delighted. For my part, I'm a bit worried that it may be a bit overly-Ephrussi-centered.

I hope all is well with you and that our paths cross before too long. Thanks for any time you manage to put in on this and for your help on so many fronts. With best regards, Dick
P.S. That Stryer biochemistry text is a big help; it is filling in a number of gaps in my education.

On the Beginnings of Somatic Cell Hybridization:
Boris Ephrussi and Chromosome Transplantation

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Two papers published in GENETICS in November 1966 represent a key step in a decade of research in the laboratories of BORIS EPHRUSSI (1901-1979) -- research that helped transform mammalian genetics, especially human genetics. These papers, co-authored with MARY WEISS, then a graduate student in Ephrussi's laboratory at Western Reserve University in Cleveland (WEISS and EPHRUSSI 1966 a,b), provided the first detailed reports of the formation of viable and self-perpetuating hybrids between somatic cells of two different species: mouse and rat (preliminary reports in EPHRUSSI and WEISS 1965; EPHRUSSI 1966). Such hybrids contributed crucially to the development of somatic cell genetics and soon provided an important tool for efforts to gain detailed information about the organization of genetic information on human chromosomes (WEISS and GREEN 1967).

Although the techniques described in these papers played an important role in the development of human formal genetics, this outcome was quite distant from Ephrussi's own scientific goals. His primary interest in constructing such "zoological oddities" as interspecific hybrids was to develop tools for analyzing the processes of determination, differentiation, and regulation in development (including their bearing on oncogenesis). We will show that the work on interspecific hybrids was a natural culmination of investigations that occupied Ephrussi throughout his career and how the investigations described by WEISS and

EPHRUSSI (1966 a,b) grew out of the Ephrussi's lifelong effort to develop tools for understanding fundamental developmental processes (see BURIAN et al. 1991; SAPP 1987, Chap. 5).

We will particularly emphasize Ephrussi's strategic use of methods involving variations on the theme of transplantation. Working with a great variety of organisms, he consistently found ways to explant, implant, or otherwise transfer organs, tissues, cells, and nuclei into foreign organismal environments, combining these techniques with what he called "the genetical tool". He used the behavior of the transplant in the new context to test hypotheses about its regulation and control of its destiny, and about how it influenced or regulated its host. In this respect, his work with somatic cell hybrids is best understood as a way of transplanting chromosomes, chromosome arms, or blocks of genes into a genetically and cytoplasmically foreign context. Although it fell short of the ideal of transplanting single genes, it was a natural extension of Ephrussi's approach and allowed him to gain insights (and develop tools for others to gain insights) into complexities of development that had eluded him ever since Zallen and Burian

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his early work with tissue culture and with sea urchin development as a young researcher in Paris in the 1920s.

HARNESSING TRANSPLANTATION

From the start of his scientific training in France in 1920 as a Russian migr, Ephrussi studied the initiation and regulation of embryological processes by intracellular and extracellular factors. A major strand of his early research concerned the effect of temperature on development of fertilized sea urchin eggs (e.g., EPHRUSSI 1923, 1932). In this work, he employed a relatively new apparatus, a micromanipulator. Robert Chambers, an American biologist, had developed an accurate manipulator, enabling one to alter single cells by inserting (or extracting) small quantities of substances into (or from) them. In Paris in April 1925, Chambers instructed LOUIS RAPKINE, a fellow student and a close friend of Ephrussi's, in its use. Rapkine, interested in chemical processes in the cell, employed the micromanipulator in a series of studies on cellular physiology during developmental change to probe the chemical state within individual cells. He and Ephrussi, working singly and together at the Coll Biological Station, studied chemical changes that occurred during the course of sea urchin development (e.g., EPHRUSSI and RAPKINE 1928). Ephrussi thus became familiar with the operation of the instrument and the opportunities it offered to track developmental changes by probing and altering internal and external cellular environments.

Ephrussi's second dissertation (two were then standard in France) was a project on tissue culture (EPHRUSSI 1933a, see also EPHRUSSI 1935a). Despite difficulties associated with the early unsatisfactory tissue culture techniques, Ephrussi concluded from this work and two explantation studies of brachyury in mice (EPHRUSSI 1933b, 1935b), that intrinsic factors -- i.e. genes -- play a key role in development.

HARNESSING GENETICS

In the next phase of his career, Ephrussi coupled his embryological concerns to a firm conviction that one must understand the role of genes to decipher embryological processes. Supported by a Rockefeller Foundation fellowship, Ephrussi went to Caltech in 1934-5 to learn genetics within the intellectual empire of T. H. MORGAN. While there, Ephrussi arranged a

collaboration with GEORGE BEADLE, who joined him in Paris in the fall of 1935. They aimed at a genetic analysis of development, with Beadle at first contributing genetic expertise and Ephrussi the insights and techniques of embryology. Their strategy was to subject a single species to both genetic and embryological attack. Since such traditional embryological organisms as sea urchins and frogs are ill-suited for standard genetic analysis, Ephrussi and Beadle decided to apply experimental embryological techniques to a genetic organism par excellence -- *Drosophila melanogaster*. They were encouraged by STURTEVANT, who provided

some leads from his work on flies mosaic for the vermilion mutation (STURTEVANT 1920, 1932). This work suggested that a diffusible substance, present in the wild type, could compensate for the absence of the wild-type product of the vermilion gene.

But could one do experimental embryology with *Drosophila*? *Drosophila* larvae seemed to be too small to permit use of the standard embryological technique of transplantation of parts of a developing embryo to learn about influences of location and of adjacent tissues on development. And difficulties in identifying imaginal disks added further complications. However, Ephrussi, aware of the implantation experiments of CASPARI, KHN, and PLAGGE on *Ephestia* (see e.g., CASPARI 1933; KHN et al. 1929, 1932) and well aware of the capabilities of the micromanipulator, was able to forge that instrument into a tool that allowed implantation of imaginal disks into *Drosophila* larvae. As EPHRUSSI and BEADLE described the procedure they developed:

The essential part of the technique ... is the actual operation of injection of the desired tissue by means of a micro-pipette. We have used the technique in implanting gonads and various imaginal disks. ... The assembly that we use is that of the standard Chambers' micro-injection apparatus (EPHRUSSI and BEADLE 1936, pp. 218, 219, 221).

Striking results were obtained by implanting imaginal disks of various genotypes, fated to form eyes, into genetically foreign larvae. Ephrussi and Beadle demonstrated the sequential involvement of the substances present in flies with wild-type vermilion and cinnabar genes in the terminal portion of a pathway leading to the production of the brown eye pigment normally found in *Drosophila*. These and other results obtained by implanting various imaginal discs and organs, and injecting hemolymph, provided some insights into the pathways by which genes affect phenotypic characteristics by controlling the production of diffusible substances (see BURIAN et al. 1988, pp. 389-400). Starting from this basis, BEADLE and TATUM, working with *Neurospora* and using more standard genetic approaches, were able to connect gene function with the production of specific enzymes as codified in their "one-gene : one-enzyme" hypothesis.

YEAST (AND CYTOPLASMIC) GENETICS

After World War II, Ephrussi (who spent most of the war as a refugee scientist at Johns Hopkins University) returned to France to reinstitute research aimed at disentangling the various influences, nuclear and cytoplasmic, on development. This time, Ephrussi eschewed the transplantation of cells and tissues between organisms, though he assigned his student PIOTR SLONIMSKI a thesis based on transplantation of sea urchin nuclei -- an attempt that was unsuccessful (interview, RB and P. SLONIMSKI, Nov. 1984). Given the failure of these efforts, he explained his

choice of a new experimental organism as follows:

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[W]hat is needed is direct genetic analysis of somatic cells, for the assumed functional equivalence of irreversibly differentiated somatic cells, however plausible, is only an hypothesis. Crosses between such cells being impossible, only nuclear transplantation from one somatic cell to another, or grafting of fragments of cytoplasm, could provide the required information; such experiments however will have to await the development of adequate technical devices. In the meantime, the closest approximation to the evidence we would like to have is provided by the study of lower forms which propagate by vegetative reproduction and possess no isolated germ line (EPHRUSSI, 1953, p. 5; also in EPHRUSSI 1958, p. 37).

He selected the yeast *Saccharomyces cerevisiae* as a model system -- that is, as a surrogate for his real concern with the development of distinct cell types with differing functions in higher organisms. He had the good fortune to stumble onto the ability of acriflavine to induce cytoplasmically inherited respiratory incompetence in yeast (EPHRUSSI 1949). The resultant 'petite' mutation, so-called because of the small colony size, became a major object of study, playing a formative role in mitochondrial genetics (see BURIAN et al. 1991; EPHRUSSI 1953 for an early review; SAPP, 1987, Chap. 5). With this, Ephrussi managed to mimic the effects of transplantation, crossing wild-type with the respiration-deficient petite strains. This placed various nuclear genes in genetically distinct cytoplasms. Using such rearrangements of cellular parts with the full panoply of genetic and biochemical techniques, Ephrussi and his group at the Institut de Biologie Physicochimique (the Institut Rothschild in Paris) and later at the CNRS at Gif-sur-Yvette studied the contribution of the cytoplasm to cell phenotype and pursued the interactions between nuclear and cytoplasmic genetic endowments needed to yield an intact, functioning -- albeit single-celled -- organism. Specifically, they were able to demonstrate the necessity of genetic information on cytoplasmic particles, ultimately identified as mitochondria, for the production of numerous enzymes in the respiratory chain.

The idea of transplantation is as fundamental to the yeast experiments as it was to the *Drosophila* program, though less obviously so. In yeast the effect of transplantation was accomplished not by surgically fusing different types of tissues, but by designing sexual crosses between yeasts whose cytoplasms exhibited genetic variation independent of the nucleus.¹ Thus mating and budding, not micromanipulation, brought nuclei with defined constitutions into cytoplasmic environments with differing physiological and biochemical capabilities. And the micromanipulator still figured in some of the yeast experiments; it was used to isolate successively produced buds from individual

¹Much the same is true of many experiments around that time -- e.g., Lederberg's on transduction or Jacob and Wollman's on zygotic induction.

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yeast cells treated with acridine dyes to induce the petite phenotype. These bud analysis experiments demonstrated that the

dyes increase the rate of mutation to the petite phenotype rather than altering selection (EPHRUSSI and HOTTINGUER, 1950).

SOMATIC CELL GENETICS²

Ephrussi's exploitation of the opportunities offered by the ability to "transplant" yeast nuclei between respiratory competent and respiratory incompetent cytoplasms did not permit him to get to the heart of his concerns about development. As he frequently pointed out (e.g., EPHRUSSI 1970, pp. 19 ff.), there is an apparent conflict between the embryological concept of the restriction of developmental potentiality in differentiation and the genetic concept of the genotypic equivalence of virtually all cells of a metazoan. He hoped to understand how differences in the determination of cells in various cell lineages (which he had long thought might be cytoplasmic in origin) are created, regulated, and perpetuated and how overt differentiation is regulated and maintained. During the 1950s, as the yeast work proceeded, Ephrussi sought a new system with which to study somatic cell differentiation. To this end he visited RENATO DULBECCO's laboratory in 1959-60 to learn modern methods of handling cells in tissue culture. This choice was fortuitous since the new tool that fell into his hands for understanding somatic cell specialization depended on tissue culture.

The stimulus for this work came from a novel observation made by GEORGES BARSKI, SERGE SORIEUL and FRANCINE CORNEFERT at the Institut Gustave Roussy in Paris. BARSKI and his group were studying mouse cancer cell lines originally derived from a single mouse fibroblast cell (SANFORD et al. 1954). Two lines had evolved in tissue culture so as to display recognizably different phenotypes, chromosomal configurations, and tumor-producing abilities: the "high-cancer" line (N1), easily produced tumors, whereas the "low-cancer" line (N2) did so rather poorly. Hoping to find Pneumococcus-like transformation between the two lines (see EPHRUSSI 1970, p. 10), Barski et al. began a series of experiments on December 9, 1959, in which both cell types, N1 and N2, were grown together. After about three months of continuous co-cultivation, they found an unexpected cell type, markedly different, growing vigorously in the mixed culture (BARSKI et al. 1960, 1961). The new cells appeared to be hybrids generated by a fusion between N1 and N2 cells, with a single nucleus containing the chromosomes. The chromosome number was roughly the sum of those for N1 and N2 and the cells included chromosome types unique to each of the lines. With time in culture there was random loss of some chromosomes (2-11% in replications by EPHRUSSI and SORIEUL 1962a), especially after passage into mice where the new cell type actively produced tumors.

²The work in Ephrussi's laboratories in somatic cell genetics 1960-1970 has been usefully reviewed by EPHRUSSI (1970, 1972) and WEISS (in press).

Surprised by this result and unsure how to exploit it, Barski, who knew of Ephrussi's interests in tissue culture and somatic cell differentiation, turned to his colleague in Paris, explaining what he had found. Ephrussi was immediately fascinated with the opportunity presented by somatic cell hybridization. Should the phenomenon be reliably reproducible, it would provide a basis for genetic studies on differentiated cells that might shed light on the very questions that had driven his research for many years.

Moving Sorieul to his own laboratory, Ephrussi started to

search for somatic cell hybrids on January 3, 1961, only months after Barski's first report appeared. He set out to verify the original reports and to attempt, if he could, to convert the phenomenon into a genetic tool for probing the differentiated states of such cells. In a preliminary report, SORIEUL and EPHRUSSI (1961) wrote that "If this hope is justified hybridization may become a useful tool for the investigation of a number of problems of somatic cell genetics, of oncology and virology." In a number of subsequent publications, Ephrussi spelled out the characteristics which would allow these hybrids to meet his research needs. These included:

hybridization would have to occur often enough that cells of different genetic constitutions within a species -- normal as well as neoplastic -- could be readily mated; it would have to be possible to detect and select the hybrid cells against the background of parental cell types; hybrid cells would have to be stable and capable of persisting through many cycles of transfer in tissue culture; genes contributed by both parental sets of chromosomes would have to be functional in the hybrid cells; some form of "segregation," analogous to genetic exchange in microorganisms or recombination in sexual reproduction, would have to occur (perhaps via random chromosome loss or mitotic recombination) so that distinct gene combinations could be generated in different hybrid cells.

This last requirement is extremely important. It represents an extension of Ephrussi's transplantation methodology. By trapping different groups of chromosomes or chromosome segments in a single nucleus, somatic cell hybridization would mimic the transplantation of particular chromosomes or chromosome segments from one cell into another, allowing one to test the effects of their presence on cell functions and the regulatory controls altering the expression of their genes.

Over the next few years, while on prolonged leave at Western Reserve to establish a new laboratory, Ephrussi developed his new research program. He and his group invested a much effort to turn mouse somatic cell hybrids into a reliable system, running huge series of experiments on hybrid cells to establish control of the basic phenomena and the stability of appropriate markers. They proved that each of the desiderata listed above could be met, including, in particular, that segregation occurred through

accidental loss of chromosomes during the cycles of mitoses that followed the original cell fusion events (EPHRUSSI and SORIEUL 1962 a,b; EPHRUSSI et al. 1964).

But the system was still sub-optimal. The selection of hybrids was a major problem. Unless hybrid cells enjoyed a significant growth advantage over the parental cells (which, in one frustrating case, was finally found to occur only at 28-29°C, rather than the usual temperature employed in tissue culture incubators (SCALETTA and EPHRUSSI 1965)), one could not find or isolate them. This problem limited the range of hybrid cells available for experiment. Also, the group had only karyological markers to work with, which made the protocols extremely laborious. Worse, since there were no distinctive chromosomes in most of the crosses they wanted to carry out, fusions between different parental cells were often indistinguishable from fusions between two similar cells.

The solution to this experimental dilemma came from another laboratory. JOHN LITTLEFIELD at Harvard developed a selective

system using drug resistant biochemical mutants for thymidine kinase (TK) and hypoxanthine guanine phosphoribosyl transferase (HGPRT), each needed for incorporation of nucleosides via the salvage pathway. In a selective medium called HAT, including hypoxanthine, aminopterin (which blocks de novo synthesis of DNA), and thymidine, mouse cell strains mutant for either enzyme cannot synthesize DNA. When two lines, each mutant for one of the enzymes, are raised in a HAT medium, only TK+ and HGPRT+ cells (capable of utilizing hypoxanthine and thymidine) -- i.e., presumptive hybrids -- are able to form DNA via the salvage pathway; the others die (LITTLEFIELD 1964). This system allowed one to select hybrid cells, thus greatly expanding the search for mouse somatic cell hybrids. DAVIDSON and EPHRUSSI (1965) were able to adapt Littlefield's system of selection to produce a "half-selective" system in which only one of the parent cells is HGPRT- or TK-. The other parent can come from any mouse cell line that displays contact inhibition in cell culture, including normal diploid cells. In this modification, the biochemical mutant cannot grow in the HAT medium and the normal cells will form a monolayer on the surface of the growth vessel. Hybrid cells can then be recognized by their ability to grow in clumps on top of the monolayer, from which they can be isolated and maintained in pure culture (DAVIDSON and EPHRUSSI 1965).

Ephrussi and his coworkers applied the methods they had painstakingly developed during four years to address some larger questions about determination, differentiation, regulation of the cell cycle, and the onset and inheritance of neoplasticity. Some hints about regulatory phenomena began to emerge as they observed the gain and loss of particular antigens and enzyme bands in hybrids (e.g., SPENCER et al. 1964; GREEN et al. 1966; DEFENDI et al. 1967) and other experiments were begun to test for dominance or recessiveness, or positive or negative regulation, of neoplasticity (e.g., EPHRUSSI 1965, DEFENDI et al. 1967). The mouse hybrids with their "transplanted" chromosomes were beginning to yield interesting results, with the promise of more insights into the secrets of differentiation to come.

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INTERSPECIFIC CELL HYBRIDS

By his own account, Ephrussi was truly startled to learn from the New York Times (February 17, 1965) that HENRY HARRIS and J. F. WATKINS at Oxford had shown that inactivated Sendai virus could be used to facilitate the fusion of unlike cells, producing heterokaryons between human Hela cells and mouse tumor cells (HARRIS and WATKINS, 1965). The heterokaryons produced were not capable of division, although they manifested a few irregular mitoses and survived for up to two weeks. Ephrussi himself had earlier considered using viruses as agents to accomplish somatic cell fusion (see the speculations of EPHRUSSI and SORIEUL 1962, p. 90), so the application of inactive virus to aid fusion was probably no surprise. But what galvanized him into action was the use of fusion to cross species barriers. We have found no evidence that Ephrussi had considered creating interspecific hybrids in the four years he had devoted to somatic cell hybrids. The Harris and Watkins report changed all that. As Ephrussi himself recollects: "[I]t was Harris and Watkins' demonstration that cells of different species can be fused ... that in 1965 led Mary Weiss and me to the isolation of the first viable interspecific hybrids" (EPHRUSSI 1972, p. 23, our emphasis). And the effect was immediate. According to Weiss:

[O]ne afternoon, rushing out to his airport-bound taxi,

Ephrussi shouted to me, then a fledgling graduate student, "Order some rat fibroblasts from Microbiological Associates and set up a cross with (mouse) L cells". Within a few weeks we had the first viable proliferating interspecific hybrids (WEISS, in press).

A brief report of this work (less than 600 words), which used the half-selection technique to detect hybrids between (TK-) mouse L cells and explanted embryonic rat cells, was submitted on March 24, 1965 (EPHRUSSI and WEISS 1965). The interspecific hybrid cells, representing one cross, had been growing in culture for only about one month (about 25 cell divisions). The reports in Genetics (WEISS and EPHRUSSI 1966 a,b) were based on more substantial experience: seven different crosses between mouse and rat cells were studied and, in some cases, more than two hundred division cycles had taken place. Careful karyotypic analysis confirmed beyond doubt that interspecific hybrids were formed. As with the intraspecific hybrids, there were some early chromosome losses (mainly rat chromosomes), with subsequent stabilization of the karyotype. Enzyme studies revealed that both rat and mouse enzymes -- lactic dehydrogenase and α -glucuronidase -- were produced in the hybrids, with mouse and rat subunits yielding hybrid molecules, providing a striking marker.

These papers dramatically changed the emerging field of somatic cell hybridization. As Ephrussi and many others quickly saw, the potential uses of the techniques of cell hybridization were enormously expanded. Somatic cell hybrids between different

species vastly increased the markers that researchers could utilize since even the same enzyme would have somewhat different properties in different species, allowing the regulation and fate of the separate protein molecules in the hybrids to be accurately analyzed. Potentially, regulation of the expression of very many enzymes could now be studied, not just those few with known mutant forms maintained in cell culture. Moreover, the robustness of interspecific hybrids, their coordination of gene expression, the ability to extinguish and restore their differentiated functions, and the coordinated mitotic division of hybrid cells all pointed to the existence of similar systems of cellular control even in distantly related organisms. These results suggested that general controls of cell division and gene expression, common across species barriers, could now be explored via cell hybridization (see the speculations on control of the cell cycle in EPHRUSSI and WEISS 1967). Similar hopes were expressed with regard to processes relating to determination, differentiation, and dedifferentiation (including neoplastic transformation) of cells.

CONCLUSION

We have examined the first decade of somatic cell genetics from the perspective of one of its principal protagonists. After the field had developed to this point, the limitation to an individual's perspective is harder to justify. A new biological field had opened up, one that could no longer be dominated by the work of a small group of laboratories (WEISS in press). As Ephrussi's research program moved on, so did that of others who were drawn to this area of study. The number of different interspecific combinations grew very rapidly, mouse, Chinese hamster, Syrian hamster, rat and Chinese and human cells, etc. serving as "parents" of hybrids. In each of the resulting systems, there were a great variety of studies, formal and

biochemical. A great number of technical refinements, selective systems, enzyme systems, and approaches were introduced, placing experimental studies of the principal aspects of somatic cell genetics beyond the reach of any single laboratory.

Furthermore, the study of somatic cell hybrids was propelled into far greater prominence in genetics (with a corresponding increase in activity) by a new type of hybrid first produced by MARY WEISS and HOWARD GREEN, working at New York University School of Medicine (WEISS and GREEN 1967). They created a mouse/human hybrid using a (TK-) mouse line and embryonic human lung fibroblasts. Such hybrid cells retained the mouse chromosome complement but exhibited a substantial loss of human chromosomes. As Weiss and Green pointed out: "Study of clones containing a small number of human chromosomes should permit the localization of other human genes (WEISS and GREEN 1967, p. 1111)." Indeed that has been the case. Mouse/human hybrids, by effectively transplanting a few human chromosomes into a new cell type, have permitted detailed study of the organization of genes on human chromosomes and provided a substantial stimulus to research in human genetics -- research that previously had been

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stymied by the difficulty of conducting research on humans. The readers of this journal are certainly aware of the wide range of information that has been derived from such studies and from somatic cell genetics in general.

For his part, Ephrussi continued to work on the topics in which he was primarily interested into the late 1970s, using hybrids with teratomas to explore determination and differentiation (e.g., FINCH and EPHRUSSI 1967; KAHAN and EPHRUSSI, 1970), negative regulation of differentiated function (e.g. DAVIDSON et al. 1966; FOUGRE et al. 1972), and related topics. He continued to advocate cellular and genetic approaches over a direct attack at the molecular level (EPHRUSSI 1970, esp. p. 12). Nonetheless, he lived long enough to recognize that his transformation of transplantation into a genetic tool would take on a new and more powerful aspect in the molecular era. Indeed, we suggest that it is useful to interpret recombinant DNA procedures as a form of transplantation of individual genes or groups of genes into new cellular environments, thus facilitating detailed study of their structure, action, and regulation and the production of novel biological entities, processes, and products. Ephrussi could not have foreseen the new genetics emerging from recombinant DNA studies, but the many sorts of studies he set in motion played an important role in making such work possible.

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