PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **516th Meeting** *of the Society was held at the University of Oxford, on Thursday and Friday,* 8 *and* 9 *July* 1971, *when the following papers were presented:*

COLLOQUIUM ON 'SOMATIC CELL GENETICS'

Genetic Analysis with Human–Mouse Hybrid Cells

By W. F. Bodmer. (*Genetics Laboratory, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX*1 3*QU, U.K.*)

Chromosomal segregation after preferential loss of human chromosomes in human–mouse somatic-cell hybrids has provided the first systematic approach to the genetic analysis of somatic cells in culture. Using these techniques, Weiss & Green (1967) and others have shown that the human thymidine kinase gene is on an E group chromosome, probably no. 17. Peripheral-blood white cells (leucocytes), which can be used as the human component in such hybridizations, can provide a convenient cell source from any desired human donor. Any identifiable gene product that can be distinguished in mouse and man provides a potential genetic marker. So far, enzyme electrophoretic differences, antigens and also drug resistance have been the main marker sources. The X-linkage of the enzyme hypoxanthine/guanine phosphoribosyltransferase, which is deficient in 8-azaguanine-resistant cells and in individuals with the X-linked Lesch–Nyhan syndrome, has been confirmed. Thus, in the products of hybridization between normal human cells and 8-azaguanine-resistant mouse cells, sensitivity to 8-azaguanine and presence of human glucose 6-phosphate dehydrogenase are uniformly associated (Nabholz, Miggiano & Bodmer, 1969). A search for consistent associations between pairs of unselected markers has shown that the human genes for peptidase B and lactate dehydrogenase B are most probably linked. Lack of consistent association indicates that most of the 11 other enzymes examined, and in particular lactate dehydrogenase A and lactate dehydrogenase B, are on different chromosomes (Santachiara, Nabholz, Miggiano, Darlington & Bodmer, 1970; Ruddle, Chapman, Chen & Klebe, 1970). Such association must, however, be evaluated in the light of probable strong selective pressures influencing the evolution of hybrid clones.

Nabholz, M., Miggiano, V. & Bodmer, W. (1969). *Nature, Lond.*, **223**, 358.

Ruddle, F. H., Chapman, V. M., Chen, T. R. & Klebe, R. J. (1970). *Nature, Lond.*, **227**, 251.

Santachiara, A. S., Nabholz, M., Miggiano, V., Darlington, A. J. & Bodmer, W. F. (1970). *Nature, Lond.*, **227**, 248.

Weiss, M. C. & Green, H. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 1104.

Insertion of Small Pieces of Foreign Genetic Material into Somatic Cells

By P. R. Cook. (*Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX*1 3*RE, U.K.*)

When a chick red-blood-cell (erythrocyte) nucleus is introduced into the cytoplasm of a tissue-culture cell of the same or of a different species, it resumes the synthesis of DNA and RNA (Harris, 1965, 1967; Johnson & Harris, 1969); and when the reactivated red-blood-cell nucleus develops a nucleolus it determines the synthesis of chick-specific proteins in the hybrid cell (Harris, Sidebottom, Grace & Bramwell, 1969; Harris & Cook, 1969; Cook, 1970). Within 3–4 days the heterokaryons formed by cell fusion enter mitosis and the red-blood-cell nuclei can no longer be distinguished as discrete entities. The fate of the red-blood-cell nuclei was initially obscure, but recent studies have shown that when such heterokaryons enter mitosis the red-blood-cell nuclei undergo fragmentation by a process that has been called 'chromosome pulverization' or 'premature chromosome condensation' (Johnson & Rao, 1970). On cultivation under special conditions mononucleate hybrid cells eventually emerge from such populations of heterokaryons. These hybrid cells, though retaining many of the characteristics of the tissue-culture parental cells, nevertheless contain small amounts of genetic material of the chick parent. By use of this special application of the cell-fusion technique it is possible to incorporate small amounts of foreign genetic material into somatic cells; further, the genes incorporated in this way are expressed and replicated in the foreign environment (Schwartz, Cook & Harris, 1971).

The tissue-culture cells used for these experiments were A 9 cells (Littlefield, 1964). These are a

subline of the mouse L cell and are deficient in the enzyme inosinate pyrophosphorylase (IMP–pyrophosphate phosphoribosyltransferase, EC 2.4.2.8). They were selected by continued subculture in the presence of 8-azaguanine; the cells that have lost inosinate pyrophosphorylase cannot incorporate 8-azaguanine into RNA and are thus resistant to the lethal effects of the antimetabolite. Chick red-blood-cell nuclei, obtained from chick embryos, were introduced into the cytoplasm of A 9 cells by the use of inactivated Sendai virus (Harris & Watkins, 1965). Heterokaryons were cultivated in HAT medium, which contains hypoxanthine, aminopterin, thymidine and glycine at concentrations of 0.1 mM, 0.4 μM, 16 μM and 3 μM respectively. In this medium cells lacking inosinate pyrophosphorylase, and hence unable to synthesize RNA from exogenous sources, do not survive (Syzbalska & Szybalski, 1962). Cells possessing the enzyme do survive in this medium because the block to endogenous nucleic acid synthesis imposed by the aminopterin can be circumvented by the incorporation of exogenous precursors.

When populations of chick red blood cell–A 9 cell heterokaryons were cultivated in HAT medium, the great majority of the cells gradually died; but some clones resistant to HAT medium made their appearance within 2–3 weeks after cell fusion (Schwartz *et al.* 1971). Cells from these clones were found to contain inosinate pyrophosphorylase, and electrophoretic examination of the enzyme by the method of Cook (1970) revealed that it migrated with a mobility characteristic of chick, and not mouse, enzyme (Schwartz *et al.* 1971). It was therefore clear that these clones of cells had retained the chick gene for inosinate pyrophosphorylase and that this gene was replicated on continued cultivation *in vitro*.

Normally when mononucleate hybrid cells are generated from binucleate heterokaryons the resulting hybrid nucleus contains the chromosomes of both parents (Harris, 1970). In some cases many of the chromosomes of one or other parental nucleus are eliminated during early cell divisions, possibly at the first mitosis (Weiss & Green, 1967; Nabholz, Miggiano & Bodmer, 1969). However, the karyotypes of the HAT-resistant hybrid cells derived from chick red blood cell–A 9 cell heterokaryons contained no detectable chick chromosomes (Schwartz *et al.* 1971). This was so, even at an early stage after cell fusion. That very little chick genetic material is present in these hybrid cells is also revealed by tests for the presence of chick-specific surface antigens on their surface. Evidence has been presented that the genes determining species-specific surface antigens are widely distributed in the human chromosomal set (Weiss & Green, 1967). Although the mononucleate hybrid cells produced from the chick red blood cell–A 9 cell heterokaryons continued to produce inosinate pyrophosphorylase, no chick-specific surface antigens could be detected on the surface of these cells (Schwartz *et al.* 1971).

If the chick genetic material were fully integrated into the structure of the mouse chromosomes one might expect that the chick genes would not readily be lost from the cell population when the selection pressure against cells lacking inosinate pyrophosphorylase was removed. Indeed, if the chick inosinate pyrophosphorylase gene were integrated into the chromosome in the same way as the normal mouse inosinate pyrophosphorylase gene, one would expect that the frequency with which inosinate pyrophosphorylase activity would be lost from the hybrid cells would be similar to the frequency with which mutants lacking inosinate pyrophosphorylase are generated in mouse cells cultivated under the same conditions. The rate of production of cells lacking inosinate pyrophosphorylase was therefore measured in populations of hybrid cells and in populations of L cells (from which the A 9 mutants were originally derived). Cells lacking inosinate pyrophosphorylase appeared in the L-cell population at a frequency of less than 1 in 10^6; in the hybrid-cell population the frequency was 20%. The high frequency at which cells lacking inosinate pyrophosphorylase appeared in the cultures of hybrid cells indicates that, in the absence of selective pressure to retain inosinate pyrophosphorylase, the chick inosinate pyrophosphorylase gene is only loosely integrated into the mouse cells (Schwartz *et al.* 1971).

These experiments carried out by Schwartz *et al.* (1971) demonstrate that an amount of genetic material too small to determine the synthesis of any species-specific surface antigens can be inserted into a foreign somatic cell, and that the gene resident in this foreign environment can be expressed and replicated. Possible applications of this technique and further experiments with toad red blood cells are discussed.

The author is a Stothert Research Fellow of the Royal Society and a Platnauer Junior Research Fellow of Brasenose College, University of Oxford.

Cook, P. R. (1970). *J. Cell Sci.* **7**, 1.
Harris, H. (1965). *Nature, Lond.*, **206**, 583.
Harris, H. (1967). *J. Cell Sci.* **2**, 23.
Harris, H. (1970). *The Dunham Lectures: Cell Fusion.* Oxford: Clarendon Press.
Harris, H. & Cook, P. R. (1969). *J. Cell Sci.* **5**, 121.
Harris, H., Sidebottom, E., Grace, D. M. & Bramwell, M. E. (1969). *J. Cell Sci.* **4**, 499.
Harris, H. & Watkins, J. F. (1965). *Nature, Lond.*, **205**, 640.
Johnson, R. T. & Harris, H. (1969). *J. Cell Sci.* **5**, 625.
Johnson, R. T. & Rao, P. N. (1970). *Nature, Lond.*, **226**, 717.

Littlefield, J. W. (1964). *Nature, Lond.*, **203**, 1142.
Nabholz, M., Miggiano, V. & Bodmer, W. (1969). *Nature, Lond.*, **223**, 358.
Schwartz, A. G., Cook, P. R. & Harris, H. (1971). *Nature New Biol., Lond.*, **230**, 5.
Szybalska, E. & Szybalski, W. (1962). *Proc. natn. Acad. Sci. U.S.A.* **48**, 2026.
Weiss, M. C. & Green H. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 1104.

Chromosome Behaviour in Human–Mouse Somatic-Cell Hybrids

By S. D. HANDMAKER. (*Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, U.K.*)

The usefulness of cell fusion in the genetic analysis of somatic cells stems largely from two observations. First, Harris & Watkins (1965) demonstrated that not only like cells, as shown by Okada (1962), but also cells of widely different types, even cells from species as different as man and mouse, could be fused together by inactivated Sendai virus. Thus cell fusion can produce recombination of genetic material of different somatic cells. Secondly, Weiss & Green (1967) found that in human–mouse hybrid cells the great majority of the human chromosomes were rapidly eliminated. This preferential loss of chromosomes of one species is a characteristic pattern of behaviour of chromosomes in hybrids between cells of different species. It is the loss of chromosomes from these cells that produces the segregation necessary for genetic analysis.

The hybrid cells that have so far been used most often in genetic analysis have been human–mouse hybrids. All of these have been obtained by the use of drug-resistant markers and growth in HAT selective medium (containing hypoxanthine, aminopterin and thymidine), the procedure of selecting hybrid cells that was introduced by Littlefield (1964). There are some reasons for believing that the HAT medium may be a significant factor in the pattern of chromosome loss: (*a*) excess of thymidine produces a prolonged S-phase (E. Stubblefield, unpublished work cited by Schmid, 1965), and O'Neill & Miles (1970) have shown that in dikaryons 'pulverized' chromosomes are the last to replicate; (*b*) excess of thymidine is also reported to cause loss of chromosomes into the cytoplasm (Bootsma, Budke & Vos, 1964); (*c*) the preferential loss of human chromosomes appears to be more marked in the absence of HAT or in BUDR medium than it does in the HAT medium in which the hybrid was isolated (Weiss & Green, 1967; Matsuya, Green & Basilico, 1968).

We have now succeeded in obtaining human–mouse cell hybrids without the use of HAT medium or any other selective medium by fusing mouse Ehrlich ascites-tumour cells, which are fast-growing and do not attach to glass, with normal human diploid fibroblasts, which are slow-growing and attach to glass. The parental Ehrlich ascites-tumour cells were removed simply by changing the medium, and a hybrid was obtained that attached to glass and grew much faster than the parental human fibroblasts. Chromosome preparations were made and examined. The objectives of this cytogenetic analysis were (*a*) a consideration of the factors determining chromosome loss and (*b*) a more complete description of the behaviour of chromosomes in hybrid cells.

Cytogenetic analysis of these hybrid cells revealed the following points: (1) human–mouse cell hybrids isolated without selective medium showed the same preferential loss of human chromosomes as cells selected in HAT medium; (2) although some of the human–mouse cells retained a substantial number (15–20) of human chromosomes as long as a year after fusion, others lost all identifiable human chromosomes within a few months; (3) most of the hybrid cells were derived from more than a single chromosome complement of each parental type; (4) chromosomal rearrangements sometimes occurred in the hybrid cells after prolonged growth; (5) some of the hybrid cells showed a substantial loss of human chromosomes even in the earliest divisions after fusion; (6) the chromosome variation observed within the hybrid population was greatest shortly after fusion; (7) the evidence was against non-disjunction as the mechanism responsible for the loss of human chromosomes.

Further studies of the chromosomes in the early divisions will be discussed.

S.D.H. is a Research Fellow of the Helen Hay Whitney Foundation.

Bootsma, D., Budke, L. & Vos, O. (1964). *Expl Cell Res.* **33**, 301.
Harris, H. & Watkins, J. F. (1965). *Nature, Lond.*, **205**, 640.
Littlefield, J. W. (1964). *Science, N.Y.*, **145**, 709.
Matsuya, Y., Green, H. & Basilico, C. (1968). *Nature, Lond.*, **220**, 1199.
Okada, Y. (1962). *Expl Cell Res.* **26**, 98.
O'Neill, F. J. & Miles, C. P. (1970). *Proc. Soc. exp. Biol. Med.* **134**, 825.
Schmid, W. (1965). In *Human Chromosome Methodology*, p. 91. Ed. by Yunis, J. J. New York and London: Academic Press.
Weiss, M. C. & Green, H. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 1104.