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Chromosome Behaviour in Human-Mouse Somatic-Cell Hybrids

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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factor in the pattern of chromosome loss: (a) excess of thymidine produces a prolonged S-phase (E. Stubblefield, unpublished work cited by Schmid, 1965), and O'Neill & Miles (1970) have shown that in dikaryons 'pulverized' chromosomes are the last to replicate; (b) excess of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.

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