Interaction Properties of Cell Hybrids Formed by Fusion of Interacting and Non-interacting Mammalian Cells

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Mutant BHK cells,* derived from the hamster fibroblast line BHK 21/13 (Macpherson & Stoker, 1962), that lack inosinate pyrophosphorylase or thymidine kinase (BHK-IPP⁻ and BHK-TK⁻ cells; Marin & Littlefield, 1968) are unable to incorporate [³H]hypoxanthine and [³H]thymidine respectively when grown separately in tissue culture. However, when grown together in confluent cultures both cell mutants incorporate [³H]hypoxanthine and [³H]thymidine. This mutual phenotypic modification of BHK-cell mutants, through cell-cell contact in tissue culture, is most probably explained by the transfer of [³H]purine nucleotides and [³H]thymidine nucleotides through intercellular junctions, which, unlike the normal cytoplasmic membrane, are permeable to these nucleotides (Bürk, Pitts & Subak-Sharpe, 1968; Pitts, 1971; J. D. Pitts, unpublished work).

Mutant L cells, derived from the mouse fibroblast line L 929 (Sanford, Earle & Likely, 1948), namely L-IPP⁻ and L-TK⁻ cells (Littlefield, 1966), on the other hand do not interact in this way and remain phenotypically distinct when grown together in confluent cultures. Further, the inability of such mutant L cells to interact is dominant in mixed cultures of L cells and BHK cells (Pitts, 1971a).

The interaction properties of cell hybrids formed between BHK cells, which do interact, and L cells, which do not interact, have now been studied. Hybrids were formed between BHK-TK⁻ cells and L-IPP⁻ cells by fusion with inactivated Sendai virus (Harris & Watkins, 1965) and selected by growth in medium supplemented with aminopterin, thymidine and hypoxanthine.

Cloned hybrid BHK-L cells were phenotypically wild-type, incorporating both [³H]hypoxanthine and [³H]thymidine, and interacted in mixed confluent cultures with mutant BHK cells but not with mutant L cells. The hybrid cells were shown to possess both BHK-cell and L-cell cytoplasmic membrane determinants by cytotoxicity tests with rabbit anti-(hamster cell) serum and rabbit anti-(mouse cell) serum, in the presence of guinea-pig complement, and measuring cell lysis by the uptake of Naphthalene Black.

These results are consistent with the concept that cells that fail to interact in tissue culture have lost some membrane function essential to both donor

and recipient cells for the formation of the inter-cellular junctions required for nucleotide exchange.

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Histone F2c and Erythrocyte Maturation

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It has been suggested that the erythrocyte-specific histone F2c is involved in gene restriction during the terminal maturation of these cells (Neelin, Callahan, Lamb & Murray, 1964). It is clearly of interest to determine the point of differentiation at which the histone appears in chromatin. Dick & Johns (1969) have shown that the proportion of histone F2c relative to the other histones is the same in reticulocytes from anaemic birds as in mature cells from normal birds.

In the present study the histones from erythrocytes of the chick embryo, from adult hen bone marrow and from erythrocytes in anaemic hens were examined.

The histones were extracted from nuclei prepared from these cells, whole histone with 250mm-hydrochloric acid, or lysine-rich histone, containing only histone F1 and F2c, with 5% (w/v) perchloric acid. The relative amounts of the histones were measured by densitometry after separation of the proteins on polyacrylamide gels and staining with Naphthalene Black.

In chick embryos at between 4 and 20 days of incubation the cell population in the circulating blood changes from mainly immature cells of the secondary generation of erythrocytes to mainly mature cells (Lucas & Jamroz, 1961). Analysis of the histones during this period shows no change in the proportion of histone F2c as compared with mature erythrocytes from adult birds.

Whole cells or nuclei from bone marrow or from anaemic hens, labelled *in vitro* with [³²P]phosphate, were separated by zonal centrifugation on sucrose density gradients either by rate or by equilibrium sedimentation, the former for cells, the latter for nuclei, as described by Mathias, Ridge & Trezona

* Abbreviation: BHK cells, baby-hamster kidney cells.