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cytoplasmic membrane, are permeable to these nucleotides (Bürk, Pitts & Subak-Sharpe, 1968; Pitts, 1971; J. D. Pitts, unpublished work).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.

(1969). This gave fractions of differing maturity as judged by the incorporation of [32 P]phosphate into RNA and DNA.

Histone F2c was present in all the fractions obtained in these studies and, when due allowance had been made for non-erythroid-cell contamination no significant change in the proportion of this histone relative to that of the other histones could be established. It was found, however, that the incorporation of [32 P]phosphate into the lysine-rich fraction declined with maturation, confirming the result obtained by Adams, Vidali & Neelin (1970).

These studies indicate that the function of histone F2c, if related to terminal maturation, may depend on its phosphate content. It seems possible, however, that it may be involved at an earlier point in differentiation than has so far been suggested, since it appears to be present at the earliest stages of erythroid development analysed in these experiments.

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Changes in Rat Liver Non-histone Nuclear Proteins during Nitrosamine Carcinogenesis

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It has been reported (Harbers, Lederer, Sandritter & Spaar, 1968) that when rats are given diethylnitrosamine in their drinking water at 3 mg/kg per day then liver tumours are produced after 180 days. At this low dose no cirrhotic changes were observed in the liver, but in the nuclei a doubling of heterochromatin content, accompanied by a corresponding decrease in euchromatin, was found to occur after about 40 days of dosage with the carcinogen. As this probably indicates a profound change in the chromatin structure that may lead to neoplasia, investigations were made to ascertain whether any change in the composition of the nuclear proteins occurs at this point. Initial studies had indicated that no change occurred in the composition of the histones after administration of the carcinogen. This communication is concerned with preliminary attempts to investigate the non-histone proteins of the liver cell nucleus.

Various methods have been described for the extraction of the non-histone nuclear proteins. These usually involve several stages, using combinations of dilute buffers, dilute and concentrated sodium chloride solutions, detergent mixtures and phenol (see Hnilica, 1967). A method has been described (Gronow, 1969) by which practically all of the nuclear non-histone protein can be extracted by using 8M-urea-50mM-sodium phosphate buffer, pH 7.6. Proteins in this mixture have been separated into about 22 components by using the technique of isoelectric focusing in polyacrylamide gel (pH 3-10 Ampholine). Separation on the basis of molecular weight by using sodium dodecyl sulphate-polyacrylamide-gel electrophoresis (Elgin & Bonner, 1970) gave about 18 bands. When these techniques were applied to rat liver nuclear proteins extracted with other solvents (e.g. dilute and concentrated saline) it was found that each fraction contained, in various amounts, components present in others.

Nuclei prepared by the Chauveau, Moulé & Roueller (1956) technique from the livers of rats given diethylnitrosamine for 50 and 70 days were extracted with 8M-urea-50mM-sodium phosphate buffer, pH 7.6. The Coomassie Blue-stained isoelectric-focusing polyacrylamide gels and sodium dodecyl sulphate-polyacrylamide gels of these extracts looked identical with the controls. However, when the thiol groups of these proteins were labelled with [14 C]-*N*-ethylmaleimide, an increase in the specific radioactivity of the proteins from the 70-day-treated animals was found. Components focusing on the pH 7-8 region were found to have a marked increase in thiol content.

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Lipase Activity against 1,2-Diglyceride in Rat Erythrocyte Membranes

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Erythrocyte membranes prepared from fresh heparinized rat blood were treated with phospholipase C (from *Clostridium welchii*; Sigma Chemical Co., St Louis, Mo., U.S.A.). Lipids were then