STUDIES ON CELL COMMUNICATION WITH ENUCLEATED HUMAN FIBROBLASTS

R. P. COX, M. R. KRAUSS, M. E. BALIS, and J. DANCIS

From the Division of Human Genetics, Departments of Medicine, Pharmacology, and Pediatrics, New York University Medical Center, New York 10016, and the Sloan Kettering Cancer Center, New York 10021

ABSTRACT

Metabolic cooperation, the correction of the mutant phenotype in cells deficient in hypoxanthine phosphoribosyltransferase (HPRT-) by intimate contact with normal cells (HPRT+), represents a form of cell communication that is easily studied with radioautography. In the present study it was found that the formation of cell junctions needed for communication does not require protein synthesis nor is it under the immediate control of the cell nucleus. Enucleated normal cells efficiently communicate with HPRT- mutant cells. The effectiveness of enucleated cells as donors in metabolic cooperation provides evidence that it is the transfer of small molecules, nucleotide, or nucleotide derivatives that is responsible for correction of the mutant phenotype. Karyoplasts (nuclei with small amounts of cytoplasm surrounded by a plasma membrane) are unable to efficiently communicate with intact cells. The utilization of [3H]hypoxanthine by communicating mixtures of HPRT+ and HPRT- human cells is not significantly different than in the normal cells alone. Metabolic cooperation, as studied, involves a redistribution of purine-containing compounds among communicating cells.

Communication between cells by physical contact of plasma membranes constitutes a fundamental property of many cells and is required for a number of biologically important cell-to-cell interactions. Current evidence suggests that contact-mediated intercellular communication requires the formation of a specialized membrane structure—the gap junction (11). Gap junctions are seven-layered structures composed of polygonal lattices of 70-80 Å subunits. Freeze-fracture techniques for studying cell surface topography have demonstrated that these junctions are present as localized plaques that are distinctly differentiated from regions of nonjunctional membrane (11).

Ionic coupling and metabolic cooperation have been extensively studied as functional expressions of intercellular communication, and these investigations complement those on the morphology of gap junctions (1, 8, 10, 12, 19). Metabolic cooperation is a form of cell communication in which the mutant phenotype of enzyme-deficient cells, as determined by incorporation of labeled substrates, is corrected in culture by contact with normal cells (1, 3, 4, 8, 12, 18, 24). In the present studies it was found that the protein synthesis inhibitor, cycloheximide, did not interfere with the formation of those junctions required for metabolic cooperation.

Prescott et al. (20) have described a method for enucleating mammalian cells by centrifugation in medium containing the fungal metabolite cytochalasin B. The enucleated cytoplasm (cytoplast) is metabolically active (20), possesses a near normal ultrastructure (26), and exhibits normal be-
tion is aided by the different degree of decolorization (23). TG-2 is a variant of BHK\(_{13}\), selected for resistance to thioguanine (50-100 \(\mu\)g/ml), and has a marked sensitivity to cytosine arabinoside (26). Cytoplasts and karyoplasts provide the opportunity to study independently the contributions of the nucleus and cytoplasm to cell communication. In the present study, normal (HPRT\(^+\)) cytoplasts and karyoplasts were used as donors in metabolic cooperation. It was found that cytoplasts efficiently communicated with mutant (HPRT\(^-\)) hamster cells but that karyoplasts did not.

Previous studies suggested that metabolic cooperation between HPRT\(^+\) and HPRT\(^-\) cells is the result of transfer of small molecules from normal to mutant cells rather than transfer of enzyme or informational macromolecules that lead to synthesis of the enzyme (5, 6, 8, 18). Evidence obtained with enucleated cells and karyoplasts provides strong support that it is the transfer of small molecules, nucleotides, or nucleotide derivatives that corrects the mutant phenotype of HPRT-deficient cells.

The uptake of labeled hypoxanthine in cooperating mixtures of HPRT\(^+\) and HPRT\(^-\) cells was measured to determine whether the utilization of labeled purine was increased to compensate for the enzyme deficiency in the mutant cell. The results indicated that there was no increase in \(^{[3}\)H\]hypoxanthine utilization.

### MATERIALS AND METHODS

**Cells**

Human diploid fibroblasts derived from normal male subjects (HPRT\(^+\)) and patients with Lesch-Nyhan syndrome (HPRT\(^-\)) were established in culture by explanting skin as previously described (5, 7). Hamster cells were the established baby Syrian hamster kidney cell line BHK\(_{13}\) clone 13 which has a diploid male karyotype (23). TG-2 is a variant of BHK\(_{13}\) \(C_{13}\), selected for resistance to thioguanine (50-100 \(\mu\)g/ml), and has a marked deficiency of HPRT (16). Both hamster cell lines were the generous gift of Dr. John W. Littlefield, Johns Hopkins Hospital, Baltimore, Md.

Morphological differences between cells of different species in cell mixtures permit the identification of donor and recipients on an individual cell basis. This identification is aided by the different degree of decolorization after staining with May-Grünewald-Giemsa's stain (6, 8, 9). As shown in Fig. 1, human cells are lighter staining and have a nucleus with three or four small nucleoli. Hamster cells are darker staining with a nucleus containing one or several large nucleoli. Occasionally, a cell cannot be unequivocally identified and is therefore not scored in analyzing results.

**Media and Cultures**

Cells were grown in Waymouth's medium (25) containing 10% fetal calf serum and antibiotics (penicillin 50 U, streptomycin 50 \(\mu\)g, and kanamycin 30 \(\mu\)g/ml). Cultures were carried in flat-bottomed flint glass bottles and were harvested, subcultured, and prepared for radioautography as previously described (6, 9).

**Enucleation of Cells with Cytochalasin B**

Enucleation of human diploid fibroblasts was carried out by minor modifications of the methods of Prescott et al. (20) and Goldman and Pollack (13). Cells were grown on dishes punched out of plastic trays (Limbro no. 96 CV-TC) with a 12-mm cork borer and coated with bovine collagen (a gift from Dr. Richard Kronenthal of the Ethicon Co., Inc., Somerville, N. J.). The disks were inserted cell side down into 15-ml Corex centrifuge tubes containing 2.5 ml of complete Waymouth's medium with 10 \(\mu\)g/ml of cytochalasin B (Aldrich Chemical Company, Inc., Milwaukee, Wis.). They were incubated for 10 min in a 37\(^\circ\)C water bath and then placed in a warmed (35\(^\circ\)C) Lourdes model AX centrifuge. The cells were spun at 11,500 rpm (17,000 \(g\)) for 12 min in an incubator at 35\(^\circ\)C. After centrifugation the disks were transferred to 35-mm plastic Petri dishes. They were washed once with Hanks' balanced salt solution and then were incubated in fresh Waymouth's medium without cytochalasin B. After 1- to 2-h incubation, the disks containing the cytoplasts were washed in Hanks' balanced salt solution, and the cytoplasts were detached from the collagen substrate with 0.25% trypsin and 0.1% EDTA (Grand Island Biological Co., Grand Island, N. Y.). When cells were observed to round up, the trypsin-EDTA solution was removed and the cytoplasts were taken up in complete medium and inoculated into Leighton tubes containing 6 \(\times\) 30 mm cover slips upon which hamster cells were attached. The cytoplasts and hamster cells were cocultured in complete Waymouth's medium containing \(^{[3}\)H\]hypoxanthine and were prepared for radioautography (see legends to figures and tables).

Karyoplasts, separated from cells during centrifugation in medium with cytochalasin B, were recovered along with a few intact detached cells from the bottom of the centrifuge tube by centrifuging the medium, after removing the plastic disks, in a clinical centrifuge for 10 min at 1,600 rpm. They were washed once in complete medium and then suspended in fresh Waymouth's medium without cytochalasin B and were inoculated either into Leighton tubes containing 6 \(\times\) 30 mm cover slips or into Lab Tek culture slides containing attached hamster cells. The karyoplasts and hamster cells were cocultured...
Effect of cycloheximide on cell communication. HPRT+ hamster cells growing on cover slips were incubated for 1 h with 20 μCi/ml of [3H]hypoxanthine. Lesch-Nyhan fibroblasts were then inoculated on top of HPRT+ hamster cells. Cultures were incubated for 6 h with 20 μCi/ml of [3H]hypoxanthine in the absence (a) and presence of cycloheximide 10 μg/ml (b). Lesch-Nyhan fibroblasts cultured alone for 6 h in radioactive medium are shown in (c). Although cycloheximide reduces the incorporation of [3H]hypoxanthine, it does not interfere with the transfer of label as shown in Table I. Human fibroblasts (marked by arrows in cell mixtures) are lightly stained and have several small nucleoli. Hamster cells are more darkly stained and have nuclei with larger and more darkly stained nucleoli. × 900 (approx).
in complete medium with radioactive isotopes and were prepared for radioautography.

**Radioautographic Studies**

Radioactive substrates were chromatographed before use. [3H]Adenine (6.0 Ci/mmol) and [3H]uridine (37.6 Ci/mmol) were sufficiently pure to use as received, but [3H]hypoxanthine (3.0 Ci/mmol) required further purification on Dowex 50. All radioactive compounds were purchased from New England Nuclear Corp., Boston, Mass.

Radioautographic studies were carried out by adding the isotope to Waymouth’s medium containing 15% fetal calf serum. The final concentration of label depended on the cell line or cell mixture being studied. The concentrations used and the duration of incubation are described in the legends to the tables and figures. Sufficient [aH]hypoxanthine was used to produce heavy labeling (50 grains or more per nucleus) in over 70% of HPRT+ cells. Labeled cultures grown on cover slips or Lab-Tek slides were prepared for radioautography as previously described (5, 6, 9).

**RESULTS**

**Effect of Inhibiting Protein Synthesis**

The specialized cell junctions required for cell communication are ultrastructurally complex and highly ordered structures. Previous work indicated that metabolic cooperation can be demonstrated within 1 h after cells make contact (9). To determine whether de novo protein synthesis is required for the production of effective cell contacts, suspensions of HPRT- human cells were inoculated onto cover slips containing normal BHK cells that had been labeled for 1 h with [3H]hypoxanthine. Cycloheximide at a final concentration of 2 and 10 µg/ml was immediately added and the cell mixture was incubated for 6 h with 30 µCi/ml [3H]hypoxanthine. Labeled cultures grown on cover slips or Lab-Tek slides were prepared for radioautography as previously described (5, 6, 9).

**Communication Between Cytoplasts and Mutant Cells**

In all of the following experiments the cell density of cultures was low to ensure that labeled mutant cells in contact with cytoplasts could not have been fed by the occasional (10-15%) human cells that escaped enucleation. The efficiency of enucleation was determined by counting the proportion of nucleated cells on cover slips treated in parallel with the cover slips used for the experimental study. Labeled human cytoplasts were prepared by incubating normal skin fibroblasts for 18 h with 30 µCi/ml [3H]hypoxanthine before enucleation. These cytoplasts functioned efficiently as donors in metabolic cooperation when cocultured with HPRT- hamster cells in medium containing [3H]hypoxanthine (Fig. 2a). Prelabeled HPRT+ cytoplasts also transferred label efficiently to HPRT- hamster cells when cocultured in nonradioactive medium, indicating that labeled compounds can be derived from the turnover of radioactive macromolecules.

A series of experiments were carried out in which HPRT+ cytoplasts were cocultured with HPRT- cells. Isolated HPRT+ cytoplasts and isolated HPRT- cells incubated in medium with

**Table I**

| Cell type in 1:1 mixtures | Cycloheximide | <10 | 11-30 | 31-50 | >50 |
|--------------------------|----------------|-----|-------|-------|-----|
| BHK C13                  | 0              | 9   | 20    | 71    |
|                           | 2              | 6   | 51    | 25    | 18  |
|                           | 10             | 34  | 58    | 7     | 1   |
| Lesch-Nyhan              | 0              | 0   | 14    | 30    | 56  |
|                           | 2              | 2   | 62    | 31    | 5   |
|                           | 10             | 24  | 75    | 1     | 0   |

Cultures were incubated at 37°C with 35 µCi/ml [3H]hypoxanthine for 3 h. Lesch-Nyhan cells grown alone in absence of cycloheximide showed all the cells with less than 10 grains per nucleus (65% of cells had no grains over nucleus and 35% had less than five grains per nucleus).

* Numbers in the table are the percentage of cells with a given number of grains per cell nucleus. A total of 200 cells of each type was counted in each preparation.
FIGURE 2 Cell communication between HPRT+ human cytoplasts and HPRT- hamster cells. Human HPRT+ fibroblasts were enucleated by centrifuging in medium containing cytochalasin B. After recovery in medium without cytochalasin B, they were trypsinized and inoculated onto cover slips containing widely separated HPRT- hamster cells. Cytoplasts and mutant cells were cocultured for 8 h in medium containing 30 μCi/ml of [3H]hypoxanthine. (a) Prelabeled cytoplasts marked by arrows were prepared by enucleating human HPRT+ fibroblasts that had been labeled for 18 h in medium containing 30 μCi/ml of [3H]hypoxanthine. (b–e) Cytoplasts marked by arrows were prepared from unlabeled HPRT+ human fibroblasts. HPRT- hamster cells that are in contact with HPRT+ cytoplasts (a–e) are labeled, indicating efficient metabolic cooperation. (f) Isolated HPRT- hamster cells in the same preparation show background labeling. × 400 (approx).

[3H]hypoxanthine did not show labeling. However, HPRT- cells in contact with HPRT+ cytoplasts were labeled (Fig. 2b, c, d, and e). The cytoplasts remained unlabeled. Communication between human HPRT+ cytoplasts and HPRT- hamster cells is further documented and quantified in Fig. 3 which shows the frequency distribution of grains in HPRT- cells in contact with unlabeled HPRT+ cytoplasts (hatched bars, Fig. 3 A). These numbers are compared to the number of grains in isolated HPRT- cells in the same preparation (open bars, Fig. 3 A).
Figure 3  Cell communication between human HPRT+ cytoplasts or karyoplasts and HPRT- hamster cells. Frequency distribution of cells according to nuclear grain counts in 100 HPRT- hamster cells for each series. (A) Test series (hatched bars): HPRT- hamster cells in contact with unlabeled HPRT+ human cytoplasts. Control series (open bars): HPRT- hamster cells in the same preparation not in contact with HPRT+ cytoplasts. Higher frequency of nuclei with over 20 grains in test series as compared to control indicates efficient cell communication. (B) Test series (hatched bars): HPRT- hamster cells in contact with HPRT+ karyoplasts. Control series (open bars): HPRT- hamster cells in the same preparation not in contact with karyoplasts. Karyoplasts did not efficiently transfer label to mutant cells.

Communication Between Karyoplasts and Mutant Cells

Karyoplasts were prepared from normal skin fibroblasts that had been labeled by incubation for 18 h with 30 μCi/ml of [3H]hypoxanthine before enucleation. The labeled karyoplasts were cocultured with HPRT- hamster cells in medium with [3H]hypoxanthine. As shown in Fig. 4a and quantitatively in the right panel B of Fig. 3 the normal karyoplasts did not efficiently transfer label to mutant hamster cells. Prelabeled karyoplasts were used in these experiments to provide optimal conditions for metabolic cooperation since labeled compounds transferred to mutant cells can be derived from the turnover of labeled macromolecules as well as from [3H]hypoxanthine in the medium. Karyoplasts prepared from HPRT- Lesch-Nyhan fibroblasts failed to receive label from HPRT+ hamster cells (Fig. 4b).

The metabolism of radioactive pyrimidines and purines was studied in unlabeled karyoplasts prepared from normal human fibroblasts. Approximately 45% of karyoplasts incorporated [3H]-uridine and 20% incorporated [3H]hypoxanthine as demonstrated radioautographically in Fig. 4c and d. Differences in incorporation of uridine and hypoxanthine by karyoplasts may be the result of inadequate levels of cosubstrate phosphoribosylpyrophosphate required for hypoxanthine utilization or may be related to differences in the specific activity of the radioactive compounds.

Quantitative Incorporation of [3H]Hypoxanthine During Metabolic Cooperation

Confluent mixtures of normal and Lesch-Nyhan human fibroblasts incorporated approximately one-half the amount of [3H]hypoxanthine as an equal number of HPRT+ cells as shown in Table II. Radioautography showed that all the cells were labeled, indicating efficient cooperation. The results contrast with those observed when replicate flasks were labeled with [3H]adenine. The incorporation of this label involves an analogous reaction utilizing a different enzyme, adenine phosphoribosylpyrophosphate transferase, which is present in normal and mutant cells. The incorporation of adenine in mixtures of normal and HPRT- human fibroblasts approximates that of an equivalent number of normal cells (Table II). Lesch-Nyhan fibroblasts incorporate slightly more adenine than normal cells, presumably because of increased concentrations of the cosubstrate PRPP.
TABLE II

| [H]Hypoxanthine and [H]Adenine Incorporation into Mutant (HPRT⁻), Normal (HPRT⁺) and 1:1 Mixtures of Human Skin Fibroblasts |
|---------------------------------------------------------------|
| Hypoxanthine and Adenine Incorporation (cpm × 10⁻⁴)          |
| Human skin fibroblast strain | 3 h | 24 h | 48 h | 3 h | 24 h | 48 h |
| HPRT⁻            | 0.10 ± 0.01 | 0.56 ± 0.07 | 1.11 ± 0.05 | 15.57 | 84.22 | 86.66 |
| HPRT⁺            | 2.77 ± 0.04 | 25.95 ± 0.41 | 51.98 ± 0.63 | 8.43 | 57.79 | 77.68 |
| HPRT⁻ and HPRT⁺  | 1.53 ± 0.06 | 16.67 ± 0.89 | 30.68 ± 0.83 | 9.78 | 60.59 | 76.52 |

Replicate 6 × 30 mm cover slips were inoculated with 300,000 HPRT⁻ cells or 300,000 normal human cells or a mixture of 150,000 HPRT⁻ cells and 150,000 normal cells. The cell mixture was derived from the same cell suspensions as the HPRT⁻ and HPRT⁺ cells grown alone. Cover slips were incubated with either 50 μCi/ml of hypoxanthine or 5 μCi/ml of adenine in complete Waymouth’s medium for the time indicated. The cover slips were washed three times with Hanks’ buffered salt solution and fixed overnight in methanol. The fixed cover slips were extracted with 5% ice-cold TCA for 5 min and then washed in running water for 4 h. Cover slips were placed in Diotol scintillant and counted in a Packard Tricarb Spectrophotometer.

After counting in scintillant, the cover slips were washed three times in methanol and prepared for radioautography. Examination of these cover slip preparations showed that, in mixtures of HPRT⁺ and HPRT⁻ mutant cells, all cells were labeled, indicating efficient metabolic cooperation.

* Means and ranges are shown for hypoxanthine.

DISCUSSION

Gap junctions have been implicated as ultrastructural units necessary for efficient cell-to-cell communication (2, 10-12, 17). Other junctions (for example, tight junctions and septate junctions) may show some of the physiological properties of gap junctions but there is no evidence that they can function as intercytoplasmic channels. There is little known concerning the formation of low resistance junctions or the level of control of this process. However, cells in culture form low resistance junctions within a relatively brief but variable time (9, 21, 27).

In the present study metabolic cooperation between mutant HPRT⁻ and normal HPRT⁺ cells was investigated to determine the effect of protein synthesis and the presence of a nucleus on the formation of junctions. Neither protein synthesis nor the presence of a nucleus appears to be immediately required for the formation of efficient junctions needed for metabolic cooperation. These findings suggest the aggregation or assembly of preformed membrane structures to form communicating junctions. Karyoplasts are metabolically active as determined by incorporation of labeled uridine and hypoxanthine. Their inability to communicate efficiently with competent cells may be related to the limited plasma membrane surrounding the nucleus and rim of cytoplasm. Insufficient membrane sites required for junction formation may be responsible for this failure to communicate. Alternatively, damage to the plasma membrane during enucleation may reduce junction formation by karyoplasts. That cytoplasts do efficiently communicate indicates the reversibility of the inhibiting effects of cytochalasin B on communication (4, 9, 22) and the recovery from putative damage by centrifugation.

The present study provides further evidence for the view that small molecules are transferred through junctions from one cell to another. Important support for this conclusion is based on the experiments carried out with unlabeled HPRT⁺ cytoplasts cocultured with HPRT⁻ cells in medium containing [H]hypoxanthine. Radioautography revealed that the mutant cells were labeled but that the cytoplasts containing the HPRT enzyme were not labeled. Several deductions can be made from this observation. (a) The nucleus is not required for cell communication. This is contrary to a suggestion that we have previously made based on indirect evidence obtained from pulse and chase experiments (9). (b) The nucleus converts nucleotides formed by HPRT into macromolecules which are retained during fixation and the washing procedures required for radioautography. The cytoplast is unable to perform such conversions and therefore appears unlabeled in radioautographs. (c) The compounds that are visualized by

COX ET AL. Cell Communication with Enucleated Human Fibroblasts 699
IN CULTURE

RADIOAUTOPHONY

FIGURE 5 Schematic representation of metabolic cooperation. Cell communication between HPRT$^+$ and HPRT$^-$ cells (a) and between HPRT$^+$ cytoplasts and HPRT$^-$ cells (b). In culture, HPRT converts $[^{3}H]$hypoxanthine (A) to nucleotides (B) which are processed in the nucleus to macromolecules (C), some of which are transferred into cytoplasm as RNA (C'). In radioautographs, only macromolecules (C and C') are visualized since compounds (A) and (B) are washed out during preparation for radioautography.

Radioautography, presumably macromolecules, are not those transferred in metabolic cooperation. The sequence of events in metabolic cooperation are diagrammatically presented in Fig. 5. Hypoxanthine (A) is converted to nucleotide by HPRT in normal cells. The nucleotides or closely related small molecules (B) in Fig. 5, are transported into the nucleus for processing into macromolecules (C) or are transferred into communicating cells where they are similarly processed as shown in Fig. 5. The macromolecular compound C remains in the nucleus or is transported to the cytoplasm as C'. The fixation and washing procedures in radioautography extract compounds A and B, in Fig. 5, from the cells, leaving C and C' as indicators of the processes that have occurred.

This interpretation assigns to the HPRT$^+$ cell the role of supplier of nucleotide and related small molecules for the HPRT$^-$ cell in metabolic cooperation. A logical deduction would be that, under the conditions of metabolic cooperation between 1:1 mixtures of normal and mutant cells, the uptake of hypoxanthine would be that expected from an equivalent number of normal cells alone. Measurements of $[^{3}H]$hypoxanthine incorporation proved this hypothesis wrong (Table II). The utilization of hypoxanthine by normal cells was not increased during metabolic incorporation. To explain this unexpected result, it must be recalled that the mutant cells are deprived of the purine salvage pathway but are still capable of synthesizing nucleotides or their derivatives de novo. Under tissue culture conditions both HPRT$^+$ and HPRT$^-$ cells grow equally well and it may be assumed that they have similar concentrations of such essential materials as the purine-containing compounds. To maintain similar concentrations of purines in normal and mutant cells and yet permit transfer of labeled nucleotides from HPRT$^+$ to HPRT$^-$ cells, one must assume a reverse transfer of unlabeled compounds. Cell communication has been demonstrated to be bidirectional (3).

The biological role of low resistance junctions is not known. As Sheridan (21) has pointed out,

Figure 4  HPRT$^+$ human karyoplasts cultured with HPRT$^-$ hamster cells and the metabolic activity of karyoplasts. Labeled karyoplasts were prepared by enucleating, with cytochalasin B, HPRT$^+$ human fibroblasts that had grown for 18 h with 30 $\mu$Ci/ml of $[^{3}H]$hypoxanthine. The prelabeled karyoplasts and mutant hamster cells were cocultured for 8 h in medium containing 30 $\mu$Ci/ml $[^{3}H]$hypoxanthine. HPRT$^+$ labeled karyoplasts did not efficiently transfer label to mutant hamster cells as shown in (a). Arrow points to labeled karyoplast. Lesch-Nyhan karyoplasts were cultured with HPRT$^+$ hamster cells for 9 h in medium containing 20 $\mu$Ci/ml $[^{3}H]$hypoxanthine. Lesch-Nyhan karyoplasts did not receive label from normal cells as shown in (b). Arrow points to unlabeled karyoplast. The metabolic activity of normal karyoplast was determined by incubating them for 6 h with 25 $\mu$Ci/ml $[^{3}H]$uridine or 50 $\mu$Ci/ml $[^{3}H]$hypoxanthine. Approximately 45% of karyoplasts were labeled with $[^{3}H]$uridine (c) and 20% with $[^{3}H]$hypoxanthine (d). $\times$ 900 (approx).
these junctions transmit electrical signals (ionic coupling) between certain excitable cells. However, most cells connected by low resistance junctions do not produce electrical signals, and in these cells the junctions must have other functions. Since this study and others have implicated the transfer of small molecules (<1,000 mol wt) from one cell to another (2, 10, 15, 17), it seems probable that metazoans have evolved low resistance junctions in certain tissues to ensure coordinate responses of the interconnected cell system. For example, certain cyclic nucleotides and cations have been shown to control various aspects of cell growth and metabolism. The transfer of these small regulatory molecules from cell to cell would ensure similar concentrations and a more even response to modulators. It is therefore not surprising that such a fundamental process as cell-to-cell communication might be an intrinsic property of the cell membrane and not immediately dependent on the transcription or translation of genetic information.

We are grateful to Dr. Richard Kronenthal and the Ethicon Co. for bovine collagen and to Dr. Robert Pollack for advice.

Supported by research grants from the National Institutes of Health and the Samuel A. Berger Foundation.

Received for publication 23 April 1976, and in revised form 21 July 1976.

REFERENCES

1. Azarnia, R., W. J. Larsen, and W. R. Loewenstein. 1974. The membrane junctions in communicating and noncommunicating cells, their hybrids and segregants. Proc. Natl. Acad. Sci. U. S. A. 71:880-884.
2. Azarnia, R., and W. R. Loewenstein. 1971. Intercellular communication and tissue growth. V. A cancer cell strain that fails to make permeable membrane junctions with normal cells. J. Membr. Biol. 6:368-385.
3. Bürk, P., J. D. Pitts and J. H. Subak-Shappe. 1968. Exchange between hamster cells in culture. Exp. Cell Res. 53:297-301.
4. Corsaro, C. M., and B. R. Migeon. 1975. Quantitation of contact-feeding between somatic cells in culture. Exp. Cell Res. 95:39-46.
5. Cox, R. P., M. R. Krauss, M. E. Balis, and J. Dancis. 1970. Evidence for transfer of enzyme product as the basis of metabolic cooperation between tissue culture fibroblasts of Lesch-Nyhan and normal cells. 1970. Proc. Natl. Acad. Sci. U. S. A. 67:1573-1579.
6. Cox, R. P., M. R. Krauss, M. E. Balis, and J. Dancis. 1972. Communication between normal and enzyme-deficient cells in tissue culture. Exp. Cell. Res. 74:251-268.
7. Cox, R. P., M. R. Krauss, D. N. Silvers, and J. Dancis. 1972. Skin biopsy site and success of fibroblast culture. Lancet. 21:1373-1374.
8. Cox, R. P., M. R. Krauss, M. E. Balis, and J. Dancis. 1974. Metabolic cooperation in cell culture: a model for cell-to-cell communication. In Cell Communication, R. P. Cox, editor. Wiley Interscience Press, New York. 67-95.
9. Cox, R. P., M. R. Krauss, M. E. Balis, and J. Dancis. 1974. Metabolic cooperation in cell culture: studies of the mechanism of cell interaction. J. Cell Physiol. 84:237-252.
10. Furshpan, E. J., and D. D. Potter. 1968. Low-resistance junctions between cells in embryos and tissue culture. Curr. Top. Dev. Biol. 3:95-127.
11. Gilula, N. B. 1974. Junctions between cells. In Cell Communication. R. P. Cox, editor. Wiley Interscience Press, New York. 1-29.
12. Gilula, N. B., O. R. Reeves, and A. Steinbach. 1972. Metabolic coupling, ionic coupling and cell contacts. Nature (Lond.). 235:262-265.
13. Goldman, R. D., and R. Pollack. 1974. Uses of enucleated cells. In Methods in Cell Biology. Vol. 8. D. M. Prescott, editor. Academic Press, Inc., New York. 123-143.
14. Goldman, R. D., R. Pollack, and N. H. Hopkins. 1973. Preservation of normal behavior by enucleated cells in culture. Proc. Natl. Acad. Sci. U. S. A. 70:750-754.
15. Kanno, Y., and W. R. Loewenstein. 1966. Cell-to-cell passage of large molecules. Nature (Lond.). 212:629-630.
16. Nakamura, H., and J. W. Littlefield. 1972. Purification, properties and synthesis of dehydrofolate reductase from wild type and methotrexate-resistant hamster cells. J. Biol. Chem. 247:179-187.
17. Pappas, G. D., and M. V. L. Bennett. 1966. Specialized junctions involved in electrical transmission between neurons. Ann. N. Y. Acad. Sci. 137:495-508.
18. Pitts, J. D. 1971. Molecular exchange and growth control in tissue culture. In Growth Control in Cell Culture. G. E. W. Wolstenholme and J. Knight, editors. Churchill and Livingstone, London. 89-96.
19. Pitts, J. D. 1972. Direct interactions between animal cells. In Third Lepetit Colloquium on Cell Interactions. L. G. Silvestri, editor. North Holland Publishing Co., Amsterdam. 227-285.
20. Prescott, D. M., D. Myerson, and J. Wallace. 1973. Enucleation of mammalian cells with cytochalasin B. Exp. Cell Res. 74:480-485.
21. Sheridan, J. D. 1974. Electrical coupling of cells and cell communication. In Cell Communication.
22. STOKER, M. 1975. The effects of topoinhibition and cytochalasin B on metabolic cooperation. Cell. 6:253-257.
23. STOKER, M., and I. MACPHERSON. 1964. Syrian hamster fibroblast cell line BHK21 and its derivatives. Nature (Lond.). 203:1355-1357.
24. SUBAK-SHARPE, J. H., R. R. BÖRK, and J. D. PIRTS. 1969. Metabolic cooperation between biochemically marked mammalian cells in tissue cultures. J. Cell. Sci. 4:353-367.
25. WAYMOUTH, C. J. 1959. Rapid proliferation of sublines of NCTC clone 929 (Strain L) mouse cells in a simple chemically defined medium (MB 7521). J. Natl. Cancer Inst. 22:1003-1007.
26. WISE, G. E., and D. M. PRESCOTT. 1973. Ultrastructure of enucleated mammalian cells in culture. Exp. Cell. Res. 81:63-72.
27. YEE, A. 1972. Gap junctions between hepatocytes in regenerating rat liver. J. Cell Biol. 55(2, Part 2):294a (Abstr.).