Fusion of Mammalian Somatic Cells by Microsurgery

(hybridization/HeLa/fibroblasts)

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ABSTRACT Mammalian somatic-cell hybrids can be formed in a direct and precise manner by microsurgery. Fusion of cells brought about in this way permits selection of specific, identifiable cells of the same or different types or species and their fusion at telophase.

Mammalian somatic-cell fusion, a naturally occurring but relatively infrequent phenomenon in cell culture, can be induced by active viruses (1, 2), by inactivated Sendai virus (3, 4), and by chemicals such as lysolecithin (5) and glycerol monooleate (6). These agents must be added to one or both of the cell populations, and cells fuse in all possible combinations. Homokaryons, i.e., hybrid cells with nuclei and cytoplasm of the same types, and heterokaryons, i.e., hybrid cells with nuclei and cytoplasm from different types or species, are formed. The desired hybrid cells must be selected from among all other cells by one of different methods.

A more direct and precise way of fusion of cells by microsurgery, to be reported in this paper, has evolved from the study of human somatic cells in culture (7-9) and the resulting concept of cell disjunction (10) pertaining to mammalian somatic cells. Microsurgery exploits the capacity of two cells to be fused at a specific time in the cell cycle with precise control. Cells within the same population can be fused, and it is possible to fuse cells from two different populations or species without mixing or co-cultivating the two cell populations. Moreover, it is possible to fuse two cells, to retain their unfused, presumably identical, genetic counterparts, and to have the option of isolating these cells from all others for observation and study.

MATERIALS AND METHODS

Cell types used were HeLa clone (HC) (provided by Dr. Balblanian), human fetal (HF) (obtained through the courtesy of Dr. L. Ossowski), Lesch-Nyhan (LN) (obtained from Dr. R. Cox), and mouse L (L) (obtained through the cooperation of Dr. S. Silverstein).

The procedures for cell culture, for preparation of microcultures on coverslips, and for micromanipulation have been described (8, 9). The serum used was obtained from Flow Laboratories, Inc.

By cutting the 18-mm square coverslips diagonally before making microcultures of cells, it is possible to use two triangles to cover a single microsurgical chamber. Thus, two different cell populations are available without mixing or coculturing cells.

At least four clean microneedles with abruptly tapered tips (8) should be available.

Cell Fusion. A pair of cells in telophase is located on the coverslip and optically centered in the field of the oil immersion objective (8). The position of this pair is recorded. Then another pair of cells in telophase is located. With one or more microneedles, this pair is gently detached from the coverslip or from interphase cells. It will adhere to the tip of the needle that is in contact with but not penetrating the cell surface, and thus is lowered on one needle and stabilized with another. It must be lowered sufficiently to avoid hitting other mitotic cells.

TABLE 1. Microsurgery on human and nonhuman cells for fusion

Cell types*	Time in cell cycle†	Number of experiments	Fusions
(A) Homokaryons			
HC-HC	Telo-Telo	15	15
	Telo-Pro	1	0
	Telo-Pmeta	2	0
	Pro-Pmeta	1	0
	Inter-Inter	1	0
HF-HF	Telo-Telo	4	4
	Telo-Pro	2	0
	Telo-Pmeta	1	0
	Pmeta-Pro	1	0
	Meta-Pro	1	0
LN-LN	Inter-Inter	2	0
	Telo-Telo	1	1
	Pmeta-Pmeta	2	0
L-L	Telo-Telo	1	1
(B) Heterokaryons‡			
HC-L	Telo-Telo	2	2
HF-HC	Telo-Telo	1	1
HF-LN	Telo-Telo	1	1
LN-HC	Telo-Telo	1	1
L-HC	Telo-Telo	1	1

* HC = HeLa clone; HF = human fetal; LN = Lesch-Nyhan; L = mouse L.

† Telo = Telophase; Pro = Prophase; Pmeta = Prometaphase; Meta = Metaphase; Inter = Interphase.

‡ Fusions at times other than telophase were not attempted based on the results with homokaryons.

FIG. 1. The sequence of cell fusion between two pairs of HeLa cells in telophase and the subsequent fusion of two cells, one from each pair, after microsurgery. (a) The first pair of HeLa cells is centered. (b) The first pair of cells is on the right, and the second pair is on the

FIG. 2. Fusion of one of a pair of HeLa (HC) cells with one of a pair of human-fetal (HF) cells at arrow initiating heterokaryon formation. $\times 1875$.

By moving the chamber back to the recorded position slowly so as not to dislodge the second pair of cells on the needle, the first pair is again centered in the oil immersion field. The second pair of cells is then raised on the needle to attach it to the coverslip at the level of the first pair. Gentle pressure is sufficient for attachment to the coverslip; too much pressure will cause damage. With one or more clean needles, as necessary to stabilize and to prevent the cells from adhering to the needles, this pair is pivoted so that between the two pairs there is contact by only one cell from each pair.

Once the two pairs of cells are so positioned, a micro-needle is raised and advanced, in the same plane, to touch lightly the surface of one of the contiguous cells, just enough for a small amount of hyaline cell matrix (10) to be extruded at that site. The extruded matrix adheres to the abruptly tapered tip of the needle and is then gently propelled on the tip and inserted into the other contiguous cell just far enough to fuse with its matrix, visible by formation of a hyaline bridge. This is the instant of cell fusion. Fusion occurs between these two cells only; two unfused cells remain.

After fusion the needle is withdrawn carefully so as to avoid separating the fused cells and lowered.

RESULTS

Cells at stages of mitosis other than telophase may be brought into contact or subjected to the microsurgical treatment described above, but fusion does not occur (see Table 1).

In Table 1, Part A, the homokaryons formed from the different cell types are listed, and in Part B, the heterokaryons are listed. In every case where the procedure described above has been followed fusion occurred. Of 21 experiments with cells in telophase, 21 fusions resulted. No fusions occurred in 14 experiments with cells at times in the

cell cycle other than telophase. Based on these results with homokaryons (Table 1, Part A), experiments on heterokaryon formation (Table 1, Part B) were done with cells at telophase. In six experiments, six fusions occurred.

The sequence of events is illustrated for HeLa cells in Fig. 1. Fig. 2 shows fusion of other cell types indicated in the legend.

DISCUSSION

Fusion of cells by microsurgery arose from operations on individual pairs of cells in telophase (10). A drop of silicone oil formed at the surface and in contact with the hyaline cell matrix of one of the cells caused extrusion of hyaline cell matrix at that site. As the two cells separated, the cell in contact with the oil retained this hyaline cell matrix and was larger than the other cell (10). It was reasonable to assume that the hyaline cell matrix of two cells, one from each of two telophase pairs, might fuse if a common oil drop were used.

Fusion of cells by this method was unsuccessful for two reasons. (a) No matter how close the cells were, expanding the oil drop on the pipette tended to separate the matrix of one cell from that of the other and retracting the oil did not fuse them. (b) Changes in the distribution of the hyaline cell matrix within each pair might occur (10).

Fusion by microsurgery, using another way to mobilize cell matrix (10), exploits the naturally occurring mobilization of matrix at this time in the cell cycle and merely directs the position of extrusion at the cell surface. As reported here, fusion by microsurgery has the following advantages. Once the operation has been performed, the agent, i.e., the micro-needle, is no longer part of the system. Virus particles or chemicals inactivated by the addition of still other chemicals

left. Fusion (arrow) by microsurgery has just occurred, and the needle has just been lowered. $t = 0$ min. (c) The upper cell is to the right of the arrow in (b) and the lower cell is to the left of the arrow in (b). The unfused cells have been detached then attached again to the coverslip at positions close by. The hyaline bridge (arrow) has changed its appearance. $t = 5$ min, (d) $t = 7$ min, (e) $t = 8$ min, (f) $t = 11$ min, (g) $t = 13.5$ min, (h) $t = 15$ min, (i) $t = 18.5$ min, (j) $t = 23.5$ min, (k) $t = 25$ min, (l) $t = 28$ min, (m) $t = 32$ min. (n) This shows the unfused cell from one pair of HeLa cells. $t = 32$ min. (o) This shows the other unfused cell. Note that the cells in (n) and (o) are about one half the size of the homokaryon as it appears in (m). All figures $\times 1688$.

(5) remain as part of the system and may produce undefined effects.

This way of fusing cells is not pH dependent (11) or temperature dependent. In fact, room temperature most likely protracts this part of the cell cycle, allowing even closer synchrony of the cells to be fused than at 37°.

The opportunity to fuse specific cells at a specific time in a direct and precise manner provides a defined starting point for following cell interactions. The specific cells chosen may include virus-infected and uninfected cells, where the additional complication of another viral protein is not desirable. This is one of several areas of cell biology where application of fusion by microsurgery is of great potential value. Cell interactions that ensue may be analyzed by autoradiography or by electron microscopy.

Another desirable feature of this method is that once the fusion has been achieved, the three cells that result, i.e., a hybrid cell and its two unfused counterparts, can be isolated from all other cells directly and followed immediately in the same type of medium in which they have been growing (8).

The conditions for fusing cells are clearly defined and controlled, and, therefore, the results of the fusions should be more amenable to systematic study. Variations may occur that provide insights not heretofore available. Moreover, there is now an opportunity to fuse cells so that combinations of fusions, that is, cell pairs in other than 1:1 ratios, can be made in order to study gene-dosage effects or to compare the behavior of more complex with less complex cell hybrids and unfused cells.

It seems likely that fusions between cells of other types and species than those reported here are possible since fusion of cells by microsurgery exploits a property of all mammalian somatic cells in telophase.

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