Journal of Supramolecular Structure 11:33–49 (1979)
Tumor Cell Surfaces and Malignancy 9–25

Nuclear Control of Tumorigenicity in Cells Reconstructed by PEG-Induced Fusion of Cell Fragments

Jerry W. Shay and Mike A. Clark

The University of Texas Health Science Center at Dallas, Department of Cell Biology, Dallas, Texas 75235

The techniques of somatic cell hybridization have provided a valuable means of studying mechanisms of regulation of mammalian cell differentiation and transformation. Most previous studies have indicated that fusions between tumorigenic and nontumorigenic cells result in hybrid cells that are usually tumorigenic. In recent years it has been demonstrated that the phenotypic expression of tumorigenicity is at least partially due to the extensive chromosome loss that occurs in most interspecific and some intraspecific hybrid cells. In the present study we have utilized enucleation techniques that permit cells to be divided into nuclear (karyoplast) and cytoplasmic (cytoplast) cell fragments. Even though these nuclear and cytoplasmic fragments are metabolically stable for short periods of time, in our hands they ultimately degenerate. Viable cells can be reconstructed by PEG-induced fusion of karyoplasts to cytoplasts. Since reconstructed cells apparently do not segregate chromosomes, they may provide a clearer understanding of the interactions between the nucleus and the cytoplasm in the control of the expression of tumorigenicity. We have reconstructed cells using karyoplasts from the tumorigenic Y-1 cell line and cytoplasts from a nontumorigenic cell line, A-MT-BU-A1. In addition we have reconstructed cells containing Y-1 cytoplasts and A-MT-BU-A1 karyoplasts. The reconstructed cells produced were assayed for tumorigenicity by their ability to grow in soft agar and in nude mice. The results of these experiments indicate that the reconstructed cells containing a tumorigenic nucleus and a nontumorigenic cytoplasm ultimately are tumorigenic and conversely the reconstructed cells containing a nontumorigenic nucleus and a tumorigenic cytoplasm are nontumorigenic. These experiments support the concept that with these cell lines the nucleus (karyoplast) is sufficient to control the phenotypic expression of tumorigenicity.

Key words: cell enucleation, cell reconstruction, nuclear control of tumorigenicity

Hybrid cells produced by fusing tumorigenic and nontumorigenic cells from different species in most instances are tumorigenic. The tumorigenic phenotypes expressed in such hybrids have been partially explained by the extensive chromosome losses that occur in

Abbreviations used: PEG – polyethylene glycol; HAT – hypoxanthine, aminopterin, thymidine; CAP – chloramphenicol; ACTH – adrenocoricotrophic hormone; BrdU or BUDR – bromodeoxyuridine.

Received March 18, 1979; accepted May 25, 1979.

0091-7419/79/1101-0033$03.20

interspecific and some intraspecific hybrid cells. There have been reports that such fusions cause suppression of the tumorigenic phenotype but in these cases chromosome loss was not extensive [1]. In intraspecific hybrids there is little chromosome segregation and when tumorigenic and nontumorigenic cells from the same species are produced, the nontumorigenic phenotype is frequently but not always retained [1, 2]. These studies suggest that there is some factor present in nontumorigenic cells that is capable of suppressing the tumorigenicity of the other cells and when this factor is lost, as a result of chromosome loss or possibly some epigenetic factor, the tumorigenic phenotype is expressed. Thus, the conflicting results from different experiments may simply reflect the chromosomal instability of the hybrid cells. Interspecific whole-cell fusion studies are probably not ideal for investigating certain aspects of the etiology of the suppression or expression of the tumorigenic state.

In an attempt to overcome the difficulties with the interpretation of such experiments, we and others have been developing techniques for the enucleation of mammalian cells in culture, using the drug cytochalasin B in combination with mild centrifugation [3–5]. Such techniques permit the separation of cells into nuclear (karyoplast) and cytoplasmic (cytoplast) fragments [6], which are incapable of regenerating into whole cells under our conditions unless recombined by standard hybridization techniques. Such reconstructed cells are viable, capable of indefinite growth in cell culture [5, 7], and do not appear to segregate chromosomes. Analysis of reconstructed cells may provide a clearer understanding of the nuclear and cytoplasmic contributions to certain aspects of the expression or suppression of the tumorigenic state.

In the present study we report the reconstruction of a tumorigenic karyoplast with a nontumorigenic cytoplast. In addition, using the same cell lines we report a new technique for identifying reconstructed cells of a nontumorigenic karyoplast with a tumorigenic cytoplast. The results of these experiments indicate that with these cell lines the nucleus (karyoplast) is sufficient to control the phenotypic expression of tumorigenicity.

METHODS

Cell Lines

The tumorigenic Y-1 line was originally derived from a murine adrenal tumor [8]. The nontumorigenic A-MT-BU-A1 cell line (hereafter designated AMT) was originally derived from MT-29240 in Dr. Coon's laboratory and is a murine transplantable tumor that arose spontaneously in a female Balb/c mouse. The AMT cell line is contact-inhibited and is chloramphenicol- and BrdU-resistant, and although it contains intracisternal A virus particles, it is not tumorigenic as tested by lack of growth in soft agar and nude mice. (We thank Drs. Malech and Wivel for providing the AMT cell line). Both parental cell lines, hybrids, and reconstructed cells were found to be free from mycoplasma contamination by three different assays [9–11].

Enucleation

The Y-1 cells are flat and epitheloid, while the AMT are fibroblastoid. Both are strongly adherent to Falcon 3013 25-cm^2 tissue culture flasks (Falcon Plastics, Oxnard, California) and are therefore easily enucleated with a high efficiency by means of techniques previously described [12]. Briefly, almost confluent flasks of Y-1 and AMT cells are

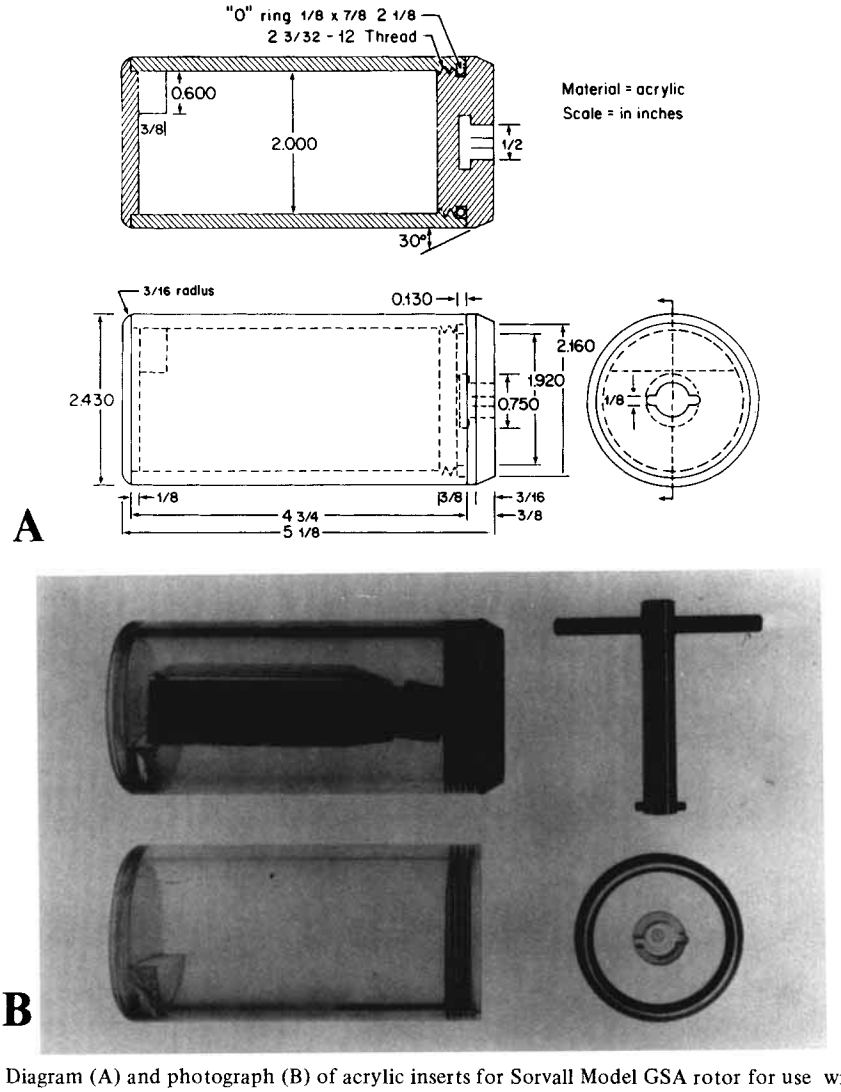


Fig. 1. Diagram (A) and photograph (B) of acrylic inserts for Sorvall Model GSA rotor for use with culture flask enucleation procedure.

completely filled with Dulbecco's modified Eagle's medium (DMEM) containing 10 μg/ml of cytochalasin B and centrifuged in a GSA rotor for 20–30 min at 20,000g at 37° in acrylic holders (Fig. 1a, b) using a Sorvall RC5 superspeed centrifuge. Equally successful enucleation is accomplished without the acrylic inserts by placing 150 ml of H_2O into the GSA rotor opening and placing the flasks directly into the rotor. Enucleation efficiencies of 95–99% or better are obtained with these cell lines without substantial cell detachment. A second centrifugation can be undertaken that results in even higher efficiencies of enucleation. The resulting procedure produces a population of Y-1 karyoplasts and cytoplasts and AMT karyoplasts and cytoplasts. Using the procedures described by Lucas and Kates [39], the amount of cytoplasm retained in the Y-1 and AMT karyoplasts ranges from 2% to 6%, as determined by the amount of ribosomal RNA.

Fusion

The karyoplasts and cytoplasts are mixed and fused using polyethylene glycol 400 MW as previously described for whole cells [13]. Essentially the mixture of karyoplasts and cytoplasts (2×10^6 cells) are centrifuged at 500g for 2 min in a 15-ml conical centrifuge tube. Most of the culture medium is removed without disturbing the pellet, 1 ml of a 50% PEG-400MW solution, which is liquid at room temperature, is carefully added to the pellet, and the cells are lightly mixed for 1 min at room temperature. The solution is then diluted with 10 ml of complete growth medium and immediately centrifuged at 500g for 2 min. The supernate is removed, 2 ml of complete growth medium is added, and centrifugation is repeated. The resulting fused cell fragments are then plated out in culture flasks at densitites of less than 2×10^4 cells per cm^2.

Selection of Hybrids and Reconstructed Cells

Three types of experiments were undertaken and are diagrammatically illustrated in Figures 2–4. The first experiment we undertook was to fuse whole Y-1 cells with whole AMT cells. As previously mentioned, the AMT cells have both nuclear (BrdU-resistant, HAT^S) and cytoplasmic (chloramphenicol-resistant, CAP^r) genetic markers. The Y-1 cells are HAT^r and CAP^S. As is illustrated in Figure 2, after fusion the heterokaryons, homokaryons, and unfused parentals are plated out in HAT medium containing 50 μg/ml of

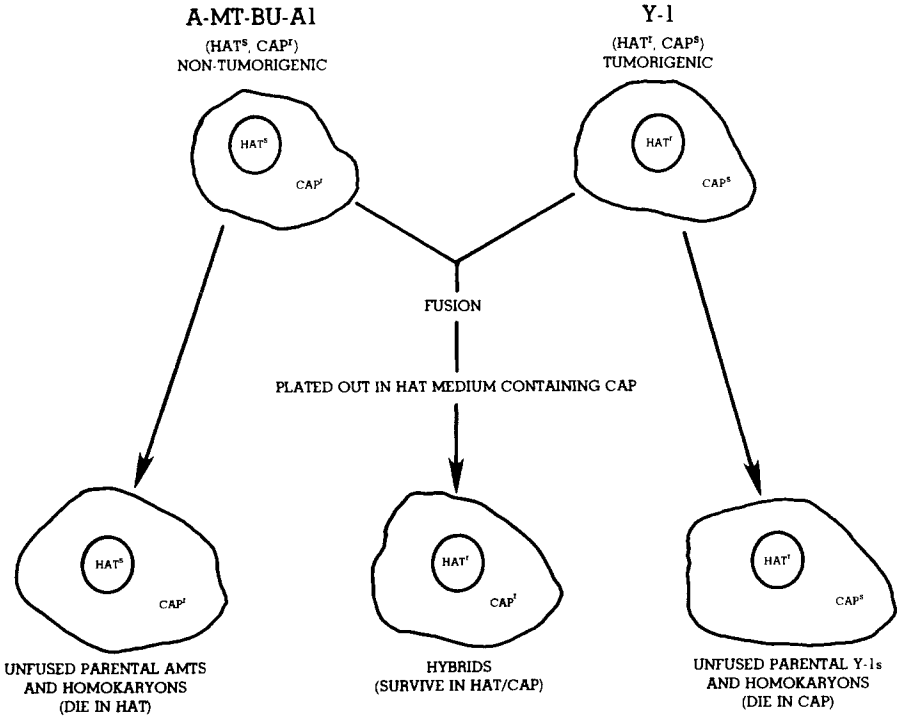


Fig. 2. Schematic representation of the experimental design to select whole-cell hybrids between the nontumorigenic AMT and tumorigenic Y-1 cells. The unfused parental cells and homokaryons die in HAT/CAP, whereas the heterokaryons survive.

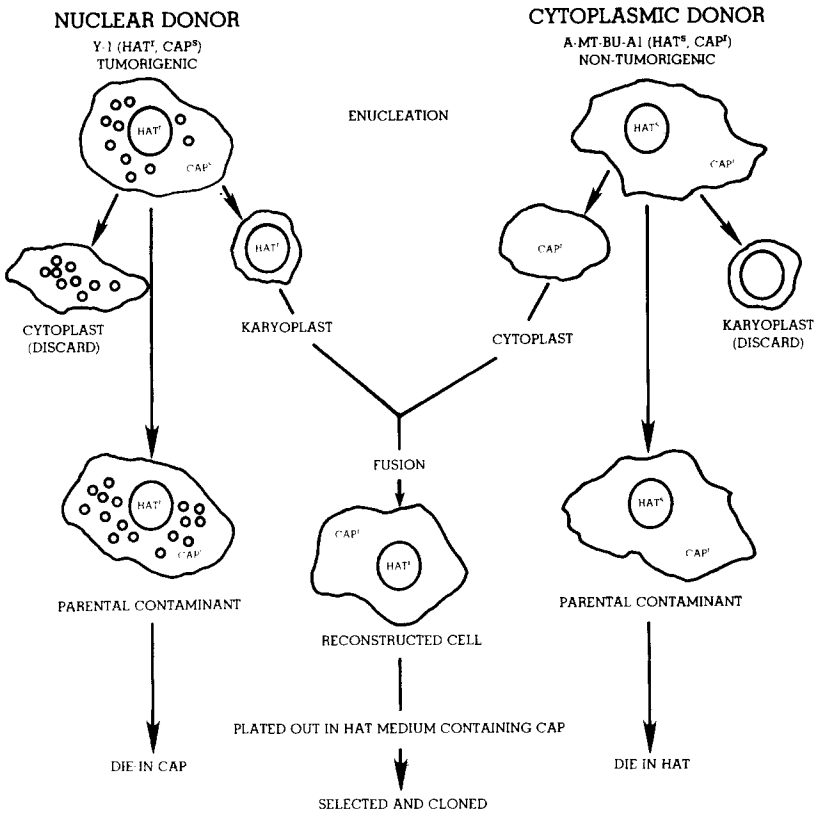


Fig. 3. Schematic representation of the experimental design to select reconstructed cells between karyoplasts derived from the tumorigenic Y-1s and cytoplasts derived from the nontumorigenic AMT cells. See Methods section for a more complete description of the selection procedures.

chloramphenicol. Under these selection conditions only the hybrid cells will survive in both HAT and CAP. The unfused AMT cells will die because of the HAT, while the unfused Y-1 cells will be killed by the CAP. Karyotyping after selection indicated the hybrid nature of the surviving clones.

The reconstruction experiment combining Y-1 karyoplasts to AMT cytoplasts used the selection procedure illustrated in Figure 3. The Y-1 cells were incubated prior to enucleation in the presence of latex spheres, as previously described [7, 41]. The Y-1 karyoplasts fused to AMT cytoplasts survive in HAT medium containing CAP, while the small percentage of whole Y-1 cells and AMT cells die in the presence of CAP and HAT, respectively. Immediately after fusion the single reconstructed cells were isolated on glass fragments and placed in multiwell chambers. Only the reconstructed cells not containing latex spheres were considered reconstructed, since both cytoplasmic hybrids (cybrids) and whole-cell fusions would contain latex spheres and would survive in HAT/CAP. The clones not containing latex spheres that grew were then analyzed for chromosome constitution to separate whole-cell hybrids from reconstructed cells, and then only reconstructed cells were further analyzed for the tumorigenic properties.

The reconstruction experiments fusing the AMT karyoplasts to Y-1 cytoplasts are illustrated in Figure 4. Essentially BrdU-resistant AMT karyoplasts are fused to CAP^s

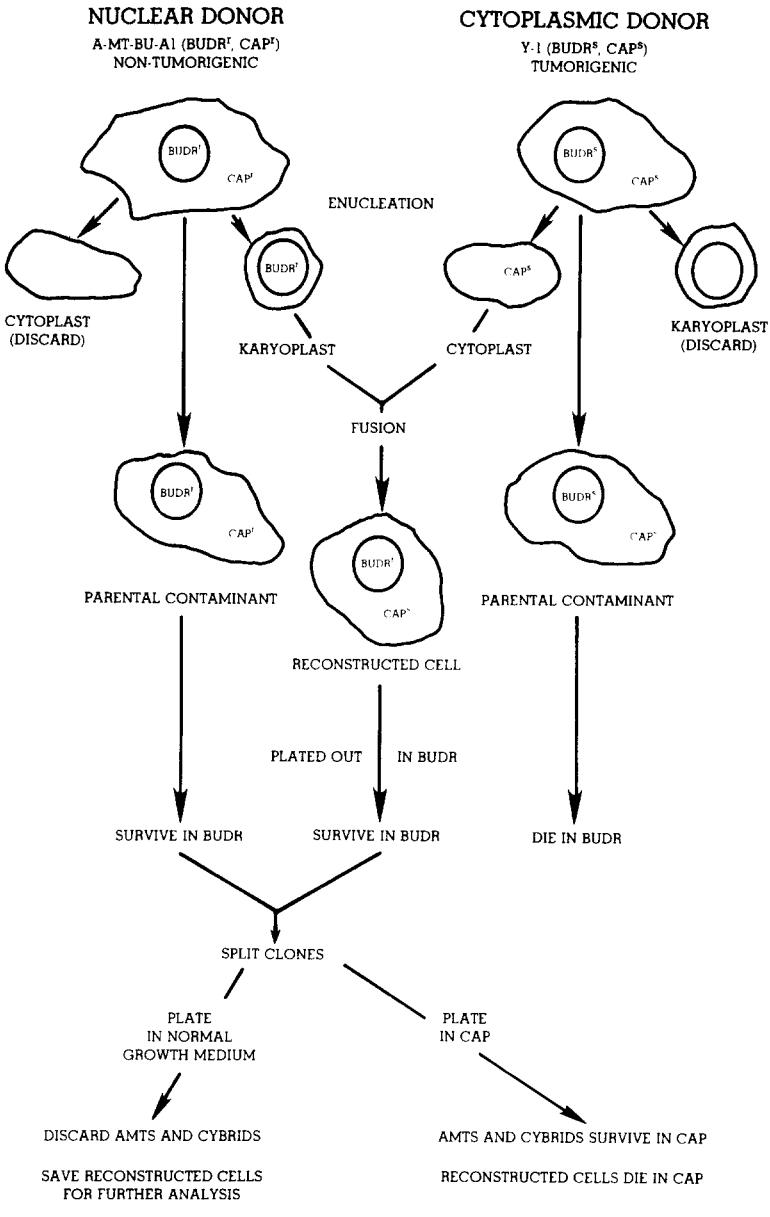


Fig. 4. Schematic representation of the experimental design to select reconstructed cells between karyoplasts derived from the nontumorigenic AMTs and cytoplasts derived from the tumorigenic Y-1 cells. See Methods section for a more complete description of the selection procedures.

Y-1 cytoplasts. The Y-1 cells that have not enucleated and whole-cell hybrids die in the presence of BrdU. The surviving BrdU-resistant reconstructed cells, AMT parentals, and cybrids (AMT whole cells × Y-1 cytoplasts) are then isolated and each growing clone is split; one half of each clone is placed in complete growth medium and the other half is placed in growth medium containing CAP. A clone that survives in medium containing CAP must have been derived from either a whole AMT cell or a cybrid. Clones that die in

CAP (but have survived the BrdU treatment) must be reconstructed cells. Thus the AMTs and cybrid clones are identified and eliminated from further study. The replicate CAP-sensitive clones, which have been maintained in normal growth medium, are then analyzed.

Electron Microscopy

The isolated hybrid and reconstructed cells were analyzed not only for their tumorigenic properties but also for their ultrastructural properties, by means of transmission electron microscopy. The AMT cells contain intracisternal A virus particles (IAP), which are excellent morphologic markers. These particles are not shed from the cells but are only transmitted vertically (that is, by mitosis) [14, 15]. The techniques for electron microscopy are standard procedures previously described [3, 16]. Thin sections were examined on a JEOL 100-B transmission electron microscope and the surface morphology of cells was observed in a JEOL U-3 scanning electron microscope.

Cell Cycle Analysis

Cell cycle analysis was done using the method described by Crissman and Tobey [17]. The cells were observed and analyzed with a Becton-Dickinson cell sorter (FACS III) using a laser wavelength of 488 nm.

In Vitro and In Vivo Tumor Assays

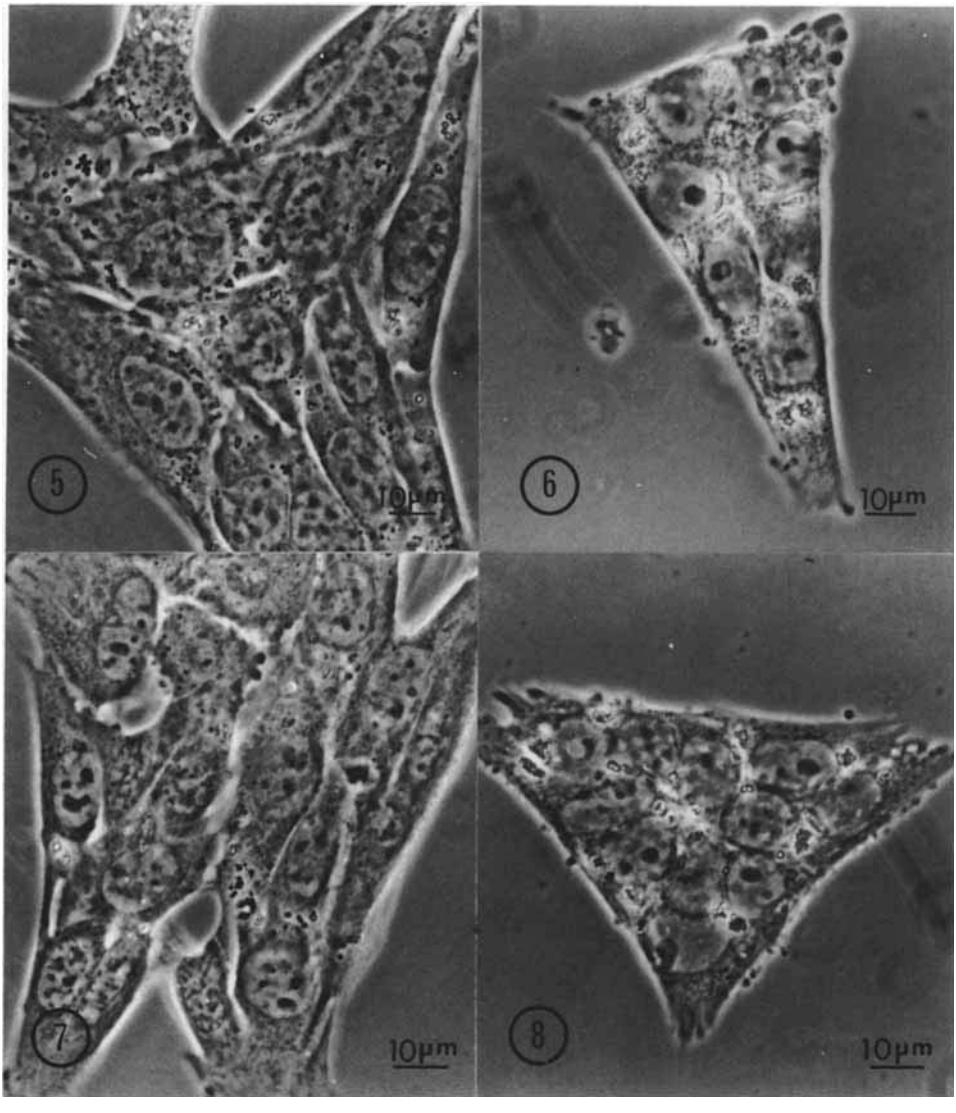
The parental cell lines and clones of hybrids and reconstructed cells were studied for their ability to grow in soft agar by the technique described by Miller et al [18]. This procedure consisted of first adding 10 ml of DMEM plus 10% fetal calf serum plus 3% agar into a 10-cm petri dish. After this solution had solidified another 10 ml of DMEM plus 10% fetal calf serum plus 1.5% agar and 1,000 cells were poured on top of the solidified mixture. These dishes were then incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO_2 for two weeks. At the end of this time discrete clones of cells were readily identified and counted.

We also tested the ability of cells to grow and produce tumors in nude mice. The nude mice used in this experiment were obtained from Dr. John Porter (University of Texas Health Science Center at Dallas) and were 2–3 months old. Cells to be injected were trypsinized from the growth substrata, the trypsin was then neutralized with complete growth medium and the cells were diluted to a final concentration of 2×10^6 per ml. A total of six animals were used to test each cell line and reconstructed clone. Two animals were inoculated with 0.5×10^6, 1×10^6, or 2×10^6 cells. The cells were injected subcutaneously using a 21-gauge needle. When tumors reached approximately 1 cm (usually 3–4 weeks) the animals were sacrificed, and the tumor was excised and placed in cell culture. Once the resulting cultures were of sufficient size they were tested for their ability to respond to ACTH and to grow in HAT, BUDR, and CAP. In all instances the cells originating from the tumor were found to have the characteristics of the cells injected. If the animals failed to have a tumor in 8 weeks we considered the clone non-tumorigenic.

RESULTS

Experimental Design

The experimental designs for these studies are illustrated in Figures 2–4, as described in the Methods section. Essentially, hybrids were produced and selected be-

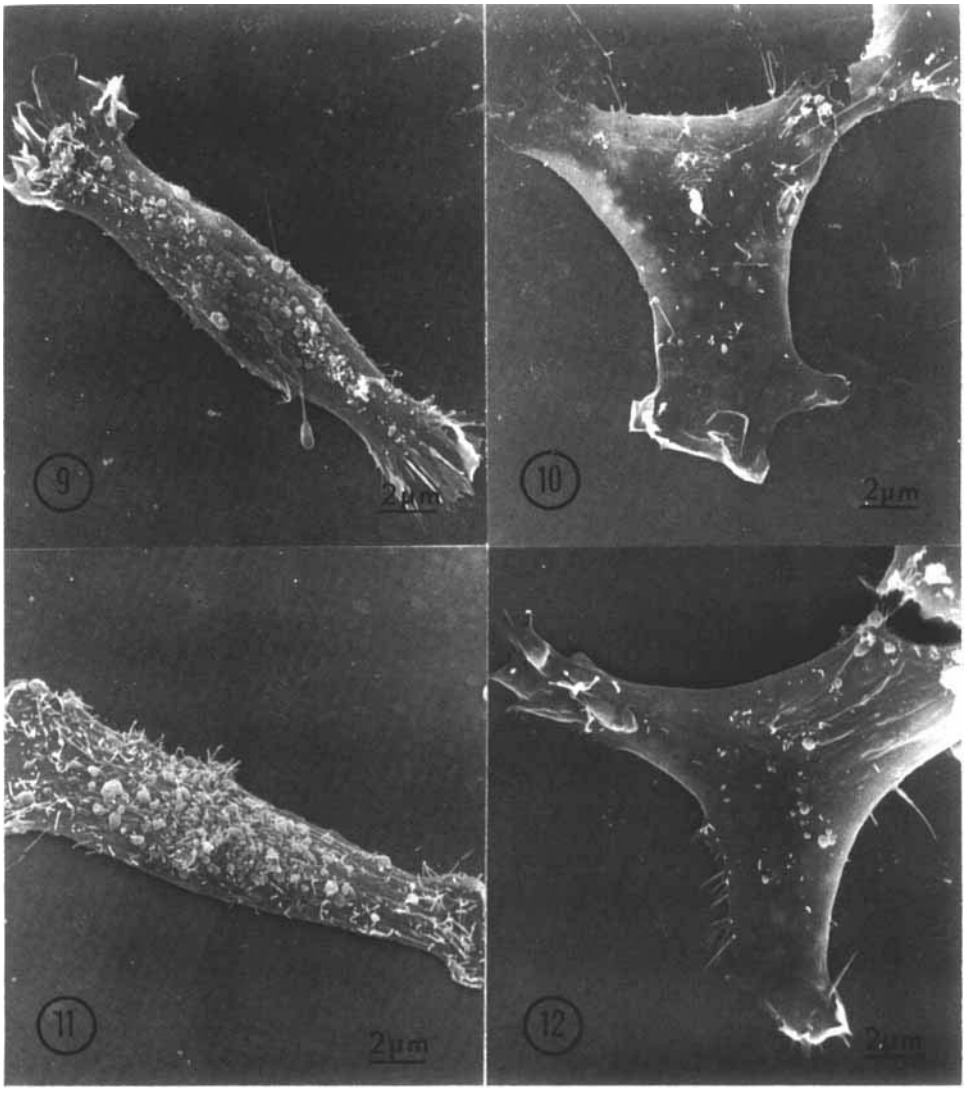


Figs. 5–8. Phase-contrast photomicrographs of the parental AMTs (Fig. 5), Y-1 (Fig. 6), and the reconstructed cells AMT(k) × Y-1(c) (Fig. 7) and Y-1(k) × AMT(c) (Fig. 8). Note that the overall morphology of the reconstructed cells resembles the morphology of the nuclear donor. ×700.

tween the nontumorigenic AMT and tumorigenic Y-1 cells (Fig. 2). The cell reconstruction experiments are illustrated in Figures 3 and 4. Reconstructed cells containing a tumorigenic nucleus and a nontumorigenic cytoplasm were produced and identified as illustrated in Figure 3. Reconstructed cells containing a nontumorigenic nucleus and a tumorigenic cytoplasm were produced and identified as illustrated in Figure 4. After selection the hybrids and reconstructed cells were further analyzed for morphology (light microscopy and transmission and scanning electron microscopy) and for in vitro and in vivo tumor production.

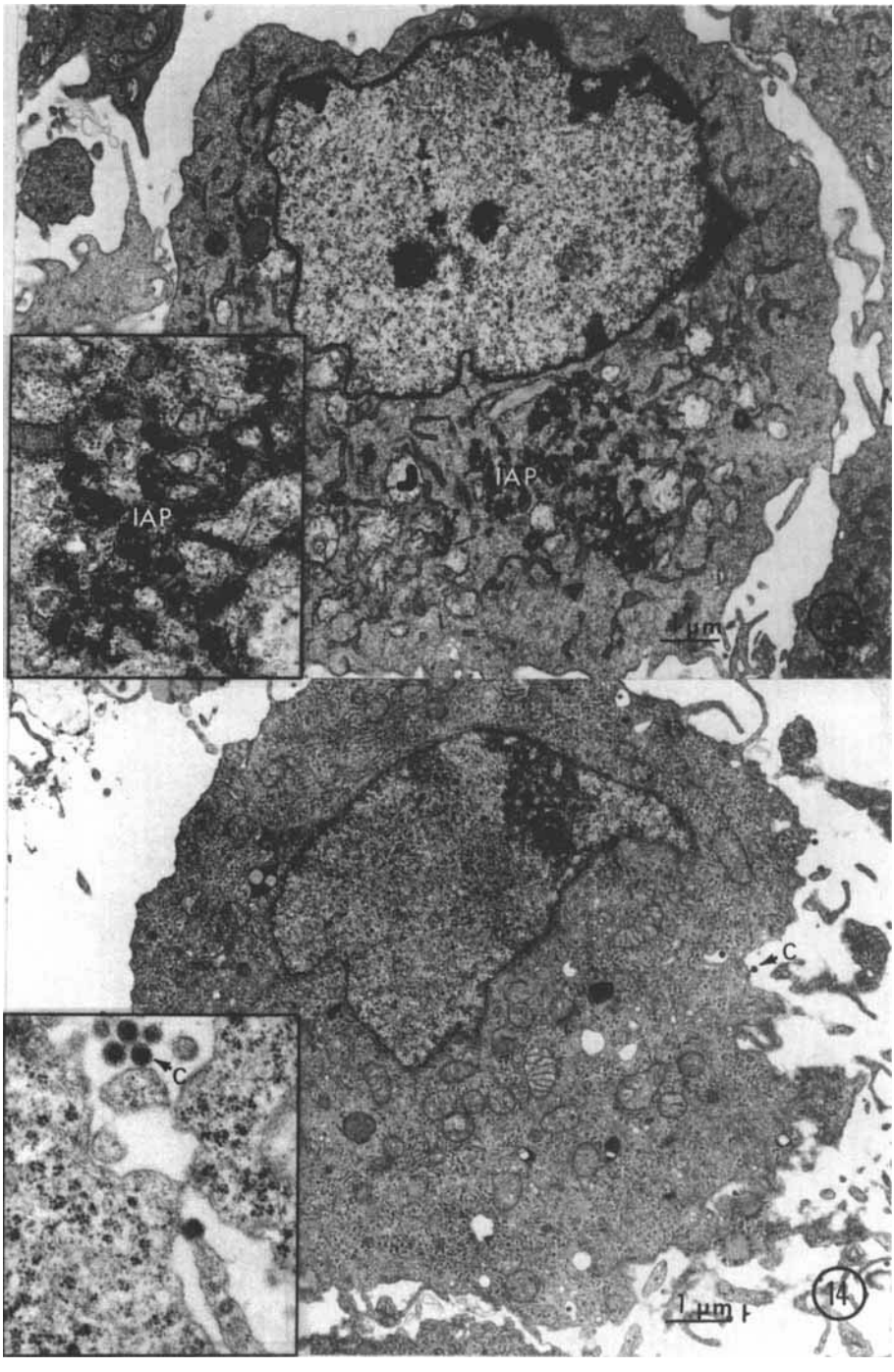
Morphology

Phase contrast. Figures 5 and 6 illustrate parental AMT (Fig. 5) and Y-1 (Fig. 6)



Figs. 9–12. Scanning electron micrographs of the parental AMT (Fig. 9), Y-1 (Fig. 10), and the reconstructed cells AMT(k) × Y-1(c) (Fig. 11) and Y-1(k) × AMT(c) (Fig. 12). As with the phase-contrast photomicrographs, the reconstructed cell surface topography resembles the morphology of the nuclear donor. ×1,600.

cells. The nontumorigenic AMT cells are fibroblastic in morphology and have a modal chromosome number of 65 (Fig. 5), while the tumorigenic Y-1 cells are epithelioid in morphology and have a modal chromosome number of 40 (Fig. 6). A minimum of 25 chromosome spreads were counted for each cell line. The AMT cells frequently contain numerous nucleoli, while the Y-1 cells usually contain a single prominent nucleolus. The reconstructed cells consisting of an AMT karyoplast and a Y-1 cytoplast, designated AMT(k) × Y-1(c), are depicted in Figure 7 and have a modal chromosome number of 65 while the reconstructed cells consisting of a Y-1 karyoplast and an AMT cytoplast, designated Y-1(k) × AMT(c), are depicted in Figure 8 and have a modal chromosome number of 40. As the photomicrographs in Figures 7 and 8 clearly illustrate, the overall morphology



Figs. 13, 14. Transmission electron micrographs of AMT parentals (Fig. 13), containing intracisternal A particles (IAPs) (insert), and of Y-1 parentals (Fig. 14), containing C-type virus particles (insert). ×9,000.

of the selected reconstructed cells is essentially identical to that of the nuclear donor; the morphology of the AMT(k) × Y-1(c) reconstructed cells resembles the AMT parental morphology, while the morphology of the Y-1(k) × AMT(c) reconstructed cells resembles the Y-1 parental morphology.

Scanning electron microscopy. Figures 9–12 illustrate the surface topography and overall cell shape of the parental and reconstructed cells. AMT cells and reconstructed cells containing an AMT(k) × Y-1(c) are depicted in Figures 9 and 11. The fibroblastic shape of isolated cells is clearly different from the Y-1 cells. The surfaces of the fibroblastic cells contain considerably more blebs and microvilli than the epithelioid cells. Y-1 cells and reconstructed cells containing a Y-1(k) × AMT(c) are depicted in Figures 10 and 12. The epithelial shape of isolated cells is similar to those seen in clusters (Figs. 6, 8), and the surfaces of the cells contain a few blebs and microvilli. Figures 7, 8, 11, and 12 indicate that the shape and surface features of the reconstructed cells are essentially controlled by the nucleus. Even though enucleated cells (cytoplasts) can maintain the shape of the whole cell for short periods of time, it is clear that once rescued by another nucleus the cytoskeletal elements within the cytoplasm do not appear to maintain their independence but are directed by the host nucleus.

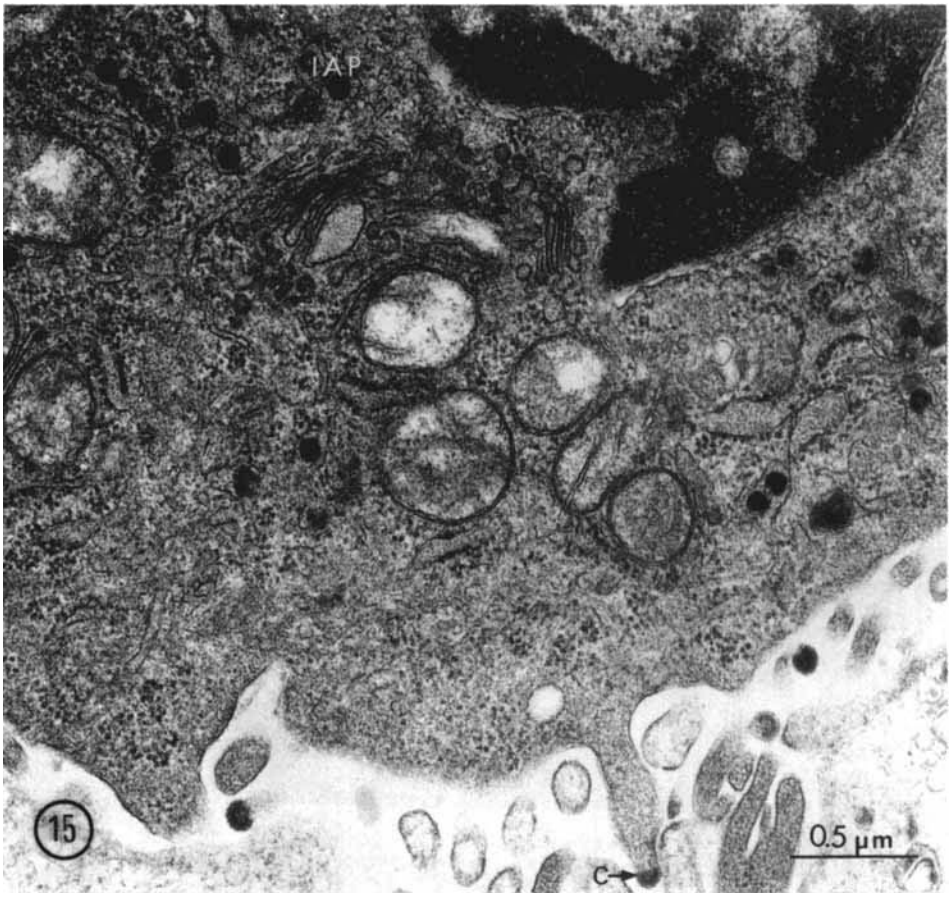
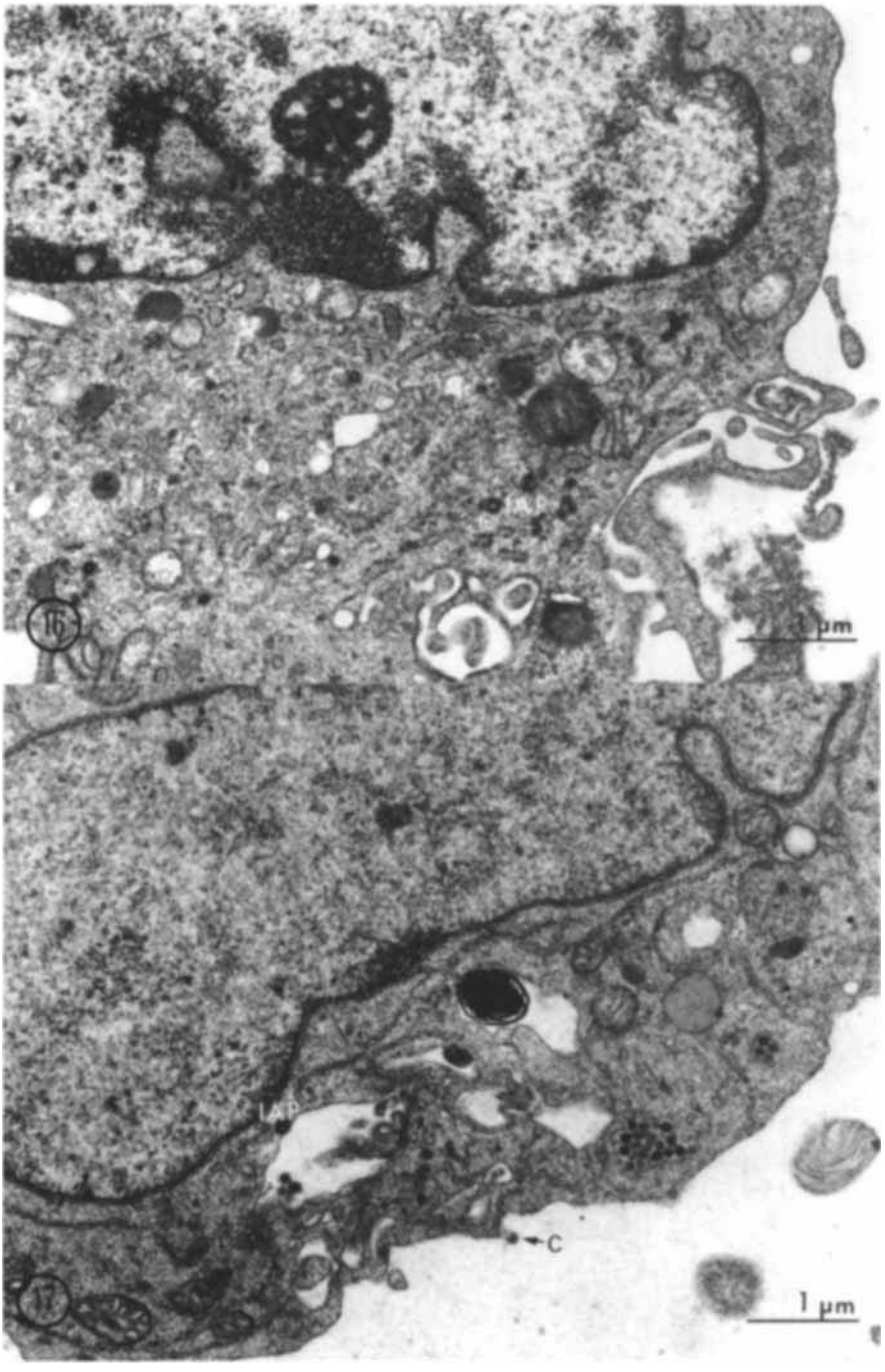


Fig. 15. Transmission electron micrograph of a hybrid cell produced by fusing AMTs to Y-1 cells. Note the presence of both intracisternal A and C-type virus particles. ×36,000.



Figs. 16, 17. Transmission electron micrographs of the reconstructed cells. The AMT(k) × Y-1(c) reconstructed cells (Fig. 16) contain intracisternal A particles, but C-type particles have not been observed. The Y-1(k) × AMT(c) (Fig. 17) contain both types of particles. ×17,000.

Transmission electron microscopy. The most striking ultrastructural difference between the Y-1 parentals and the AMT parentals is shown in Figures 13 and 14. The Y-1 cells contain numerous C-type virus particles, which are shed from the cell surface (Fig. 14 and insert) and are both vertically and horizontally transmitted. The AMT cells, though originally derived from a murine mammary tumor, are nontumorigenic in vivo but contain numerous intracisternal A-type virus particles (Fig. 13). These A-type virus particles reside within the cisternae of the endoplasmic reticulum and are only vertically transmitted (ie, by mitosis), as shedding does not occur. As previously reported, cocultivation of AMT cytoplasts with other whole cells does not result in transfer of intracisternal A particles to the other cells. However, when whole-cell hybrids are produced by AMT and Y-1 cells, both types of particles are present (Fig. 15). The ultrastructure of the reconstructed cells are depicted in Figures 16 and 17. The AMT(k) × Y-1(c) reconstructed cells contain intracisternal A particles, but C-type particles in these cells have not been observed (Fig. 16). It is possible that the nuclei in these reconstructed cells cannot incorporate the C-type virus genome but definitive information on this is not yet available. The Y-1(k) × AMT(c) reconstructed cells contain both C-type and intracisternal A-type virus particles (Fig. 17). However, considerably fewer intracisternal A-type virus particles were observed in the reconstructed cells than in the parental AMTS.

In Vitro and In Vivo Tumor Assays

The parentals, hybrids, and reconstructed cells were studied for their ability to grow in soft agar. In six different experiments in which 1,000 Y-1 parentals or Y-1(k) × AMT(c) originating from a single pure clone were placed in soft agar, 22–26% of the cells would develop into viable colonies consisting of greater than 50 cells in 3 weeks. This is in contrast to the results obtained from the AMT parentals and AMT(k) × Y-1(c). When 1,000 of these cells were placed in soft agar, none of the cells developed into viable colonies consisting of greater than 50 cells in 2–3 weeks. In each instance the reconstructed cell behaved identically with the parental cell line from which the nucleus was derived.

The AMT × Y-1 whole-cell hybrid clones varied widely in their ability to grow in soft agar. Some of the hybrid clones behaved like the AMT cells in that they failed to grow in agar while some of the clones grew just as efficiently as, but none more efficiently than, Y-1 cells. In addition a few of the clones grew in soft agar with an intermediate efficiency.

When Y-1 cells or Y-1(k) × AMT(c) cells were injected into nude mice, each of the six mice injected developed large tumors in 4 weeks even at the lower inoculation. This is in direct contrast with the results obtained from the inoculation of AMT cells or AMT(k) × Y-1(c) cells. In these instances none of these mice produced tumors even when allowed to survive for 8 weeks. These data correlated very closely with the soft agar data.

Hybrid cells formed from the fusion of an AMT × Y-1 were also tested for their ability to produce tumors. For this experiment we chose three different clones. One clone had a plating efficiency in soft agar of 25%, another had a plating efficiency of less than 0.5%, and the third had a plating efficiency of 5% in soft agar. Approximately 2×10^6 cells taken from each clone were injected into nude mice. By 8 weeks only the mouse that was injected with cells taken from the clone that grew best in soft agar produced a tumor. The other two clones did not produce tumors by 8 weeks.

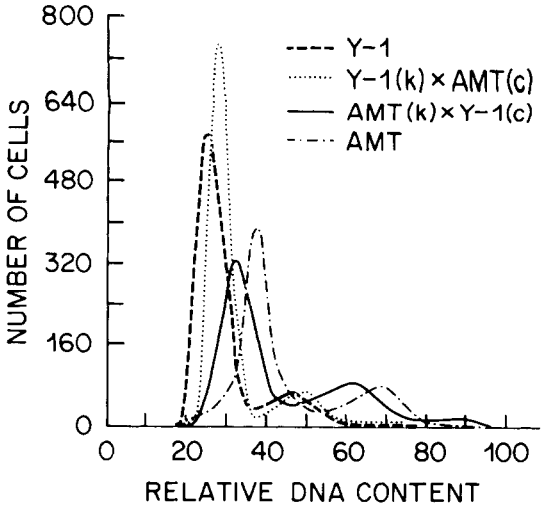


Fig. 18. Cell cycle analysis of the parental and reconstructed cells by means of a fluorescence-activated cell sorter. As to the percentage of cells in various stages of the cell cycle, the reconstructed cells lie between the parental cells, but overall the reconstructed cells have a cell-cycle profile closer to that of the nuclear donor.

Cell-Cycle Analysis

The results obtained from the cell sorter showing the distribution of DNA content of the reconstructed and parental cells illustrated are in Figure 18. The larger peaks represent the various cell lines in the G_1 phase of the cell cycle (prior to DNA replication), while the smaller peaks represent cells in the G_2 or M phase of the cell cycle. The cells in S phase (during DNA replication) lie between the two peaks. The parental Y-1 and AMT cells differ dramatically in the percentage of cells in various stages of the cell cycle, while the reconstructed cells lie between the parentals. Even though the reconstructed cells behave somewhat differently from the parentals, they do have a cell-cycle profile closer to that of the nuclear donor. The approximate percentage of the parental and reconstructed cells in various stages of the cell cycle is as follows: Y-1 [G_1 = 84%, S = 7%, G_2 + M = 9%]; Y-1(k) × AMT(c) [G_1 = 81%, S = 6%, G_2 + M = 13%], AMT(k) × Y-1(c) [G_1 = 65%, S = 12%, G_2 + M = 23%]; AMT [G_1 = 69%, S = 11%, G_2 + M = 20%].

DISCUSSION

Several approaches to elucidating the genetic basis of tumorigenesis are available but the most popular has been the production of somatic cell hybrids between tumorigenic and nontumorigenic cells. More recently the use of cytoplasmic hybrids (cybrids) has also been available for studying certain aspects of tumorigenesis. Both of these methods have provided useful information concerning the control mechanisms in the expression or suppression of tumorigenesis but few widely supported generalizations have been derived from such studies. Technical developments for producing reconstructed cells (eg, nuclear transplantation) have also been progressing in recent years and this report illustrates the utility of using such reconstructed cells for analyzing nuclear and cytoplasmic control mechanisms in the expression or suppression of the tumorigenic state. The following discussion will review some of the results of fusion experiments involving whole cells and cytoplasmic hybrids, which will illustrate that, even though these approaches result in the

acquisition of useful information, elucidation of the mechanisms involved in tumorigenesis have not been unequivocally resolved with these techniques.

There have been numerous reports on the fusion and characterization of hybrid cells produced between tumorigenic and nontumorigenic cells [1, 2, 19–34] and this discussion is not meant to be exhaustive but only to highlight some of the main points. The most important variable in fusing whole cells in attempting to study the underlying genetic basis of tumorigenesis has been the selection of appropriate cell types. Essentially two types of experiments have been utilized. The first involves interspecific hybrids that rapidly segregate chromosomes, and the results of these experiments have not elucidated any generalized mechanisms. Most but not all interspecific hybrids produced by fusing a tumorigenic with a nontumorigenic cell result in expression of the tumorigenic phenotype. These experiments cannot clearly differentiate between instability of the chromosome constitution in such hybrids and specific genetic or epigenetic factors that may be involved in the control of tumorigenesis events. The second type of whole-cell hybrid experiment involves intraspecific crosses, which segregate fewer chromosomes and have provided interesting insights into certain aspects of the expression of the tumorigenic state. Even though there are exceptions to the following statement it does appear to have substantial support from a variety of laboratories. Essentially in intraspecific crosses between tumorigenic and nontumorigenic cells there is an initial suppression of the tumorigenic state provided by some factor in the nontumorigenic cells. This suppression is then removed when a certain chromosome or possibly some epigenetic factor is lost. This was illustrated in a recent report by Sager and Kovac [2], who produced intraspecific hybrids both of which had a stable diploid chromosome constitution, and their results indicated an initial suppression of the tumorigenic state followed by a reexpression of the tumorigenic state along with chromosomal instability. These studies with whole-cell hybrids are consistent with the ones we report in this communication in that intraspecific hybrids are capable of expressing both the tumorigenic and nontumorigenic phenotype. This type of experiment does not, however, allow one to distinguish between genetic and epigenetic factors that may be of fundamental importance. Since hybrids containing almost complete sets of chromosomes from the parental cells can either suppress or express the tumorigenic phenotype, experiments have recently been reported in which cytoplasmic hybrids have been used to try to determine if epigenetic factors may be involved in either the suppression or expression of the tumorigenic state [15, 35, 36].

In brief, the results of such experiments are as follows: Using diploid intraspecific cells, Howell and Sager [35] showed that fusing a nontumorigenic cell with a tumorigenic cytoplasm did not result in expression of the tumorigenic phenotype. They did interpret some of their results to indicate that when a tumorigenic cell was fused with a nontumorigenic cytoplasm, a partial suppression occurred in some of the cybrid clones, indicating that the cytoplasm may be capable of transmitting some suppression factor. Ziegler [36], on the other hand, reported that the cell cytoplasm did not have any effect on the supression or expression of the tumorigenic state in cybrids, which would indicate that independent cytoplasmic control does not play a major role in tumorigenesis. However, Ziegler did report that the cytoplasm could affect the saturation density of cybrids, which is consistent with the observations we previously reported [15]. In those experiments,we fused tumorigenic murine SV-403T3 cells to nontumorigenic murine A-MT-BU-A1 cytoplasms and the resulting cybrids grew to higher saturation densities than the parental SV403T3 cells, and were capable of making tumors in nude mice at lower inoculation densities than the SV-403T3 cells. As with the whole-cell hybrids,

broad generalizations concerning the control of the tumorigenic state with cytoplasmic hybrids have not appeared. Again the choice of cells lines and selection procedures for studying these phenomena appear to be of major importance.

In this report we have illustrated the techniques for producing reconstructed cells between tumorigenic and nontumorigenic parental components and have analyzed these reconstructed cells for their ability to grow in soft agar and in nude mice. As far as we were able to determine, this is the first report of the use of reconstructed cells to analyze factors that may be important in controlling the expression or suppression of the tumorigenic phenotype, even though several reports and procedures are available for producing such reconstructed cells [7, 37, 38, 40, 41]. The results of these initial experiments are consistent with the idea that the nucleus (karyoplast) is sufficient to control the expression or suppression of the tumorigenic state.

ACKNOWLEDGMENTS

This study was supported in part by US Public Health Service funds (HL20643, HL00422) and a grant from the Muscular Dystrophy Association.

The excellent photographic assistance of Ms Mary Tobleman is gratefully acknowledged. We also wish to thank Mr. Mike Glover for his help in the design and construction of the acrylic inserts used for the enucleation experiments and Dr. Woodridge E. Wright for valuable discussions.

REFERENCES

1. Stanbridge EJ: Nature 260:17, 1976.
2. Sager R, Kovac PE: Somat Cell Genet 4:375, 1978.
3. Shay JW, Porter KR, Prescott DM: Proc Natl Acad Sci USA 71:3059, 1974.
4. Wright WE, Hayflick L: Exp Cell Res 74:187, 1972.
5. Prescott DM, Myerson D, Wallace J: Exp Cell Res 71:480, 1972.
6. Shay JW, Porter KR, Prescott DM: J Cell Biol 59(2):311a, 1973.
7. Veomett G, Prescott DM, Shay J, Porter KR: Proc Natl Acad Sci USA 71:1999, 1974.
8. Buonassisi V, Sato G, Cohen AI: Proc Natl Acad Sci USA 48:1184, 1962.
9. Schneider EL, Stanbridge EJ, Epstein CJ: Exp Cell Res 84:311, 1976.
10. Brown S, Teplitz M, Revel JP: Proc Natl Acad Sci USA 71:464, 1974.
11. Chanock RM, Hayflick L, Barile MF: Proc Natl Acad Sci USA 48:41, 1962.
12. Veomett G, Shay J, Hough PVC, Prescott DM: In Prescott DM (ed): "Methods in Cell Biology." New York: Academic, 1976, pp 1–6.
13. Clark MA, Crenshaw AH, Shay JW: Tissue Culture Assoc Man 4:801, 1978.
14. Malech HL, Wivel NA: Cell 9:383, 1976.
15. Shay JW, Peters TT, Fuseler JW: Cell 14:835, 1978.
16. Shay JW, Gershenbaum MR, Porter KR: Exp Cell Res 94:47, 1975.
17. Crissman HA, Tobey RA: Science 184:1297, 1974.
18. Miller CL, Fuseler JW, Brinkley BR: Cell 12:319, 1977.
19. Lyons LB, Thompson EB: J Cell Physiol 90:179, 1976.
20. Foa C, Simonetti J, Berebbi M, Fischer P, Vetterlein M: Oncology 35:58, 1978.
21. Jha KK, Cacciapuoti J, Ozer HL: J Cell Physiol 97:147, 1978.
22. Marshall CJ, Dave H: J Cell Sci 33:171, 1978.
23. Stanbridge EJ, Wilkinson J: Proc Natl Acad Sci USA 75:1466, 1978.
24. Bregula U, Klein G, Harris H: J Cell Sci 8:673, 1971.
25. Harris H, Miller OJ, Klein G, Worst P, Tachibana T: Nature 223:363, 1969.
26. Jonasson J, Povey S, Harris H: J Cell Sci 24:217, 1977.
27. Jonasson J, Harris H: J Cell Sci 24:255, 1977.

28. Klein G, Bregula U, Wiener F, Harris H: J Cell Sci 8:659, 1971.
29. Miller CL, Fuseler JW, Brinkley BR: Cell 12:319, 1977.
30. Straus DS, Jonasson J, Harris H: J Cell Sci 25:73, 1977.
31. Wiener F, Klein G, Harris H: J Cell Sci 8:681, 1971.
32. Wiener F, Klein G, Harris H: J Cell Sci 12:253, 1973.
33. Wiener F, Klein G, Harris H: J Cell Sci 15:177, 1974.
34. Wiener F, Klein G, Harris H: J Cell Sci 16:189, 1974.
35. Howell AN, Sager R: Proc Natl Acad Sci USA 75:2358, 1978.
36. Ziegler ML: Somat Cell Genet 4:477, 1978.
37. Ege T, Krondahl U, Ringertz NR: Exp Cell Res 88:428, 1974.
38. Ege T, Ringertz NR: Exp Cell Res 94:469, 1975.
39. Lucas JJ, Kates JR: Cell 7:397, 1976.
40. Krondahl U, Bols N, Ege T, Linder S, Ringertz NR: Proc Natl Acad Sci USA 74:606, 1977.
41. Shay JW: Proc Nat Acad Sci USA 74:2461, 1977.