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Genetics

A general high-efficiency procedure for production of microcell hybrids

(somatic cell genetics/chromosome transfer/gene mapping)

R. E. K. FOURNIER

Department of Microbiology and the Comprehensive Cancer Center, University of Southern California, Los Angeles, California 90033

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ABSTRACT **The relative efficiency of microcell-mediated chromosome transfer vs. somatic cell hybridization has been determined. The prolonged mitotic arrest generally used to micronucleate donor cells also reduced the fusion efficiency to 1/10th–1/5th of that in whole cell hybridizations. Here we report an alternative micronucleation protocol, involving sequential treatment of the donor cells with Colcemid and cytochalasin B, which yielded micronucleated cells that hybridized with the same efficiency as whole cells. The enucleation, purification, and fusion steps of the microcell procedure have also been refined. By using these modifications the practical yield of microcell hybrid clones can be increased 50- to 100-fold.**

Microcell-mediated chromosome transfer is a parasexual genetic technique that can be used to transfer intact chromosomes from one mammalian cell to another (1, 2). This approach is conceptually and operationally distinct from traditional somatic cell hybridization (3) in that only a fraction of the donor cell genome is introduced into the recipients at the time of fusion. Furthermore, unlike gene transfer systems that use either isolated metaphase chromosomes (4, 5) or purified DNA (6–8) as the donor material, microcell transfers yield clones containing intact donor-derived chromosomes that can be analyzed by cytogenetic tests. This procedure allows the generation of cell lines that are especially powerful gene mapping tools. These cell lines are referred to as microcell hybrids and have proved useful not only for mapping cellular genes (9–11) but also for studying the chromosomal sites of integration of foreign DNA (12–14).

The first essential step of microcell-mediated chromosome transfer is to induce cultured donor cells to become micronucleate by prolonged mitotic arrest (15). This step partitions the chromosome complement into discrete subnuclear packets (micronuclei). The micronuclei can be physically isolated from the cells by centrifugation in the presence of cytochalasin B according to standard enucleation procedures (16). The particles so produced have been termed microcells (17); they consist of a single micronucleus and a thin rim of cytoplasm surrounded by an intact plasma membrane (18). Fusion of isolated microcells with intact recipients generates microcell heterokaryons, which, under appropriate selective conditions, may proliferate to yield microcell hybrid clones. The simplest microcell hybrids contain only a single donor-derived chromosome; other clones contain a small number of such chromosomes (1, 10, 12, 14).

In their original report concerning microcell hybridization (1), Fournier and Ruddle transferred chromosomes from several murine cell lines into mouse, Chinese hamster, and human recipient cells. Cells from a variety of rodent species, and from both established cell lines and primary fibroblast cultures, have been employed successfully as donors in microcell hybridization experiments. Recently, the technique has been extended to include diploid human donor cells (19) as well as particular established lines of human origin.

The efficiency of microcell hybridization is a serious practical problem. For many genetic experiments, particularly those requiring monochromosomal microcell hybrids, the chromosome transfer technique as originally described (1, 2) is satisfactory. However, a variety of potentially interesting studies require the production of large collections of microcell hybrid clones for screening purposes. The difficulty in generating such a collection of clones is compounded in certain cases in which the particular somatic cell cross is itself relatively inefficient—e.g., in fusions involving highly differentiated cell types. These considerations motivated the experiments described here.

Specifically, we asked: What is the efficiency of a microcell hybridization relative to a whole cell fusion using the same parental cells? What steps in the microcell transfer procedure account for any observed loss of efficiency? Can these steps be modified? How many independent microcell hybrid clones can be generated feasibly in a single experiment? In this report, the results of a series of quantitative hybridization experiments are presented, and a modified protocol is described with which 50–100 microcell hybrid clones per experiment are routinely generated.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. Two mouse L-cell derivatives were used (20). A9 lacks hypoxanthine phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) and adenine phosphoribosyltransferase (APRT; AMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7); LMTK$^-$ lacks thymidine kinase (TK; ATP:thymidine 5′-phosphotransferase, EC 2.7.1.21). E36b1 is a HPRT$^-$, ouabain-resistant cell line derived from Chinese hamster lung fibroblasts (21). AuxB1 is an auxotrophic Chinese hamster ovary cell that requires glycine, adenine, and thymidine [gat$^-$ (22)]. Fu5 is a wild-type, H4IIEC3-derived rat hepatoma (23), and RG6A.tgA is a HPRT$^-$ rat glioma. Two human cell lines were employed, both deficient in HPRT activity. These were D98/AH2 and HT1080-TG^R. Cell lines were cultured as described (1).

Micronucleation of Donor Cells. Donor cells for microcell transfer experiments were micronucleated by one of two procedures. The first method involved prolonged mitotic arrest: exponentially growing populations of cells were treated with Colcemid (0.02 μg/ml) for 1–2 cell generations (1, 2). An alternative micronucleation protocol involved sequential expo-

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Abbreviations: HPRT, hypoxanthine phosphoribosyltransferase; TK, thymidine kinase; HAT, hypoxanthine/aminopterin/thymidine; PHA-P, phytohemagglutinin P; PEG, polyethylene glycol.

sure to Colcemid (0.02 μg/ml, 2–16 hr) and cytochalasin B (0.5–5.0 μg/ml, 2–6 hr).

Enucleation of Micronucleate Cells. Cells attached to plastic "bullets" were employed for enucleation. The bullets were cut from tissue culture plates, were approximately 24 × 86 mm, and had one rounded end.

For some experiments, cells growing on plastic bullets were micronucleated by exposure to Colcemid at 0.02 μg/ml for 48 hr. Alternatively, populations of cells previously micronucleated either by prolonged mitotic arrest or by sequential Colcemid/cytochalasin B treatment were seeded directly onto bullets to which concanavalin A had been covalently linked (24).

Micronucleate cells attached to bullets were enucleated by centrifugation at 27,000 × g for 30 min in α modification of Eagle's minimal essential medium + cytochalasin B (10 μg/ml) at 34°C. Eight bullets were processed per fusion experiment. After enucleation, the microcell pellets were suspended in α minimal essential medium and pooled.

Purification of the Microcell Preparation. The crude microcell preparation was purified by filtration through Uni-Pore polycarbonate membrane filters (Bio-Rad). The microcells were suspended in a minimum volume of 20 ml and divided into two to four aliquots, which were filtered separately. The polycarbonate filters were mounted in 25-mm Swinnex adaptors (Millipore), and the microcell suspensions were pushed through the filters by using sterile syringes. For most preparations, two filters were employed in series. These were mounted in separate adaptors and had pore sizes of 8 μm and 5 μm, respectively.

Fusion Protocol. Isolated microcells were fused with intact recipient cells by using a modification of the procedure of Mercer and Schlegel (25). The microcells were pelleted and resuspended in 2 ml of serum-free α minimal essential medium containing phytohemagglutinin P (PHA-P, Difco) at 100 μg/ml. This suspension was added to a washed, 70–80% confluent monolayer of recipient cells in a 25-cm^2 flask. The mixture was incubated 10 min at 37°C to allow agglutination of microcells to recipients, and the PHA-P-containing medium was removed. The monolayer was exposed to a solution of polyethylene glycol (PEG 1540, Baker) in α minimal essential medium for 1.0 min, rinsed three times with serum-free medium, and incubated in complete, nonselective medium. After 16–24 hr, the cells were distributed into 10–20 25-cm^2 flasks, and selection was applied.

The PEG concentration used for fusion was critical, and was determined empirically for each recipient cell line. The optimal concentration ranged between 35% and 50% PEG (wt/wt) but generally was 42–44%.

RESULTS

Microcell fusions are commonly less efficient than whole cell hybridizations performed under similar conditions. Two lines of indirect evidence suggested that the first step of the technique, micronucleation of the donor cells, was responsible at least in part for the loss of efficiency. First, prolonged Colcemid arrest of donor mouse cells results in a progressive and drastic reduction in microcell hybrid yield (1). Thus, micronucleation of the donor cells can be frequency-limiting for hybrid production. Second, both cell reconstruction experiments (26, 27) and cytoplast fusions (28, 29) can yield clones at frequencies approaching those of cell–cell hybridizations. These procedures involve the same experimental manipulations as microcell fusions but omit mitotic arrests. In order to test whether micronucleation alone influenced the frequency of hybrid production, intact micronucleate donor cells were fused with untreated mononucleate recipients.

Fusion of mouse LMTK$^-$ cells with HPRT$^-$ E36b1 Chinese hamster cells yielded hypoxanthine/aminopterin/thymidine (HAT)-resistant hybrid clones at frequencies of approximately 3×10^{-5} (Table 1). However, when the LMTK$^-$ cells were micronucleated prior to fusion by prolonged mitotic arrest, the hybrid yield was reduced to about 1/5th. Reversing the markers under selection yielded similar results: hybrid clones were generated with a frequency of $5.0–5.3 \times 10^{-5}$ in experiments in which HPRT$^-$ mouse A9 cells were fused with TK$^-$ RJK hamster cells. In contrast, hybridization of micronucleated A9 cells with intact RJK recipients produced clones at frequencies of $0.5–0.6 \times 10^{-5}$.

Micronucleation of one parental cell line in a standard somatic hybridization experiment thus reduced hybrid yield to 1/5th to 1/10th. Furthermore, the frequencies with which hybrid clones were generated in such crosses were similar to those observed in microcell-mediated chromosome transfer experiments using the same parental cell lines. Thus, micronucleation alone accounted for most if not all of the reduction in hybrid yield of microcell fusions as compared to whole cell hybridizations.

The donor cells used in the experiments described above were micronucleated by prolonged exposure to Colcemid. We next explored the possibility of developing a micronucleation protocol that did not depend upon prolonged mitotic arrest. Populations of metaphase cells were generated by limited exposure to Colcemid and subsequently plated in the presence of various agents known to perturb normal progress through mitosis. These treatments included storing the mitotic cells at 4°C or 25°C for various periods before replating, incubating the cells in medium without serum or at elevated pH or both, and plating the cells in the presence of various thiol compounds, mitotic arrest agents, or cytochalasin B (30). Plating mitotic cells in the presence of low concentrations of cytochalasin B induced micronucleation most effectively.

LMTK$^-$ cells were arrested in metaphase by Colcemid treatment for various periods of time, and mitotic cells were replated in the presence or absence of cytochalasin B (Fig. 1). Cells plated in the absence of cytochalasin B became progressively

Table 1. Fusion of mononucleate and micronucleate mouse cells with intact Chinese hamster recipient cells

Fusion	Exp. no.	Flasks with clones	No. of clones	Hybrid yield* ×10^5
LMTK$^-$ and E36b1	I	9/10	32	3.2
	II	9/10	24	3.0
m(LMTK$^-$)† and E36b1	I	5/10	6	0.6
	II	6/10	6	0.6
A9 and RJK	I	7/10‡	9‡	5.0
	II	8/10‡	11‡	5.3
m(A9)† and RJK	I	4/10	5	0.5
	II	5/10	6	0.6
LMTK$^-$ microcells and E36b1	Several	—	—	0.3–1.0

Mixed monolayers of mono- or micronucleate mouse cells and intact Chinese hamster recipients (donor-to-recipient ratio = 1:1.5) were fused with 50% (wt/wt) PEG 1540. After 24 hr, the cultures were redistributed and HAT selection was applied. The flasks were fixed and stained with crystal violet 14 days later. Clones with a diameter of 2 mm or greater were scored.

* Number of primary hybrid clones/number of donor cells or cell equivalents.

† m(X) indicates that cell line X was micronucleated by 48-hr exposure to Colcemid at 0.02 μg/ml.

‡ Only 1/10th of the fusion mixture was plated.

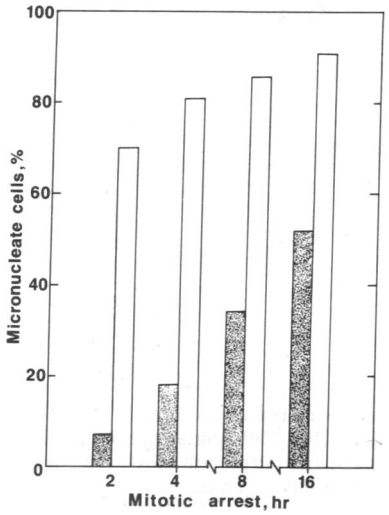

FIG. 1. Induction of micronuclei in LMTK⁻ cells. LMTK⁻ cultures were exposed to Colcemid at 0.02 μg/ml for the periods indicated. Mitotic cells were collected and replated in the presence (open bars) or absence (shaded bars) of cytochalasin B at 1 μg/ml. Four hours after replating, cells were either fixed *in situ* for Hoechst 33258 staining or harvested for staining with aceto-orcein. For each point, 500 individual cells were scored.

more micronucleate as the duration of Colcemid arrest was increased. When mitotic LMTK⁻ populations collected after the exposure to Colcemid for 2 hr were replated in α minimal essential medium + 5% fetal bovine serum, 7% of the cells became micronucleate, but mitotic populations exposed to Colcemid for 16 hr yielded 50% micronucleate cells. Cytochalasin B treatment augmented this tendency towards micronucleation after mitotic arrest. After Colcemid arrest for 2 hr, 70% of the population became micronucleate after replating in cytochalasin B at 1 μg/ml, and 16-hr Colcemid arrest followed by cytochalasin B treatment induced micronucleation in 90% of the cells.

Micronucleate cells produced by a sequential Colcemid–cytochalasin B treatment differed in a number of ways from populations in which micronucleation had been induced by prolonged mitotic arrest (Table 2). Morphologically, sequential-treatment micronucleate cells strongly resembled the mononucleate cells from which they were derived, whereas prolonged-arrest micronucleation generated populations of cells that were large and polymorphic (Fig. 2). In terms of protein content and cell size, sequential-treatment micronucleates were twice as large as normal LMTK⁻ cells, as would be expected for synchronous populations emerging from a mitotic phase in which

Table 2. Physical properties of mononucleate and micronucleate LMTK⁻ cells

Property	Mononucleate	Micronucleate	
		Sequential*	Prolonged arrest†
Mean cell diameter, μm	13.7	17.0	20.6
Mean cell volume, μm^3	1340	2550	4650
Density at confluence, no. cells/cm^2	1.3×10^5	1.2×10^5	0.5×10^5
Protein content, ng/cell	0.24	0.33	0.64
Average no. of micronuclei	—	7	8

Cell counting and sizing were accomplished with the use of a Coulter Channelyzer.

* Exponentially growing LMTK⁻ cultures were treated with Colcemid at 0.02 μg/ml for 16 hr. Mitotic cells were collected, resuspended in medium containing cytochalasin B at 1 μg/ml, and replated. Data are presented for populations collected 4 hr after plating in cytochalasin B-containing medium. In this population 92% of the cells were micronucleate.

† Exponentially growing LMTK⁻ cells were treated with Colcemid at 0.02 μg/ml for 48 hr. After this treatment 90% of the cells were micronucleate.

cytokinesis had been blocked. Prolonged-arrest micronucleates were much more heterogeneous and had mean cell volumes and protein contents 4 times greater than those of their mononucleate counterparts.

The hybridization efficiency of LMTK⁻ cells micronucleated by sequential Colcemid–cytochalasin B treatment was determined and compared to that of populations micronucleated by prolonged mitotic arrest (Table 3). The upper part of the table shows results of experiments in which intact micronucleate LMTK⁻ cells were fused with E36b1 recipients. In hybridizations involving donor cells micronucleated by a 48-hr Colcemid treatment, HAT-resistant hybrid clones were generated at frequencies approaching 1.0×10^{-5}. However, when donor cells were micronucleated by sequential Colcemid–cytochalasin B treatment, the hybrid yields were approximately 5×10^{-5}. This value was essentially identical to that of whole cell fusions employing intact untreated LMTK⁻ and E36b1 cells (see Table 1). Thus, the reduction in hybrid yield previously observed in fusions involving micronucleate donor cells (above) was not due to micronucleation *per se*, but rather to the prolonged arrest protocol that had been employed. In contrast, micronucleate populations generated by plating mitotic cells in the presence of cytochalasin B were as efficient in somatic hybridization experiments as the normal mononucleate cells from which they were derived.

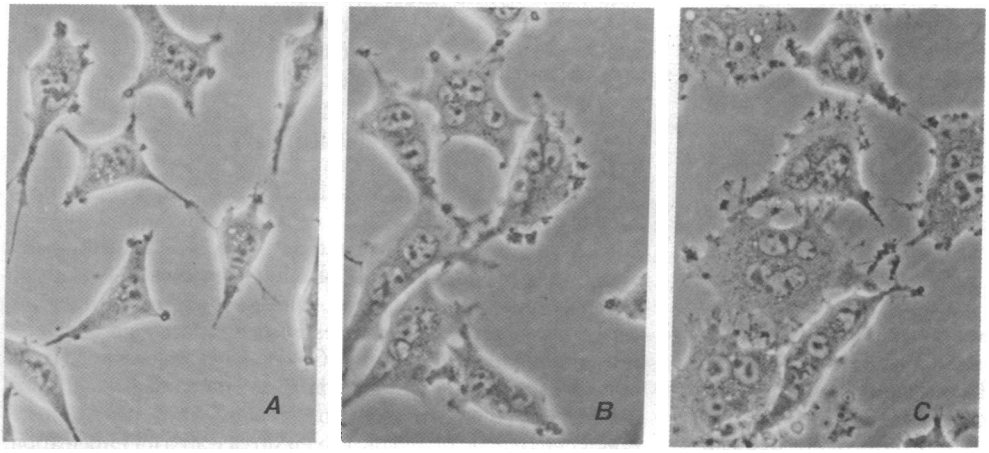

FIG. 2. Morphology of LMTK⁻ cells. (*A*) Mononucleate cells. (*B*) Micronucleate cells induced by sequential treatment with Colcemid (0.02 μg/ml, 16 hr) and cytochalasin B (1.0 μg/ml, 4 hr). (*C*) Micronucleate cells induced by prolonged Colcemid arrest (0.02 μg/ml, 48 hr). (Phase-contrast photomicrographs of living material; ×280.)

Table 3. Hybridization efficiency of LMTK$^-$ cells micronucleated by prolonged mitotic arrest vs. by sequential Colcemid–cytochalasin B treatment

Micronucleation protocol*	Exp. no.	No. HAT-resistant clones	Flasks with clones	Hybrid Yield† × 10^5
A. Whole-cell fusions: micronucleate LMTK$^-$ and E36b1				
Prolonged arrest	IA	29	10/10	0.9
	IB	9	8/10	0.9
Sequential	I	44	10/10	4.9
Prolonged arrest	II	10‡	9/10‡	1.0
Sequential	II	6‡	6/10‡	6.0
B. Microcell fusions: LMTK$^-$ microcells and E36b1				
Prolonged arrest	I	4	3/10	0.2
Sequential	I	13	8/10	1.1
Prolonged arrest	II	6	5/10	0.4
Sequential	II	11	9/10	1.3
Prolonged arrest	III	3	3/10	0.2
Sequential	III	12	8/10	0.9
Prolonged arrest	IV	4	4/10	0.2
Sequential	IV	23	9/10	1.1
Sequential	V	17	8/10	3.1

(*A*) Whole-cell fusions. Mixed monolayers of micronucleate LMTK$^-$ and intact E36b1 cells were incubated with PHA-P at 100 μg/ml for 10 min. The cells were fused by using 44% (wt/wt) PEG 1540. (*B*) Microcell fusions. Microcells were prepared, purified, and fused with E36b1 recipients as described in *Materials and Methods*. The purified microcell preparations were quantitated with a hemocytometer, and isolated microcells were scored after staining with 0.5% orcein in 50% (vol/vol) acetic acid.

* Details of the prolonged arrest and sequential treatment micronucleation protocols are given in the legend to Table 2.

† For the whole-cell fusions, number of primary hybrid clones/number of donor cells. For the microcell fusions, number of microcell hybrid clones/number of donor cell equivalents of microcells. Donor cell equivalents = (total number of microcells/modal number of micronuclei per micronucleate cell).

‡ Only 1/10th of the fusion mixture was replated in selective medium.

Micronucleate LMTK$^-$ cells produced either by prolonged mitotic arrest or by sequential Colcemid–cytochalasin B treatment were also used as donors in microcell-mediated chromosome transfer experiments. Parallel cultures of LMTK$^-$ cells were micronucleated by either protocol and enucleated as described in *Materials and Methods*. The resulting microcell preparations were purified by membrane filtration and fused with E36b1 monolayers by using PHA-P and PEG. The results of five such experiments, performed on different days, are presented in Table 3. In each case, microcell preparations derived from donor cells micronucleated by sequential Colcemid–cytochalasin B were 4- to 5-fold more efficient in terms of microcell hybrid yield than microcells prepared from donors subject to prolonged mitotic arrest. This difference in fusion efficiency was the same as that observed in fusions in which intact micronucleate LMTK$^-$ cells were hybridized with E36b1 recipients (compare parts *A* and *B* in Table 3).

To be generally useful, this micronucleation protocol must be applicable to a variety of cell lines. Experiments were therefore performed in which mitotic cells collected from a number of different cell lines were plated in the presence of cytochalasin B. As shown in Table 4, this procedure effectively induced

Table 4. Micronucleation of various cell lines by using a sequential Colcemid–cytochalasin B protocol

Cell line	% mitotic	% of cells after replating: Micronucleate	Mononucleate	Binucleate	Mitotic
LMTK$^-$	98	90	8	2	0
A9	97	89	9	2	0
E36b1	82	74	20	6	0
AuxB1	85	84	14	2	0
RG6A	87	64	20	16	0
Fu5	62	53	42	5	0
D98AH2	98	32	24	16	28
HT1080	91	67	13	16	4

Cultures were incubated with Colcemid at 0.02 μg/ml for 16 hr (6 hr for Fu5 and E36b1). Metaphase cells were collected by selective mitotic detachment and the percent mitotic cells was determined. The suspensions were replated in medium containing cytochalasin B at 1 μg/ml. The cells were scored 4 hr after replating; 500 individual cells were counted for each point.

micronucleation in all cell lines tested. For two cell lines (D98/AH2 and Fu5) it would be desirable to increase the fraction of the population that became micronucleate on replating. It is likely that this could be achieved by altering the time of exposure to Colcemid, cytochalasin B, or both. It is noteworthy, however, that this procedure induced micronucleation not only in the rodent lines tested (mouse, Chinese hamster, and rat), but also in cell lines of human origin.

DISCUSSION

By modifying our original microcell-mediated chromosome transfer technique (1) we have been able to increase the practical yield of microcell hybrid clones 50- to 100-fold. Thus, it is now a relatively straightforward matter to generate a large collection of clones that can be screened for particular phenotypes of interest.

The most significant modification is a micronucleation protocol involving sequential exposure of the donor cells to Colcemid and cytochalasin B. This treatment produces populations of micronucleate cells 5–10 times more efficient in terms of hybrid yield than micronucleate cells produced by prolonged mitotic arrest. Micronucleate cells produced by the sequential protocol are quasi-synchronous populations of G1 cells. In contrast, populations subject to prolonged mitotic arrest are highly asynchronous, with some cells having proceeded through S phase and even into a subsequent abortive mitosis after micronucleation. A significant fraction of the microcells derived from such cultures appear to be genetically inactive and do not yield viable hybrid clones upon fusion. Reducing the duration of mitotic arrest would minimize this problem, but the applicability of this approach is limited by the extreme asynchrony with which mitotic cells escape the metaphase block in the presence of Colcemid and similar agents. The sequential treatment micronucleation protocol eliminates the problem, and a relatively synchronous release from mitosis with concomitant micronucleation is achieved by plating the mitotic cells in the presence of cytochalasin B.

Micronucleation can be effectively induced in a variety of cell lines by using the sequential Colcemid–cytochalasin B protocol. These include human cell lines, which are difficult to micronucleate by prolonged mitotic arrest. Furthermore, micronucleate cells produced by sequential Colcemid–cytochalasin B treatment are approximately half as large as micronucleate cells produced by prolonged exposure to Colcemid. Thus, twice as many donor cells can be plated on a given surface area for enucleation.

At present, the general utility of this method is limited only by the ability to produce relatively pure populations of mitotic cells for plating in the presence of cytochalasin B. For either slow-growing cells (e.g., some diploid fibroblast strains) or for cultures in which selective mitotic detachment is difficult (e.g., Fu5), prolonged mitotic arrest may continue to be the most effective technique for inducing micronucleation. Even in such cases, however, the enucleation and fusion modifications described in this report are sufficient to enhance microcell hybrid yield 10- to 20-fold.

The procedures used to enucleate micronucleate populations have been modified in two respects. The simplest modification has been to use cultures attached to plastic bullets rather than small discs for enucleation. The manipulations involved are identical in either case, but the surface area that can be processed per centrifugation, and hence the number of microcells generated, is four times greater (20.2 vs. 4.9 cm^2 per tube).

For many experiments, concanavalin A-treated bullets have been employed. This has proven to be a useful technique for enucleating donor cells that attach only poorly to plastic. In addition, it has the advantage that precise numbers of donor cells can be plated for enucleation, an important consideration in quantitative fusion studies such as those described in this report.

The purification of isolated microcell preparations by using polycarbonate membrane filters (19) is simple and faster than unit gravity sedimentation techniques. However, the most stringent purification of microcells from contaminating karyoplasts and intact cells seems best accomplished with the use of unit gravity density gradients.

The microcell fusion protocol described in this report uses PHA-P as an agglutinin followed by treatment with the fusogen PEG. The efficiency of this procedure is 5- to 10-fold greater than the efficiencies of procedures employing inactivated Sendai virus or PEG in a suspension fusion protocol. In addition, it is one of the simplest fusion procedures described to date.

By using the modifications described in this report, it has routinely been possible to generate 50–200 microcell hybrid clones per experiment, and we rarely recover fewer than 20. In contrast, hybridizations employing the microcell-mediated chromosome transfer technique originally described typically produced 1–6 clones per fusion, rarely as many as 18 (10), and about 25% of experiments yielded no clones at all.

The calibration experiments in which intact mono- or micronucleate $LMTK^-$ cells were fused with E36b1 recipients have led to an unexpected observation. Evidence has been obtained that the direction of chromosome segregation was different in the two families of hybrid clones. Five hybrids formed by fusion of $LMTK^-$ and E36b1 cells have been analyzed: all five clones were Chinese hamster cells segregating mouse chromosomes. In contrast, the direction of chromosome segregation was reversed in hybrid clones generated by fusing intact, micronucleate $LMTK^-$ cells with E36b1 recipients. In all five cases so far examined, these clones had a double complement of mouse ($LMTK^-$) chromosomes and were segregating hamster chromosomes. The basis of this effect is not presently known.

In summary, a modified procedure for microcell-mediated chromosome transfer has been developed that has increased microcell hybrid yield 50- to 100-fold. These modifications have enabled us to generate large collections of microcell hybrid clones for a variety of genetic studies. This has been especially important in low-efficiency crosses—e.g., in fusions involving highly differentiated cell types. The techniques described in this report simplify the construction of microcell hybrid clones and may contribute to the general use of such hybrid cells in mammalian somatic cell genetics.

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