Construction of Microcell Hybrid Panel Containing Different Neo Gene Insertions in Mouse Chromosome 17 Used for Chromosome-Mediated Gene Transfer

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Abstract—A panel of four microcell hybrids representing different sites of insertion of the exogeneous neo gene into mouse chromosome 17 has been constructed. These constructions were based on a cotransfer of mouse chromosome 17 and neomycin resistance generated in a stepwise procedure involving (1) random insertion of the neo gene into a primary cell hybrid containing mouse chromosome 17 in a hamster cell background, (2) microcell-mediated chromosome transfer (MMCT) to segregate mouse and hamster chromosomes, and (3) identification of the mouse chromosome containing cells using a novel cell dotting procedure for mass screening at the cell colony level by molecular hybridization. Using this panel of four microcell hybrids for chromosome mediated gene transfer (CMGT), we obtained one transformat containing a chromosome fragment derived from the t-complex region located on mouse chromosome 17. It is concluded that the specific chromosome based procedure used here to generate CMGT transfectants may provide a general means to produce large numbers of transfectants containing megabase fragments covering, in principle, all regions of a given chromosome.

INTRODUCTION

Somatic cell hybrids containing a limited amount of genetic material (e.g., one or a few chromosomes) from one parent have been a useful approach to mammalian genetics in particular human and mouse genetics. Such hybrids have been used for chromosomal mapping of many isozymes and DNA restriction fragments (1, 2). Hybrid cells have also been used for functional studies of chromosome-specific factors in the control of malignancy (3) and expression of tissue-specific genes (4, 5).

Microcell-mediated chromosome transfer (MMCT) techniques have proved an efficient means to produce hybrids containing only one or a few chromosomes from one parent (6, 7). However, only very few chromosomes carry genes that can be efficiently selected for (e.g., mainly HPRT, TK, and APRT). Thus, the selection for and subsequent fixation of a specific chromosome in the generation of microcell hybrids is possible only in very few cases.

One approach to circumvent this problem is to use parental cells with defined translocations involving chromosomes that carry selectable genes in addition with the one of interest (3, 8). An alternative and more general approach is to introduce exogenous selectable genes into the chromosomes of interest. A number of such cloned genes (including bacterial and mammalian) has become available
that can be used for selection of stable chromosomal integrations. Particularly useful are those that confer a "dominant" selection gene and thus are not relying on complementation of a recessive mutation in the recipient cell. The *E. coli* neomycin-resistance (*neo*) gene from transposon Tn5 confers resistance of mammalian cells to the antibiotic G418. Inserted into mammalian SV40 based or retroviral expression vectors (9, 10), the *neo* gene can be efficiently expressed in mammalian cells.

Hybrid cells can also be produced containing only subchromosomal fragments of continuous DNA. This is efficiently obtained using isolated metaphase chromosomes transferred by so-called chromosome-mediated gene transfer (CMGT) techniques (11). Such hybrid transfecants are useful for establishing subchromosomal mapping, and furthermore, as a favourable starting material for the molecular cloning of any gene residing on the fragment (12–15). However, this elegant approach is currently limited to cases where the fragments either carry inherent selectable genes or when fragments carrying inserted selectable genes can be identified on the basis of cell surface marker genes residing on the fragment (16, 17).

In the present study we demonstrate a general approach using the MMCT technique to construct a panel of microcell hybrids carrying *neo* gene insertions at four different sites in mouse chromosome 17, which proved efficient to produce CMGT transfecants carrying fragments derived from the t-complex residing on this chromosome.

**MATERIALS AND METHODS**

**Cell Lines.** E36 is a HPRT-deficient Chinese hamster cell line (18). R44 is a mouse-hamster hybrid cell line containing mouse chromosome 17 as the single mouse component (19). R44neoA and R44neoB are pSV2neo (10) transfected R44, and R44rneoB is pRSVneo (20) transfected R44. All three R44 transfecants represent large pools (over 500 clones) of individual stable neo transfected cells (Höglund, unpublished).

**Microcell-Mediated Chromosome Transfer (MMCT).** The procedures used for micronucleation, enucleation, and microcell hybridization were essentially as described elsewhere (7, 21). Briefly monolayers of R44neoA, B, and R44neoB were treated with colcemid (Demecolin; Sigma) 50 ng/ml for 16 h before the cells were trypsinized and transferred to plastic "bullets." After attachment the cells were centrifuged at 15,000 g for 40 min in α-MEM medium containing cytochalasin B (Sigma) 10 μg/ml. Microcell pellets were suspended and filtered through polycarbonate filters (Nucleopore) in a series using 8- and 5-μm pore sizes. Yields of 1–5 × 10⁶ microcells were obtained in each experiment. After treatment in phytohemagglutinin (P) (Difco) 100 μg/ml (22) for 15 min, the microcell suspensions were added to monolayers of E36 recipient cells at a ratio of two donor cell equivalents per recipient cell. After adhesion, cell fusion was induced by the addition of a 45% PEG-1540 solution (polyethylene glycol, mol wt 1540, Riedel de Haen) for 100 sec. After a thorough rinsing, the fusion-treated cells were incubated overnight in α-MEM supplemented with 10% FBS before being plated out at appropriate cell densities. G418 (Gibco/BRL)-containing medium (800 μg/ml) was added 36 h after fusion and renewed twice a week.

**Mass Screening of Microcell Hybrids on Cell Dot Blots.** The selection of microcell hybrids were performed in 96-well microtest plates (Nunc), allowing an efficient handling using a Titerette multichannel pipet system (Flow). After primary outgrowth, hybrid colonies were replated into three replicate microtest plates. Two of the plates were used for cell dotting analysis, while the third plate was frozen down at −70°C in complete medium supplemented with 10% DMSO for later reactivation of relevant clones. After outgrowth in the two replica microtiter plates, colonies of