Review

Monochromosomal Hybrids and Chromosome Transfer: A Functional Approach for Gene Identification

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Abstract. *Functional complementation of cellular defects has been a valuable approach for localizing causative genes to specific chromosomes. The complementation strategy was followed by positional cloning and characterization of genes for their biological relevance. We herein describe strategies used for the construction of monochromosomal hybrids and their applications for cloning and characterization of genes related to cell growth, cell senescence and DNA repair. We have cloned RNaseT2, GluR6 (glutamate ionotropic receptor kainate type subunit 2-GRIK2) and protein tyrosine phosphatase, receptor type K (PTPRK) genes using these strategies.*

The sequencing of the human genome has been an important milestone in modern biology (1, 2). This process involved characterization of clones with insert sizes ranging from a few thousand bases to a million bases in plasmid, bacteriophage, phagemid, cosmid, bacterial artificial chromosome and yeast artificial chromosome vectors (3-8). These cloned DNA segments, spanning across specific regions of human chromosomes, were subsequently sequenced and assembled together to generate contiguous

stretches of genomic sequences. This entire process involved contributions from numerous laboratories either individually or as part of large consortia. A variety of methods were used to generate the starting DNA for creating genomic libraries. One such method for library preparation involved purification of individual human chromosomes based on their sizes (9-12). The purification of individual chromosomes was facilitated by using monochromosomal/ somatic cell hybrids (13-15).

In light of the utility of somatic cell hybrids in the pre-genomic era, we present a historical perspective of chromosome transfer strategies, and construction and applications of monochromosomal hybrids for identifying a variety of important genes. Despite the availability of the complete human genome sequence, monochromosomal cell hybrids remain an important resource for investigating the functional significance of various genomic regions. Here we have emphasized the production and the value of monochromosomal hybrids for cloning of genes responsible for specific cellular functions.

Transfer of Chromosomes into Recipient Cells

The success in creating hybrid cells paved the way to develop experimental strategies for investigating the expression and functional significance of human genes. It was known in 1962 that mammalian cells can fuse together to form polynuclear cells (16), and hybrid cells had been derived from mouse and man by 1965 (17). It is noteworthy that only a few human chromosomes were retained in the hybrid cells when human and mouse cells were fused together (18). These observations indicated the potential utility of hybrid cells for mapping genes onto various human chromosomes. The presence of multiple human chromosomes in a unique combination in clones of segregating mouse-human hybrid cells facilitated mapping of genes to specific human chromosomes (19).
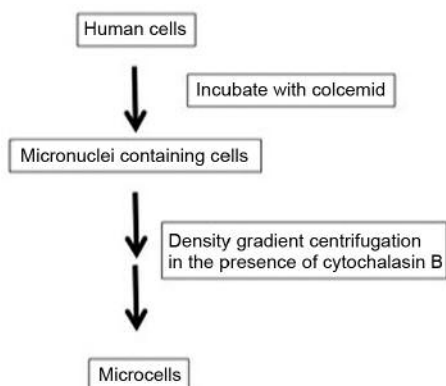
Figure 1. *Schematic for isolating microcells. The human cells were transfected with cloned XGPRT gene, and subsequently cultured in a selection medium containing mycophenolic acid and xanthine. Further treatment of these cultured human cells with colcemid leads to micronuclei formation, which are released as microcells in the presence of cytochalasin B. The microcells are separated by density-gradient centrifugation.*

Figure 2. *Strategy for fusion of microcells with recipient mammalian cells. The microcells were fused with the recipient mouse A9 cells in the presence of polyethylene glycol, and monochromosomal hybrids were isolated by culturing the fused cells in the selection medium containing either G418 or a mixture of mycophenolic acid and xanthine.*

Based on the packaging of eukaryotic genetic material as a well-defined compact chromatin structure in metaphase (20), it was predicted that chromosomes can be transferred from donor cells to recipient cells. McBride and Ozer took advantage of such organized packaging of chromatin and showed the transfer of functional genes using metaphase chromosomes from Chinese hamster fibroblasts into mouse A9 cells (21). The mutant status of HPRT gene in A9 cells allowed the selection of clones for the presence of specific donor chromosome (22). These experiments demonstrated unambiguous transfer and stable expression of donor HPRT gene in the recipient cells, and set the basis for using chromosome transfer as a feasible tool for gene identification (23). It warrants mentioning that this was the conclusive demonstration of the transfer and expression of functional genes in mammalian cells.

Chromosome Transfer in Mammalian Cells

The initial success of chromosome transfer experiments was followed by the transfer of a variety of human and rodent genes into mutant recipient cell lines (24, 25). The frequency of transfer, however, was very low in the presence of poly-lysine buffer used by McBride and Ozer (21). The limitations posed by low frequency transfer were subsequently addressed by applying specific modifications to the chromosome transfer methodology. Spandidos and colleagues (26) were able to marginally improve the transfer frequency using a buffer system containing calcium phosphate. Miller and Ruddle (27) employed a modified protocol that included treatment with 10% dimethyl sulfoxide 4.25 h after mixing the metaphase chromosomes with the recipient cells.
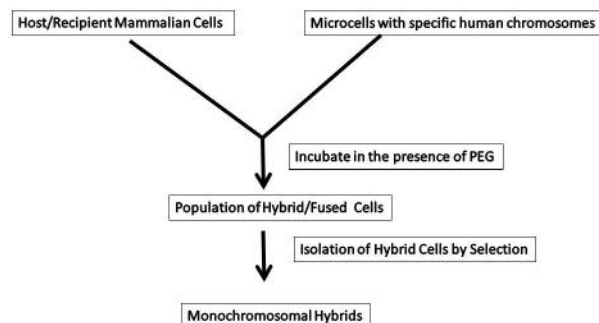
This modification significantly improved transfer frequency as well as the size of the transferred chromosomal fragments. Although the transferred chromosomal fragments were readily detectable in the recipient cells (27), the fate of the transferred DNA was not clear. Using a sequential transfer strategy, Athwal and McBride (28) first demonstrated that the transferred genetic material integrates at random into the recipient cell chromosomes. The random integration of human thymidine kinase (TK) and adenine phosphoribosyl transferase (APRT) genes provided a method for integration of a selectable marker in recipient cells (29-31). Subsequently, the introduction of cloned selectable prokaryotic genes such as xanthine guanine phosphoribosyltransferase (XGPRT) and neomycin resistance gene into mammalian cells provided a highly efficient method for chromosome tagging (32).

Monochromosomal Hybrids

The standardization of protocols for high efficiency chromosome transfer set the stage to accomplish gene mapping and functional complementation of cellular defects. Initial strategies were aimed at establishing a panel of somatic cell hybrids by somatic cell hybridization and random elimination of a majority of human chromosomes from fused nuclei of human and rodent hybrids (33-35). This hybridization technique was subsequently improved for establishing monochromosomal hybrids by employing fusion of micronuclei (36-38). Micronuclei arise due to partitioning of chromosomes during prolonged arrest of cells in mitosis. These micronuclei that contain a single or few chromosomes can be subsequently purified by disruption of microfilaments followed by centrifugation (38). The isolated micronuclei surrounded by plasma membrane are termed "microcells". A flow chart of this strategy is shown in Figure 1.
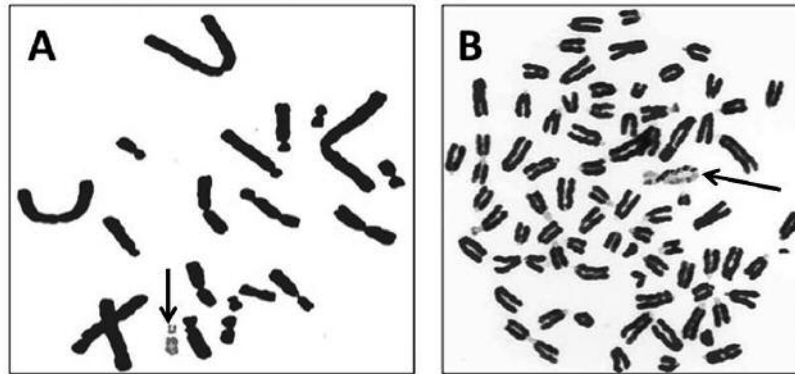
Figure 3. *Identification of transferred human chromosome by Giemsa 11 staining. Panels A and B show the presence of a single human chromosome (lightly stained) in Chinese hamster background (panel A) and mouse background (panel B). The arrow shows lightly stained human chromosome.*

The microcells are size-fractionated and subsequently fused with rodent cells in the presence of polyethylene glycol (PEG) for delivering the human chromosomes into the recipient cells. The advantage of the microcell-based chromosome transfer is that it does not depend on spontaneous elimination of transferred chromosomes for obtaining monochromosomal hybrids (38). The fusion product is cultured in a selection medium to facilitate the growth of cells containing the chromosome tagged with a marker. This protocol, as schematized in Figure 2, shows a single step production of monochromosomal hybrids.

Characterization of Monochromosomal Hybrids

The monochromosomal hybrids were characterized by using several different methods to ascertain that each hybrid contains a single specific chromosome. These methods included characteristic karyotypes and banding patterns obtained by Giemsa 11 and Hoechst staining (39). An illustration of Geimsa staining in Figure 3 shows metaphase chromosomes of a hybrid that contained human chromosome 5 in the rodent background.

The initial identity of a specific chromosome present in a monochromosomal hybrid was determined by zymographic analysis of cell lysates, which involves biochemical detection of isozymes encoded by specific human chromosomes. The presence of single human chromosome(s) in the hybrid cells was further confirmed by fluorescence *in situ* hybridization (FISH) and probing Southern blots of the hybrid cell DNA with human DNA. The existence of human chromosome specific DNA in the hybrid cell is evident from the signal shown in Figure 4. The chromosome transfer method described here has yielded a panel of hybrid cell lines each containing a single different human chromosome and thus representing the entire human genome. Table I presents the details of 22 cell

lines characterized for the presence of each of the 21 autosomes and the X chromosome.

The human chromosomes in the hybrid cell lines shown in Table I exist in the background of mouse A9 cells. A similar hybrid panel has also been produced in Chinese hamster CHTG49 cells. The human chromosomes present in these monochromosomal hybrids are tagged with a selectable marker GPT (guanine phosphoribosyltransferase) (41). Similarly, neomycin resistance gene (neo) was integrated in chromosomes 10 and 20 as a selectable marker. A large number of hybrid cells were characterized, and the 20 cell lines shown in the table contain a single GPT-tagged chromosome. RA4A7 and RA20A hybrid cell lines harbor neomycin-tagged chromosomes. It warrants mention that GPT and neo markers were important for selecting the somatic cell hybrids that retained specific human chromosomes.

As previously described, these hybrids were characterized by Giemsa banding (Figure 3), FISH (Figure 4) and PCR karyotyping (41). Southern hybridization with human DNA prepared by Alu-PCR of a hybrid cell DNA was used to exclude the presence of a specific chromosome in other hybrid cells. Figure 5 demonstrates the characteristic hybridization of hybrid cells containing chromosome 2. These techniques confirmed the presence and identities of individual human chromosomes in monochromosomal hybrids.

Applications of Monochromosomal Cell Hybrids

Somatic cell hybrids have been useful in the pre- as well as post-genomic era. These hybrids have been used for gene mapping and identification of senescence genes, growth/tumor suppressing genes and DNA repair genes. The following sections highlight representative examples of such applications of monochromosomal hybrids.

Figure 4. *Fluorescence in situ hybridization. Metaphase chromosomes were hybridized with fluorochrome-tagged total human DNA and visualized under a fluorescence microscope. The bright yellow signal indicates the transferred human chromosome among Chinese hamster metaphase chromosomes.*

Table I. *A panel of somatic cell hybrid cell lines with specific human chromosome(s) in a rodent background*.*

Cell Line	Chromosome(s)	Host	Marker
RA1A	1	A9	GPT
RA2-1	2	A9	GPT
RA3A	3	A9	GPT
RA4A7	4 &7	A9	GPT
RA5A	5	A9	GPT
RA6A	6	A9	GPT
RA7A	7	A9	GPT
RA8A1	8	A9	GPT
RA9	9	A9	GPT
RA10	10	A9	Neo
RA11	11	A9	GPT
RA12A	12	A9	GPT
RA13A	13	A9	GPT
RA14A	14	A9	GPT
RA15A	15	A9	GPT
RA16A	16	A9	GPT
RA17A	17	A9	GPT
RA18A	18 & 5	A9	GPT
RA19A	19	A9	GPT
RA20A	20	A9	Neo
RA21	21	A9	GPT
RAXA	X	A9	GPT

*Similar monochromosomal hybrid cell lines are also available in Chinese hamster CHTG49 background.

Gene Mapping

Monochromosomal hybrids provided an extremely convenient methodology for mapping of any unique DNA or cDNA fragment to a specific chromosome (42-46). The map positions of specific genes were further refined by using subchromosomal hybrids (38, 42). Furthermore, monochromosomal hybrids were used to transfer known chromosomes/ chromosomal regions to recipient cells to identify genes that complement mutant phenotypes related to cell growth and DNA repair. This proved to be a highly productive approach to characterize genes involved in cell senescence, tumor suppression and DNA repair, as described below.

Senescence Genes

The normal cells have a finite life span, which culminates into cessation of proliferation and acquisition of characteristic morphological changes. In contrast, cells cultured from many tumors can proliferate indefinitely. It was reasoned that these immortal cells arise by mutations in certain genes that under normal circumstances are responsible for senescence. If indeed mutant genes were to be present in tumor cells then complementation of these defective genes by chromosome transfer should restore the normal phenotype of the cell. In the absence of any knowledge about the identity of these genes, transfer of specific chromosomes using monochromosomal hybrids was a logical and an ideal choice to identify the complementing gene(s). In fact, chromosome transfer in immortal cells has led to restoration of senescence, and such chromosome transfer experiments have allowed identification of senescence loci on several different chromosomes. Chromosome transfer has been particularly useful for characterizing chromosomes 6 and 16 for their abilities to impart senescence to immortal cells (47). The confirmation of these observations has been obtained by restoring senescence of immortal cells upon introduction of a specific region of chromosome 6 (48, 49). These results are supported by observations indicating the loss of chromosome segments in SV40-transformed human fibroblasts (50). We have observed similar effects upon introduction of chromosome 16 into breast and ovarian tumor cells (51). Figure 6 shows senescent phenotype of immortal cells by the presence of senescence associated (SA) β-galactosidase activity in transformed cells containing a normal chromosome 16. Upon transfer of chromosome 16, the cells acquire characteristic enlarged morphology as shown in Figure 7.

It warrants mentioning that the region of chromosome 6 responsible for attributing senescence to transformed cells encodes RNaseT2 and GluR6 (52). We have shown that RNaseT2, contrary to other observations (53), by itself does not cause senescence, but modulates cell proliferation (52). Thus, GluR6 (52, 54) appears to be an attractive senescence
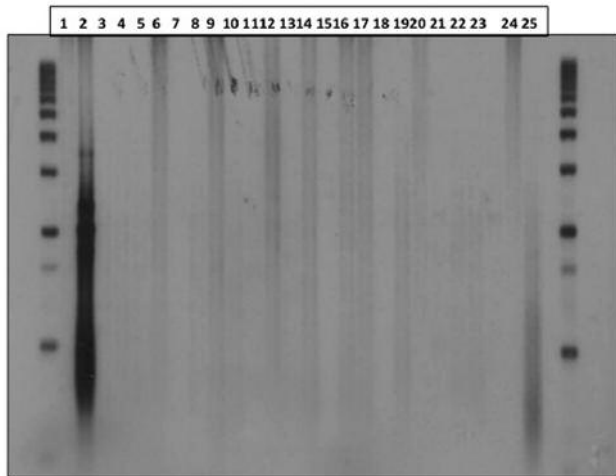
Figure 5. *Southern blot analysis of DNA from monochromosomal hybrids. Genomic DNA isolated from 24 different somatic cell hybrids was digested with a restriction enzyme and the digested DNA separated by electrophoresis on an agarose gel. The separated DNA was transferred from the gel to a nylon membrane. The membrane blot was hybridized to a mixture of 32P labeled chromosome 2 specific human DNA. The blot was washed and then exposed an X-ray film. Intense signal is visible in the lane containing the DNA isolated from the chromosome 2 hybrid. Lane 25 represents control human DNA.*

Figure 6. *Senescence associated (SA) β-galactosidase expression in senescent human breast cancer cells (MCF7) after transfer of a normal chromosome 16. Chromosome 16 was transferred to human cancer cells by microcell-mediated procedure. Panel A shows native cancer cells, and panels B and C show SA β-galactosidase expressing senescent cells after the transfer of chromosome 16.*

gene for further consideration. The confirmation of GluR6 involvement will be the first proof-of-principle demonstration of chromosome transfer strategy for the identification of senescence genes, which will have significant implications for investigating aging process. In addition to GluR6, we have also identified PTPRK as a putative cell senescence gene on chromosome 6 (unpublished observation).

Tumor/Growth Suppressor Genes

Aberrant regulation of cell growth and proliferation is implicated in the initiation, development and metastasis of human cancers. Growth suppression is an important arm of regulatory network for controlling cell proliferation. As previously discussed, chromosome transfer remains a valuable strategy for functional identification and positional cloning of genes involved in human cancers. The identification and cloning of RNAseT2 (52) represents an illustrative example of the application of chromosome transfer strategy. The modulation of cancer cell phenotypes following chromosome transfer (55-58) indicates the presence of responsible genes on specific chromosomes. Based on these observations, we believe that chromosome transfer is a productive approach for identifying genes involved in growth/tumor suppression.
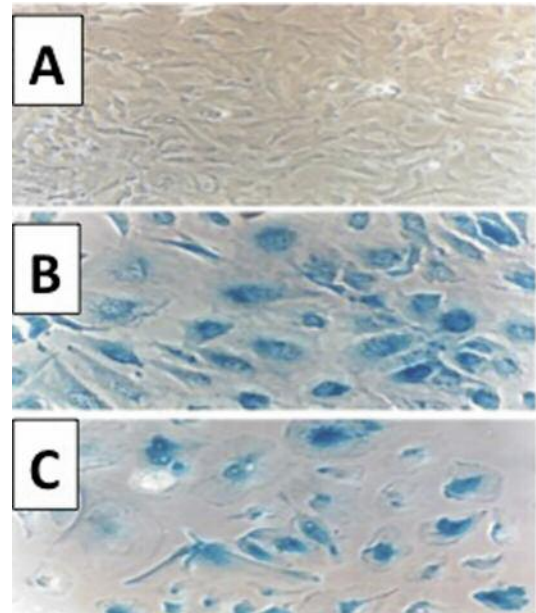

Figure 7. *Enlarged morphology of senescent cells. The picture shows enlarged senescent cells after introduction of a normal human chromosome 16 into MCF7 cells.*

DNA Repair Genes

Chromosome transfer strategy has also been successful for identifying DNA double strand break and excision repair genes. In particular, the complementation of genes defective in xeroderma pigmentosum (XP), an extensively investigated genetic defect, has been assigned to seven different

complementation groups XP-A through XP-G. While the genes for XP complementation groups B, C, A, E, G, F and D are located on chromosomes 2, 3, 9, 11, 13, 16 and 19, respectively, the double strand break repair genes are assigned to chromosomes 2 and 8. Functional complementation of these defects has been confirmed and some of these genes cloned by applying chromosome transfer technique (59-66).

Monochromosomal Hybrids and Genotoxicity of Environmental Chemicals

Monochromosomal hybrids have also been used for evaluating environmental chemicals for their ability to cause aneuploidy, chromosome breaks and gene mutations (67, 68). The experimental strategy involves exposure of monochromosomal hybrid cells to a test chemical followed by survival of cells in a selection medium. This selection process circumvents the need for tedious cytogenetic analysis for aneuploidy and chromosomal breaks (67, 68). This assay is amenable to automation by combining it with current devices available for cell and colony counting.

Future Directions

It is noteworthy that chromosome transfer technique is being improved for better efficiency and a variety of biomedical applications (69-74). In the post-genomic era, however, the focus of geneticists and molecular biologists is shifting from genes to proteins, an area termed "proteomics" (75). In addition to numerous other applications of monochromosomal hybrids, we propose a novel set of experiments aimed at isolating cell surface proteins encoded by different human chromosomes. Monochromosomal hybrids represent the fractionation of human genomic components i.e. individual human chromosomes present in rodent background. The proteins expressed in each hybrid will represent a mixture of mouse and human proteins encoded by a specific human chromosome. Cell surface proteins from hybrid cells can be used for raising antibodies that will subsequently be fractionated to isolate human specific antibodies. These proteins can then be sequenced by mass spectrometry, and the cell surface proteins corresponding to individual chromosomes can be exploited for diagnostic and therapeutic purposes against a variety of human diseases. This strategy can also be modified for isolating, characterizing and discovering miRNAs as well as long noncoding RNAs corresponding to individual human chromosomes. We recognize that it is a challenging task, but once accomplished it will be extremely useful.

In our opinion, chromosome transfer strategy has enormous promise for identifying tumor suppressor genes, and deciphering the involvement of senescent cells in modulating the phenotypes of malignant and premalignant cells. In tumor biology, the senescent cells may be akin to dormant cells, which influence the behavior of cancer cells. Senescence cells are implicated in enhancing inflammation associated with cancer progression (76), and these inflammatory responses may promote malignant transformation and suppress immune surveillance (77). These hypotheses can be tested by first converting tumor cells into senescent cells by introduction of specific chromosomes into tumor cells and then seeding these cells in a milieu of pre-malignant cells or malignant cells to observe the molecular, morphological and growth/proliferation changes in the target cells. Such experiments would also allow assessment of combinatorial effects of genes on cellular phenotypes.

Finally, cells engineered by chromosome transfer can lend themselves to investigating transcriptomic (mRNAs and miRNAs) and proteomic changes associated with cellular behaviors. With the advent of massively parallel sequencing strategies and rapid mass spectrometry, such experiments can yield a wealth of information on the molecular influence of intact normal chromosomes in genetically-defective cells. It should be noted that transfer of intact chromosomes is preferable than introduction of individual cDNA constructs. The transfer of intact chromosomes would allow assessing the effects of specific genes in the context of associated control regions of the genome. This would eliminate ectopic effects frequently arising due to random integration of cDNA constructs. Despite the availability of complete sequencing of the human genome and its coding complement, chromosome transfer strategy remains a valuable tool to validate the biological significance of coding as well as non-coding regions of the human genome.

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