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Somatic Cell Hybridization in Cytogenetic Analysis

For many years, human geneticists dreamed of finding a way to study segregation and recombination in somatic cells, in order to overcome the difficulties of genetic analysis with small families, few genetic markers, limited availability of meiotic material, and no experimental matings. Somatic cell genetics became a powerful way to do this, with the discovery of preferential loss (segregation) of human chromosomes in interspecific human-rodent somatic cell hybrids. The analysis of such hybrids has made it possible to correlate the loss or retention of a specific chromosome or chromosome segment with the loss or retention of a specific gene product or gene, allowing the gene to be mapped to that chromosome or segment. Enormous strides have been made using this approach. It is still one of the most widely used methods for mapping genes and for demonstrating multiple genetic causes of a disease phenotype (*genetic heterogeneity*). Somatic cell hybrids have many other uses in cytogenetic analysis. It is

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therefore helpful to understand somatic cell hybridization and how it is achieved experimentally.

Cell Fusion

Spontaneous fusion of cells occurs in the normal development of bone, muscle, and placental trophoblast tissues. Spontaneous fusion also occurs between cancer cells or between cancer cells and normal cells (Kovacs, 1985), but it is unclear whether cell fusion plays any role in the origin of the polyploid and aneuploid chromosome constitutions seen in cancer (Chapter 26). Spontaneous fusion of nonmalignant lymphocytes and fibroblasts has so far been observed only in Bloom syndrome (Chapter 24) (Otto and Therman, 1982). The mechanism of spontaneous cell fusion is still poorly understood.

The observation that inactivated parainfluenza (Sendai) virus can induce cell fusion, and the discovery that this can lead to mononucleated hybrid cells, has caused a revolution in cytogenetics and the rapid development of the field of somatic cell genetics (reviewed by Harris, 1995). Cell fusion is now induced mainly with polyethylene glycol (PEG), but electrofusion also works with some cell types (Cervenka and Camargo, 1987). Cell fusion is a widely used tool in complementation analysis (below and Chapter 24) and gene mapping (Chapter 29), and in the creation of hybridomas, lymphocyte hybrids that produce virtually limitless amounts of monospecific (*monoclonal*) antibodies. Their use has advanced many fields, including cytogenetics.

Complementation Analysis in Heterokaryons

When two cells fuse, the hybrid at first has two nuclei in a common cytoplasm. These *heterokaryons* can persist for some time, allowing various types of studies to be carried out. The one most important for cytogenetics is called *complementation analysis*. This is used to determine whether a particular abnormal recessive phenotype that is observed in two unrelated individuals is caused by a mutation in the same gene or by mutations in different genes. Cells cultured from one person are fused with cells from the other person to produce heterokaryons. These are then tested for the abnormal cellular phenotype. If this is still present, the same gene is mutant in the two individuals. However, if the phenotype is

now normal, each cell type has supplied the gene product missing in the other cell type; that is, each has complemented the other. This almost always means that different gene loci are mutant in the two individuals. (Very rarely, the mutations may involve the same gene locus but be so far apart that a mitotic recombination between them produces one normal gene copy and one that has both mutations.) This approach has been used to show that mutations in many different genes are responsible for each of several chromosome breakage syndromes (Chapter 24).

Premature Chromosome Condensation and Allocycly

When a cell whose chromosomes are in metaphase is fused with a cell in interphase, the nuclear membrane of the interphase nucleus breaks down (disassembles) and the chromosomes condense, a process called premature chromosome condensation (PCC). The condensation becomes visible some 15–20 min after the fusion and reaches its peak in 1 h (Rao, 1982; Sperling, 1982). The appearance of PCC depends on the stage of the cell cycle at the time of the fusion (Fig. 23.1). PCC chromosomes in G0 or early G1 cells are short, but become longer and thinner as G1 progresses (Fig. 23.1). PCC chromosomes in S-phase cells appear pulverized, or fragmented. However, even at this stage, the chromosomes are continuous; the gaps in them represent the segments that are replicating, as demonstrated by autoradiography after tritiated-thymidine incorporation (Fig. 23.2). PCC chromosomes in G2 cells resemble long, thin prophase chromosomes, which gradually shorten (Fig. 23.3). They can be banded with the usual techniques but sometimes appear spontaneously banded (Fig. 23.3 bottom). The spontaneous banding resembles replication banding. The late-replicating segments have not completed their condensation and remain stretched out.

PCC induced by cell fusion can be used to study chromosomes during interphase. Since interphase chromosomes are longer than mitotic chromosomes, their DNA and RNA synthesis patterns can be analyzed in greater detail (Sperling, 1982). PCC can be used to study the chromosomes of nondividing differentiated cells, such as neurons. PCC can be useful in studying chromosome breakage. With ordinary cytological techniques, chromosome breakage can be studied, at the earliest, several hours after its induction, whereas with PCC, breaks can be analyzed after only 20–30 min. This yields a better estimate of the

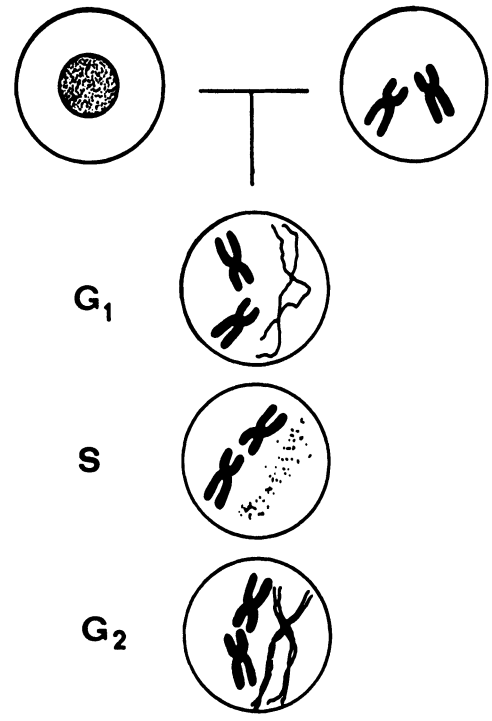

Figure 23.1. Fusion of an interphase and a metaphase cell leads to PCC formation. In G1, the PCC chromosomes are thin and single; in S, they appear "pulverized," in G2, they resemble prophase chromosomes.

original breakage rate, because breaks tend to be repaired. Analysis of PCC at different times after treatment demonstrates a gradual decrease of visible chromosome aberrations (Hittelman et al., 1980).

An otherwise normal metaphase plate may contain one or a few chromosomes that resemble PCC chromosomes in G1, S, or G2 (Figs. 20.1 and 23.2). Such *allocyclic* (out-of-phase) chromosomes are very rare in normal, untreated cells, although ring chromosomes are often allocyclic. The incidence of allocyclic chromosomes is increased by exogenous agents that break chromosomes (Rao, 1982) and in Bloom syndrome (Otto et al., 1981), which results from an endogenous chromosome-breaking genotype (Chapter 24). One hypothesis is that an allocyclic chromosome has undergone a mutation in a hypothetical condensation center that renders it unable to keep up with the rest of the chromosomes during mitosis and therefore forms a micronucleus (Otto et al., 1981). This might

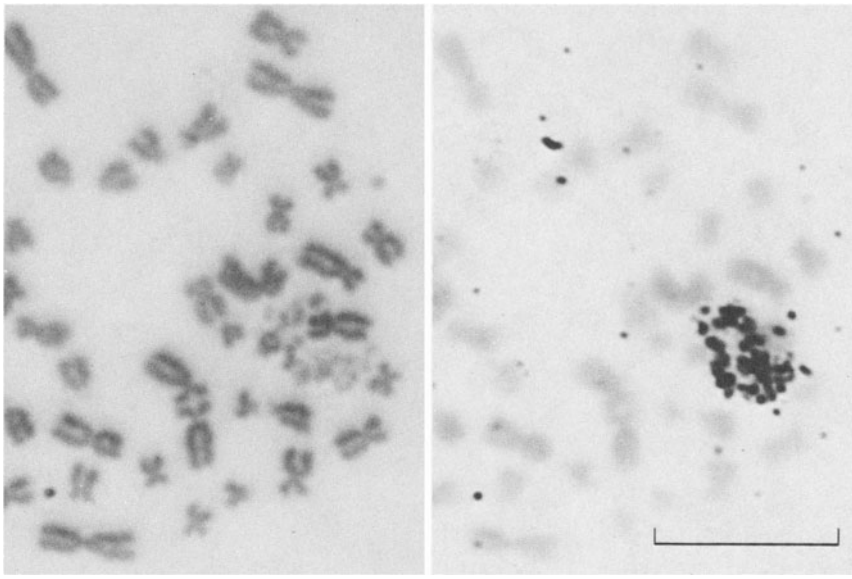

Figure 23.2. Part of a tetraploid lymphocyte metaphase from a patient with Bloom syndrome with an allocyclic chromosome before (left) and after (right) autoradiography. [^{3}H]Thymidine was added to the cell culture 4 hours before fixation. A mitotic chiasma is also present (Otto et al., 1981).

account for the correlated presence of micronuclei and allocyclic chromosomes (Obe and Beek, 1982).

Somatic Cell Hybrids

When both nuclei in a heterokaryon enter mitosis synchronously, they form a single mitotic spindle and distribute a complete set of chromosomes from each nucleus to each pole, producing identical *synkaryons*, or hybrid cells (Fig. 23.4). In order to study hybrid cells, one must be able to propagate selectively only those cells that contain chromosomes from both parents. Generally the human cells used in human-rodent cell mixtures have limited capacity for growth in culture; they will quickly be swamped out by the growth of the parental rodent cells and of the rodent-human hybrid cells. A selection procedure must be used to allow rodent-human hybrid cells to overgrow the rodent cells. The means used include culture media in which one cell type cannot grow or grows pref-

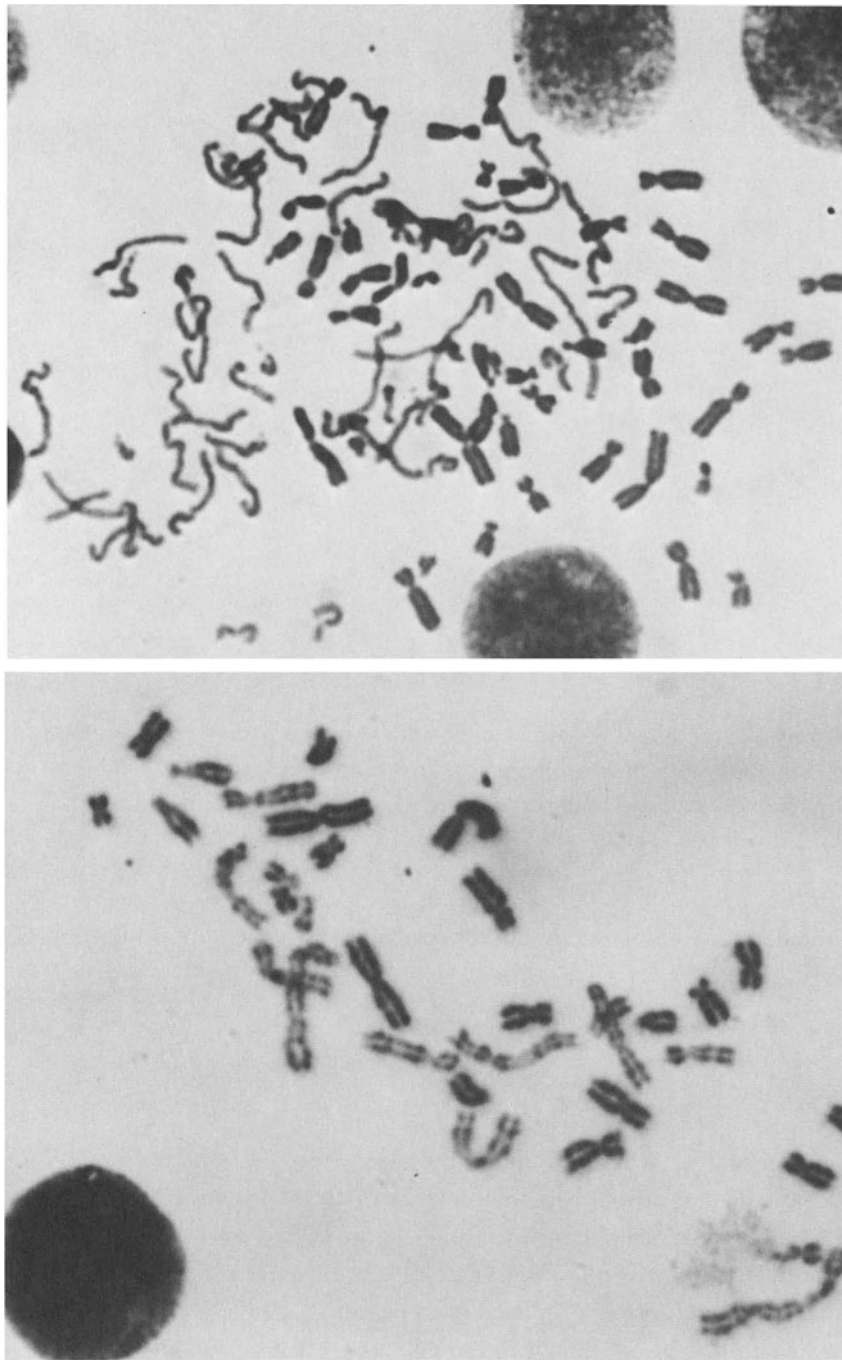

Figure 23.3. Spontaneous fusion of two Bloom syndrome lymphocytes leads to formation of prematurely condensed chromosomes (PCC). (Top) PCC in G1. (Bottom) Naturally banded PCC in G2 (Otto and Therman, 1982).

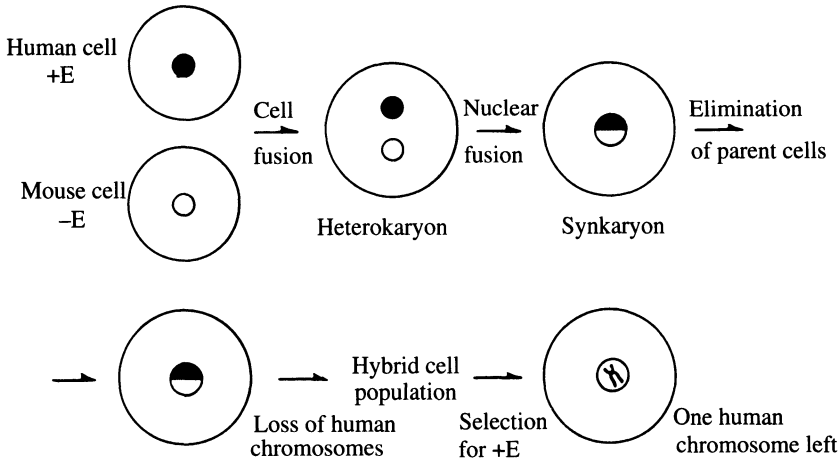

Figure 23.4. Assignment of a gene coding for the enzyme E to a specific human chromosome through fusion of a human cell (nucleus black) with a mouse cell (nucleus white) followed by hybrid cell selection.

erentially and selective killing of one cell type with toxins, antibodies, or viruses to which the other cells are resistant. Human cells can be killed with diphtheria toxin or the cardiac glycoside ouabain, to which the mouse and hybrid cells are resistant. In early studies, the rodent cell line usually had a recessive phenotype, such as a nutritional deficiency, that could be complemented by a wild-type human gene product. By growing the cells in culture medium devoid of the required nutrient, one could select against the parental rodent cells and allow selective growth of hybrid cells containing a specific human chromosome or chromosome segment carrying the gene.

Preferential Human Chromosome Loss from Rodent-Human Hybrids

Weiss and Green first pointed out the preferential loss of human chromosomes from human-mouse cell hybrids more than 30 years ago. Cell hybridization can be regarded as a parasexual process in which meiotic segregation is replaced by random chromosome loss. Neither the reason for nor the exact mechanism of the process of chromosome elimination is understood, but it plays an important role in gene mapping. A large number of human chromosomes are lost fairly

quickly and pretty much at random; a variable number of the remaining human chromosomes are lost more slowly. Selective retention of a specific human chromosome can be brought about by the use of a selectable marker. A mouse parental line that is deficient in either hypoxanthine-guanine phosphoribosyl transferase (HPRT) or thymidine kinase (TK) can be fused with normal human cells and the newly hybridized cells grown in hypoxanthine-amethopterin-thymidine (HAT) medium. These enzymes are required for cell survival in HAT medium. Only the hybrid cells that have retained a human chromosome carrying the corresponding functional gene (*HPRT* on the active X or *TK* on a chromosome 17, respectively) will survive. A number of selectable marker systems have been developed that enable one to select for the retention or the elimination of a specific chromosome. New sets of positive/negative selectable markers are continually being developed, some by introducing (*transfecting*) into a random chromosome a gene conferring resistance to neomycin, hygromycin, puromycin, or blasticidin S (Karreman, 1998). This permits selective retention or elimination of this chromosome from hybrid cells.

It would be useful if the human DNA in interspecific hybrid cells could be isolated. A novel way to do this is to clone the human DNA preferentially into yeast artificial chromosomes by a technique called *transformation-associated recombination cloning* (Larionov et al., 1996). This is achieved by cotransformation of yeast with (1) a linearized plasmid containing a human repeat sequence plus a yeast centromere and a yeast telomere and (2) a large fragment of DNA from the hybrid cell. Only fragments containing the same human repeat are able to recombine with the repeat in the linearized plasmid to form a circular yeast artificial chromosome (YAC). This method has been used for selectively cloning the human DNA in hybrids containing a single human chromosome and radiation hybrids containing only 5 Mb of human DNA (Larionov et al., 1996).

Although human chromosomes are preferentially lost from the commonly produced rodent-human hybrids, there are significant exceptions to this generalization. Croce discovered that fusion of cells of the established HT1080 human colon cancer cell line with freshly isolated murine diploid cells led to preferential loss of murine chromosomes. These hybrids, and the corresponding ones that preferentially lose human chromosomes, enabled Miller et al. (1976) to show that transcription of both human and mouse ribosomal RNA genes is dependent upon a species-specific transcription factor not carried on an acrocentric chromosome. Marcus et al. (1976) produced one human-mouse hybrid that accumulated multiple copies of the human Y chromosome despite losing almost all the other human chromosomes. This hybrid was instrumental in the

initial cloning of single-copy DNA sequences from the Y chromosome (Bishop et al., 1983).

Induced Chromosome Breakage: Radiation Hybrids

At least one of the parental cell types used in generating most human-rodent hybrids is malignant. The structural chromosome instability of malignant cells, which is associated with defective DNA damage checkpoints (Chapter 26), continues in the hybrid cells and is probably responsible for the spontaneous breakage that has long been observed in these hybrids. Induced chromosome breakage is a much more useful mapping tool. Massive chromosome-shattering doses of ionizing radiation are administered to human cells just prior to fusion with rodent cells (Goss and Harris, 1977) or to hybrid cells containing a single human chromosome (Cox et al., 1990) before fusion with rodent cells to produce hybrids containing tiny human chromosome fragments. The former method is useful for mapping any chromosome, the latter for mapping a single chromosome. For general chromosome mapping, a radiation dose of 10,000 rads (100 Gy) is satisfactory, giving fragment sizes from 2 Mb to entire chromosome arms. For high-resolution mapping or positional cloning, higher doses are preferred; 25,000 rads (250 Gy) yields DNA fragments that are mostly shorter than 3 Mb (Siden et al., 1992).

Microcell Hybrids

Selectable markers are useful in constructing chromosome-specific microcell hybrids. The neomycin resistance gene, *neo*, is a very useful dominant selectable marker. It can be introduced into diploid human fibroblasts using a retroviral vector and will integrate at random into any chromosome. These fibroblasts can then be converted into microcells that are fused to rodent cells to make microcell hybrids. By selecting those that are resistant to neomycin, one obtains microcell hybrids with a specific human chromosome containing the *neo* gene (Lugo et al., 1987). Using a series of seven such hybrids, each containing a different human chromosome, Ning et al. (1993) showed that PCR amplication of human sequences in the hybrids, using a LINE1 oligonucleotide primer, produced such a distinctive pattern of DNA fragment sizes for each chromosome that it con-

stituted a "PCR karyotype." This can be used to check the human chromosomes present in other hybrids.

The hygromycin phosphotransferase gene confers resistance to hygromycin and sensitivity to gancyclovir. Speevak et al. (1995) introduced this gene into random chromosome sites in human diploid fibroblasts, which were then used to generate microcell hybrids containing a single human chromosome. Growth of these hybrids in hygromycin (and confirmation by gancyclovir killing) enabled them to isolate a series of chromosomes containing this dually selectable marker gene.

Chromosome and Gene Transfer: Transgenomes and Transgenes

Cells in culture have the ability to take up whole chromosomes or fragments of chromosomes from the medium and incorporate them into their genomes. More important is their ability to undergo transfection, incorporating much smaller DNA fragments, ranging in size from YACs hundreds of kb in size to single genes (*transfection*). The introduction of single chromosomes or chromosome fragments has been instrumental in the analysis of tumor suppressor genes (Chapter 28). For example, the transfer of a normal chromosome 5 into some colorectal carcinoma cells inhibits the overexpression of the c-*MYC* gene that is characteristic of these tumors and abolishes their tumorigenicity. This indicates that disruption or mutation of a gene on chromosome 5 is important in colon carcinogenesis (Rodriguez-Alfageme et al., 1992). Introduction of a transgenic construct containing the catalytic subunit of the telomerase gene into human retinal epithelial cells or skin fibroblasts prevented their senescence, enabling the cells to continue to divide (Bodnar et al., 1998).

Homologous recombination is the mechanism involved in targeting genes to cells in culture. This leads to the replacement of a defective gene by a normal copy or vice versa. The chicken pre–B cell line DT40 is highly proficient for homologous recombination, whereas most human cells are poor at it. Koi et al. (1997) used microcell-mediated cell fusion to transfer single human chromosomes tagged with *pSVneo* as a selectable marker into DT40 cells. They found that targeted integration of various gene constructs into their homologous loci on the human chromosome then occurred at high frequency. This system could be very useful for

mapping and cloning genes and studying their function, and as a source of specific gene products. Gene targeting can be used to study the function of some of the large number of genes important for chromosome structure and function. For example, the p21 protein product of the cell cycle inhibitor gene *CIP1/WAF1* (Chapter 2) was shown to block cell senescence in diploid human fibroblasts, permitting their unlimited growth. To show this, the gene was inactivated by two rounds of targeted homologous recombination (Brown et al., 1997).

Somatic cell genetic approaches to other problems continue to appear. A method has been developed for preparing monosomic cell lines. The *gpt* plasmid is introduced into cultured diploid cells and allowed to integrate at random into various sites. The cells are grown with selection against retention of *gpt* and with partial inhibition of topoisomerase II to promote chromatid nondisjunction during mitosis. This has led to cell lines that are monosomic for chromosome 4, 8, or 21 and to several lines with partial monosomies or deletions (Clarke et al., 1998).

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