Cross Contamination of the Genomes in Human/Hamster Cell Hybrids by Multiple Short Recombination Events

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Abstract—We have isolated sites of de novo rearrangements from interspecific cell hybrids. Of 147,000 clones screened from a human/hamster hybrid genomic library, 14 clones were found with homology to both human and hamster repetitive DNA sequences. Five of these clones contained recombination events involving less than 13 kb of DNA, three with human DNA recombined into a section of hamster DNA, and two with hamster DNA recombined into human DNA. None of the clones involving human L1 sequences were found to be de novo transposition events, but simply short recombination or insertion events. Considering the apparent random nature of these events, they are likely to involve unique as well as repetitive sequences and also involve integration into the homologous as well as heterologous chromosome sets. These results suggest that the chromosome sets in somatic cell hybrids may be randomly contaminated with small DNA segments derived from either set of chromosomes.

INTRODUCTION

Somatic cell hybrids are widely used for isolation of genes from specific chromosomes and also for mapping genes to specific chromosomes in mammalian cells. The use of cell hybrids in assigning genes to chromosome positions requires that the human DNA content in the hybrid cell be well defined. However, interspecies somatic cell hybrids constitute a system in which there is enough "genomic shock" to induce chromosome translocations between the two sets of chromosomes and also possibly activate transposable element movement (1). This may compromise the accurate delineation of the human DNA content.

Cytological studies of the chromosomes of mammalian interspecies cell hybrids have shown many examples of large scale translocations and rearrangements (1, 2). Continuing chromosome loss is typically seen in interspecies hybrids after fusion. Specifically, in human/hamster hybrid cells, the human chromosomes are lost preferentially. It has also been suggested that some of the events occurring after cell fusion might be explained by the movement of transposable elements (1). This suggests that interspecies somatic cell hybrids may also be a useful model system for studying genomic rearrangements.

The experiments described here employed two human/hamster somatic cell hybrids, H2 and H4, derived by polyethylene glycol-induced fusion between the CHO-K1 and HT1080 cell lines. By in-situ hybridization using total human DNA as a probe, the H2 and H4 hybrids were found to contain...
four and twelve complete human chromosomes respectively (3). Hybridization of the human genomic probe was also found at a number of sites within the hamster chromosomes as well as to the intact human chromosomes. In addition to translocations between the ends of human and hamster chromosomes, some of the hamster chromosomes also appeared to contain small regions of integrated human DNA. Although the in-situ hybridization method used means that the smallest regions of human DNA that could be detected would be likely to be many megabases in size, we hypothesized that, potentially, very small regions of human DNA might also have rearranged in the hybrid DNA, either transposing between, or recombining into, the hamster chromosome set.

In this work, we aimed to isolate sites of de novo transposition or sites of recombination in mammalian hybrids. Interspersed repetitive elements in human and hamster genomes have diverged sufficiently in sequence for them not to cross-hybridize under moderately stringent conditions. We have used this limited homology between repetitive elements from different mammalian species to identify and isolate recombinant clones from interspecies somatic cell hybrids. These clones could be derived either from sites of recombination between the human and hamster chromosomes or from sites of de novo transposition events.

L1 sequences constitute the major family of long interspersed repetitive elements (LINEs) in the mammalian genome. They make up 5–10% of the genome. Hamster L1 elements are ~70% homologous to human L1 elements. All of this homology is within the two conserved open reading frames of the elements, while the 5' and 3' ends of mammalian L1 elements are totally species specific (4). The Alu sequences comprise the major family of short interspersed repeat DNAs (SINEs) in humans. On average, there would be one element every 4 kb, although the actual figure appears to vary considerably, as some regions are enriched for Alu sequences, while others are deficient (5). The B1 and B2 families are the two major families of short interspersed repeats in mice and other rodents (6). The B1 element has some sequence homology to the Alu element while the B2 element used in this study is another SINE, but shares no actual sequence homology. The use of probes enriched for dispersed repetitive elements is likely to improve the efficiency of isolation of recombination/insertion events since repetitive elements appear to be preferred sites of integration and illegitimate recombination (7, 8).

Hence, by selecting for recombinant clones containing both human and hamster repetitive sequences in a single λ clone from a genomic library of interspecific cell lines, we have been able to isolate the sites of breakpoints of a translocation or recombination events occurring between the two sets of chromosomes.

MATERIALS AND METHODS

Cell Culture and Library Construction. Two human/hamster hybrid cell lines (H2 and H4) and the two parental cell lines from which the hybrids were derived (HT1080, human and CHO-K1, Chinese hamster), were provided by R. Anderson and A. Giaccia (9). Cell lines were maintained in the α modification of Eagle's Medium +10% newborn bovine serum (Cytosystems). To extract DNA, cells were pelleted, then resuspended in cell lysis buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 10 mM EDTA, with Proteinase K at 100 μg/ml and 1% SDS). After incubation at 55°C overnight, DNA was extracted twice with phenol/chloroform, then treated with RNase A (50 μg/ml) and RNase T1 (50 units/ml) then Proteinase K (10 μg/ml). The DNA was phenol/chloroform extracted again, then ethanol precipi-