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Rapid and Precise Identification of Human Materials Using *Alu*-L1 PCR-FISH in Somatic Cell Hybrids and Establishment of Human Chromosome Mapping Panel

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We have tried to establish a chromosome mapping panel for the assignment of genes and DNA sequences on specific human chromosomes. The chromosome mapping panel was constructed by a series of HAT^r fusion hybrid clones that retain different sets of human chromosomes on the chinese hamster background. The HAT^r clones were obtained by the fusion of normal human male lymphocytes with a HPRT-deficient chinese hamster cell line, Wg3h and analyzed by the Q-banding technique for the identification of human chromosomes retained in them. In addition, we tried to exclude a possibility that the clones may contain human fragments and/or rearranged chromosomes that could not be precisely identified by the conventional Q-banding analysis. That is, we amplified human-specific DNA sequences in the man-chinese hamster hybrids by polymerase chain reaction (PCR) using primers directed to the human *Alu* and L1 consensus sequences. The PCR-amplified DNAs were biotin-labelled by additional PCR using bio-11-dUTP or by nick translation, and then were hybridized to nomal human male metaphases. So far, we have examined 24 hybrid clones using the fluorescence in situ hybridization (FISH) technique and have characterized previously unidentified chromosomal materials. Human genetic resources such as the chromosome mapping panel and the man-chinese hamster hybrids containing specific human chromosomes, which have been generated through this experiment, allow rapid mapping of human genes, and the interspersed repetitive sequence-PCR (IRS-PCR) method makes it possible to rapidly and precisely identify human materials in man-rodent hybrids.

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Status-transition of X chromosome in somatic cell-OTF9-63 hybrids

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We have established 6-thioguanine(6-TG) resistant primary and immortalized cells(genotypically $Hprt^{b\text{-}m3/a}$, $Pgk\text{-}1^{a/b}$) that undergo X chromosome reactivation when they are fused with OTF9-63(OTF9). All resulting HAT^r fusion hybrids were EC cell-like in their morphology. Synchronous patterns of X chromosome replication were uniformly observed and the expression of alleles *Hprt* and *Pgk-1* carried on the inactive X chromosome were demonstrated. The reactivation of *Hprt* was accompanied by demethylation of 5′ CpG island sequences of *Hprt*. In addition, global demethylation of genomic DNA was observed in L1 and minor satellite sequences. These results demonstrate that OTF9-somatic cell fusion hybrids(OTF9 hybrids) retain the cytogenetic, biochemical and molecular characteristics of EC cells. In long term culture, OTF9 hybrids tended to reduce the number of X chromosome resulting in 1:2 ratio of X chromosomes to autosomes which parallels the results observed with EC stem cells. Finally, we examined *Xist* expression in OTF9 hybrids after the treatment of retinoic acid(RA). OTF9 hybrids express *Xist* alleles suggesting that previously active one or more X chromosomes retained in OTF9 hybrids should be inactivated. RFLP analysis of RT-PCR products of *Xist* alleles confirmed that the *Xist* transcripts were derived from previously active X chromosome. Thus, the use of the OTF9 hybrids provides an experimental system for studying the mechanisms of the X-reactivation and X-inactivation that occur in meiotic prophase of oogenesis and in early embryogenesis, respectively.