

NOTES

Homologous Recombination between Coinjected DNA Sequences Peaks in Early to Mid-S Phase

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Received 5 January 1987/Accepted 6 March 1987

We have examined the effect of cell cycle position on homologous recombination between plasmid molecules coinjected into synchronized rat fibroblasts. Recombination activity was found to be low in G₁ and to rise 10- to 15-fold, peaking in early to mid-S phase.

The ability of somatic mammalian cells to efficiently mediate homologous recombination between newly introduced DNA sequences (2, 3, 7, 8, 10, 11, 14) has stimulated great interest in the potential for gene targeting, i.e., homologous recombination between a newly introduced DNA sequence and its cognate chromosomal sequence (6, 12, 13, 15, 16). To increase the targeting frequency, it is important to identify factors, intrinsic and extrinsic, that influence the efficiency of homologous versus nonhomologous recombination. In this study, we examined the ability of the cell to mediate homologous recombination between newly introduced DNA sequences as a function of the stage in the cell cycle. A peak of homologous recombination activity was observed in early to mid-S phase. Previously, we reported that random insertion of a linear exogenous DNA sequence into the mammalian genome, a process which presumably involves nonhomologous recombination, was insensitive to the position of the cells in the cell cycle (17).

To measure the homologous recombination events, we used a transient expression assay for a functional hamster adenine phosphoribosyltransferase (*aprt*) gene (18). The assay is rapid and permits identification of recombination events in individual cells. The nonoverlapping deletion mutations of the *aprt* gene used for these assays are illustrated in Fig. 1. Plasmid p141Δ3'*aprt* contains only 5' gene information including the first three exons and introns, while plasmid pBR322Δ5'*aprt* contains *aprt* sequences excluding the 5' end of the first exon. To regenerate a functional gene, recombination must occur within the common 1,500-base-pair *Pst*I-to-*Pvu*II fragment of *aprt*. As expected, we have never observed a spontaneous reversion of these deletion mutations to the *aprt*⁺ phenotype.

Previously, we and others (1, 2, 4, 5, 9, 18) showed that recombination between cotransferred plasmids was maximal when the plasmids were introduced as linear molecules, with the site of cleavage within the common sequences resulting in the greatest stimulation of activity. Therefore, before microinjection, plasmid p141Δ3'*aprt* was linearized at the unique *Xho*I site (within exon 3 of *aprt*) and plasmid pBR322Δ5'*aprt* was linearized at the *Hind*III site (the junction of pBR322 and *aprt* sequences). In an asynchronous

population of Rat-20 cells, this combination of linear plasmids yields a recombination frequency of one recombination event per 100 cells injected (18).

Rat-20 cells, *aprt*⁻ derivatives of Rat-2 cells, were synchronized by mitotic shakeoff as previously described (17). The reversion frequency of Rat-20 cells is very low; we have never obtained a spontaneous *aprt*⁺ revertant of these cells. Therefore, the background level of *aprt* activity is negligible. The 5' and 3' deletion plasmids were coinjected at a concentration of 10 molecules each per cell nucleus at 3-hour intervals after reattachment of mitotic cells to the glass cover slips. Immediately after microinjection, cells were incubated for 24 h with [³H]adenine (10 μCi/ml, 10 mCi/mmol), washed with phosphate-buffered saline, fixed with glutaraldehyde, and dipped in NTB-2 nuclear track emulsion (Eastman Kodak Co.). After 3 to 4 days at 4°C, the cover slips were developed with Kodak Dektol. A precise recombination event was identified by the deposition of silver grains over the cells. On separate cover slips, cell synchrony was monitored autoradiographically after a 40-min pulse-label with [³H]deoxycytidine (10 μCi/ml, 28 Ci/mmol). Homologous recombination activity was initially low in early G₁, rose 10- to 15-fold by early to mid-S phase, and declined again as the cells traversed S phase and reentered G₁ (Fig. 2).

We have shown in a previous communication that expression of *aprt* activity from a plasmid encoding an intact *aprt* gene was similar after injections at all cell cycle stages (17). This observation rules out the possibilities that the cell cycle-dependent recombination events are due to either poor

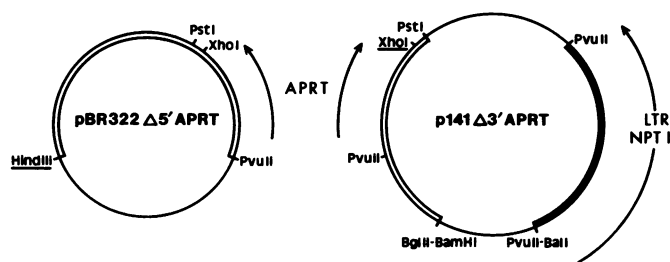


FIG. 1. Substrates for homologous recombination. Plasmids pBR322Δ5'*aprt* and p141Δ3'*aprt* were constructed as described previously (18). Plasmids were linearized at the underlined restriction enzyme recognition sites (*Hind*III or *Xho*I) before coinjection. LTR, Long terminal repeat; NPT II, neomycin phosphotransferase.

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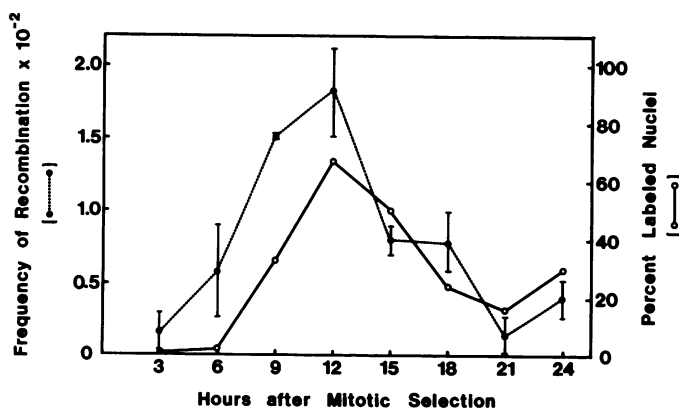


FIG. 2. Homologous recombination activity throughout the cell cycle. Cell synchrony and the frequency of recombination were assayed autoradiographically with Kodak NTB-2 nuclear track emulsion as described in the text. A total of 400 to 450 cells received injections at each time point. Each point represents the average of three experiments \pm standard deviation.

aprt expression in G₁ versus S phase or differential degradation of the substrates at various cell cycle stages.

As previously mentioned, we have shown that DNA-mediated transformation of Rat-20 cells with linear substrates was independent of the cell cycle, from which we inferred that the nonhomologous recombination machinery, which mediates random chromosomal integration, likewise was cell cycle independent (17). This observation, combined with the results of this study, suggests that early S phase is a period in the cell cycle when the ratio of homologous to nonhomologous recombination activity is the highest. These observations further indicate that the cellular machineries mediating homologous and nonhomologous recombination are not completely overlapping and therefore should be susceptible to differential inactivation as well as activation.

This work was supported by Public Health Service grant F32 GM09678 from the National Institutes of Health.

LITERATURE CITED

- Brenner, D. A., A. C. Smigocki, and R. D. Camerini-Otero. 1985. Effect of insertions, deletions, and double-strand breaks on homologous recombination in mouse L cells. *Mol. Cell. Biol.* 5:684-691.
- Folger, K. R., K. Thomas, and M. R. Capecchi. 1985. Nonreciprocal exchanges of information between DNA duplexes co-injected into mammalian cell nuclei. *Mol. Cell. Biol.* 5:59-69.
- Folger, K. R., E. A. Wong, G. Wahl, and M. R. Capecchi. 1982. Patterns of integration of DNA microinjected into cultured mammalian cells: evidence for homologous recombination between injected plasmid DNA molecules. *Mol. Cell. Biol.* 2:1372-1387.
- Kucherlapati, R. S., E. M. Eves, K.-Y. Song, B. S. Morse, and O. Smithies. 1984. Homologous recombination between plasmids in mammalian cells can be enhanced by treatment of input DNA. *Proc. Natl. Acad. Sci. USA* 81:3153-3157.
- Lin, F.-L., K. Sperle, and N. Sternberg. 1984. Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. *Mol. Cell. Biol.* 4:1020-1034.
- Lin, F.-L., K. Sperle, and N. Sternberg. 1985. Recombination in mouse L cells between DNA introduced into cells and homologous chromosomal sequences. *Proc. Natl. Acad. Sci. USA* 82:1391-1395.
- Miller, C. K., and H. M. Temin. 1983. High efficiency ligation and recombination of DNA fragments by vertebrate cells. *Science* 220:606-609.
- Robert de Saint Vincent, B., and G. M. Wahl. 1983. Homologous recombination in mammalian cells mediates formation of a functional gene from two overlapping gene fragments. *Proc. Natl. Acad. Sci. USA* 80:2002-2006.
- Rubnitz, J., and S. Subramani. 1985. Rapid assay for extrachromosomal homologous recombination in monkey cells. *Mol. Cell. Biol.* 5:529-537.
- Shapira, G., J. L. Stachelek, A. Letsou, L. K. Soodak, and R. M. Liskay. 1983. Novel use of synthetic oligonucleotide insertion mutants for the study of homologous recombination in mammalian cells. *Proc. Natl. Acad. Sci. USA* 80:4827-4831.
- Small, J., and G. Scangos. 1983. Recombination during gene transfer into mouse cells can restore the function of deleted genes. *Science* 219:174-176.
- Smith, A. J. H., and P. Berg. 1984. Homologous recombination between defective neo genes in mouse 3T6 cells. *Cold Spring Harbor Symp. Quant. Biol.* 49:171-181.
- Smithies, O., R. G. Gregg, S. S. Boggs, M. A. Koralewski, and R. S. Kucherlapati. 1985. Insertion of DNA sequences into the human chromosomal β -globin locus by homologous recombination. *Nature (London)* 317:230-234.
- Subramani, S., and P. Berg. 1983. Homologous and nonhomologous recombination in monkey cells. *Mol. Cell. Biol.* 3:1040-1052.
- Thomas, K. R., and M. R. Capecchi. 1986. Introduction of homologous DNA sequences into mammalian cells induces mutations in the cognate gene. *Nature (London)* 324:34-38.
- Thomas, K. R., K. R. Folger, and M. R. Capecchi. 1986. High frequency targeting of genes to specific sites in the mammalian genome. *Cell* 44:419-428.
- Wong, E. A., and M. R. Capecchi. 1985. Effect of cell cycle position on transformation by microinjection. *Somatic Cell Mol. Genet.* 11:43-51.
- Wong, E. A., and M. R. Capecchi. 1986. Analysis of homologous recombination in cultured mammalian cells in transient expression and stable transformation assays. *Somatic Cell Mol. Genet.* 12:63-72.