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NOTES

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

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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 G_1 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 *Hin*dIII 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 [^{3}H]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 [^{3}H]deoxycytidine (10 μCi/ml, 28 Ci/mmol). Homologous recombination activity was initially low in early G_1, rose 10- to 15-fold by early to mid-S phase, and declined again as the cells traversed S phase and reentered G_1 (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

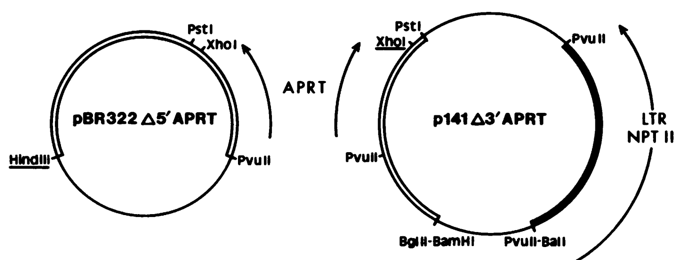

FIG. 1. Substrates for homologous recombination. Plasmids pBR322Δ5′aprt and p141Δ3′aprt were constructed as described previously (18). Plasmids were linearized at the underlined restriction enzyme recognition sites (*Hin*dIII or *Xho*I) before coinjection. LTR, Long terminal repeat; NPT II, neomycin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

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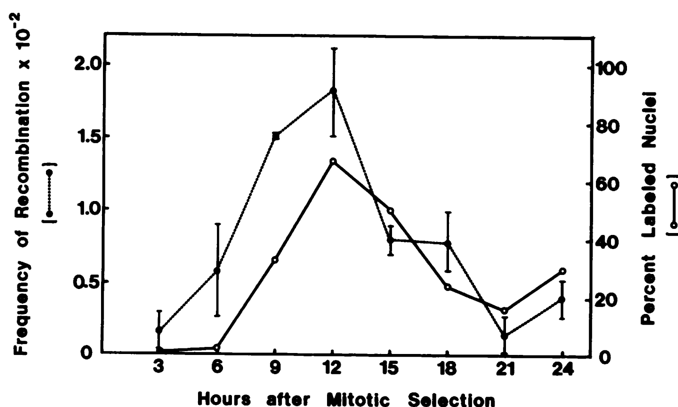

FIG. 2. Homologous recombination activity throughout the cell cycle. Cell cynchrony and the frequency of recombination were assayed autoradiographically with Kodak NTB-2 nuclear track emulsion as described in the text. A total of 400 to 450 cells received injections at each time point. Each point represents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

aprt expression in G_1 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.

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