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Interchromosomal recombination is suppressed in mammalian somatic cells

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Homologous recombination occurs intrachromosomally as well as interchromosomally, both in mitotic (somatic) cells as well as meiotically in the germline. These different processes can serve very different purposes in maintaining the integrity of the organism and in enhancing diversity in the species. As shown here, comparison of the frequencies of intra- and interchromosomal recombination in meiotic and mitotic cells of both mouse and yeast argues that interchromosomal recombination is particularly low in mitotic cells of metazoan organisms. This result in turn suggests that the recombination machinery of metazoa might be organized to avoid the deleterious effects of homozygotization in somatic cells while still deriving the benefits of species diversification and of DNA repair.

Keywords: interchromosomal recombination/intrachromosomal recombination/mammalian somatic cells

Introduction

Homologous recombination brings both benefits and burdens to the organism and to the species. Interchromosomal recombination can increase diversity and thus the robustness of the species. Intrachromosomal recombination, such as between sister chromatids, as well as interchromosomal recombination, can correct errors of replication and other damage. For single celled organisms interchromosomal recombination is equally beneficial whether it occurs in association with meiosis or prior to meiosis in vegetatively growing cells. In contrast, recombination of alleles in the somatic cells of a multicellular organism confers no corresponding advantage and can, in fact, be deleterious. For example, recombination between homologous chromosomes has been implicated as a mechanism of homozygotization of recessive oncogenes seen in some human cancers (Lasko *et al.*, 1991). Also, recombination involving repetitive sequences on heterologous chromosomes can result in gene disruption or dysregulation. The well-designed metazoan might therefore be expected to suppress interchromosomal recombination in its somatic cells, while maintaining their capacity for intrachromosomal recombination.

This expectation can be tested by comparing the rates of inter- and intrachromosomal recombination in meiotic and mitotic cells of both complex and simple eukaryotes. In order to obtain a direct comparison of intra- and interchromosomal recombination for the same locus and somatic cell type, we have measured recombination for the immunoglobulin heavy chain (IgH) locus of a mouse hybridoma cell line. Our results indicate that both interchromosomal and ectopic recombination are ~10^4-fold less frequent than observed previously for intrachromosomal recombination for this same locus and cell line (Baker, 1989; Bautista and Shulman, 1993). This 10^{-4} ratio of inter- to intrachromosomal recombination in the hybridoma cell line is ~1500-fold less than has been found for mouse spermatids (Murti *et al.*, 1992, 1994) and 500-fold less than for yeast mitotic cells (Lichten and Haber, 1989; Haber *et al.*, 1991; Petes *et al.*, 1991); it is similar to that reported for transgene recombination in CHO cells (Godwin *et al.*, 1994). These comparisons are therefore consistent with the hypothesis that interchromosomal recombination is particularly suppressed in somatic cells of metazoan organisms. As well, comparison of the frequencies of allelic and ectopic recombination implies that interchromosomal recombination in somatic cells is not facilitated by the long regions of identity which are shared by homologous chromosomes.

Results

The system which we have used in this investigation is based on the mouse hybridoma cell line Sp6, which secretes IgM(κ) specific for the hapten trinitrophenyl (TNP) and makes use of two Sp6-derived mutant cell lines, N114, which bears a TGA termination codon in the heavy chain variable (V_HTNP) region, and igm482, which has a 2 bp deletion in the μ constant (Cμ) region (Figure 1A). These markers are separated by ~10 kb in the natural IgH locus. Individual wild-type Sp6 cells make plaques on TNP-coupled erythrocytes, whereas the mutants do not. Fusion of these mutant cell lines generates a hybrid cell in which interchromosomal recombination between the N114 and igm482 mutant alleles can be assayed by the appearance of plaque forming cells (PFC). Similarly, transfection of the 4.3 kb Sp6-derived wild-type Cμ segment into the igm482 cell line creates transformants in which recombination between the ectopic wild-type Cμ segment and the endogenous μ gene can yield PFC (Figure 1B). For comparison Figure 1C depicts the IgH locus in hybridoma cell lines which were used previously to measure intrachromosomal recombination between the Cμ segments (Baker, 1989; Baker and Read, 1995).

Measurement of interchromosomal allelic recombination

As described in Materials and methods, two hybrid cell lines, H5 and H9, bearing both the N114 and igm482

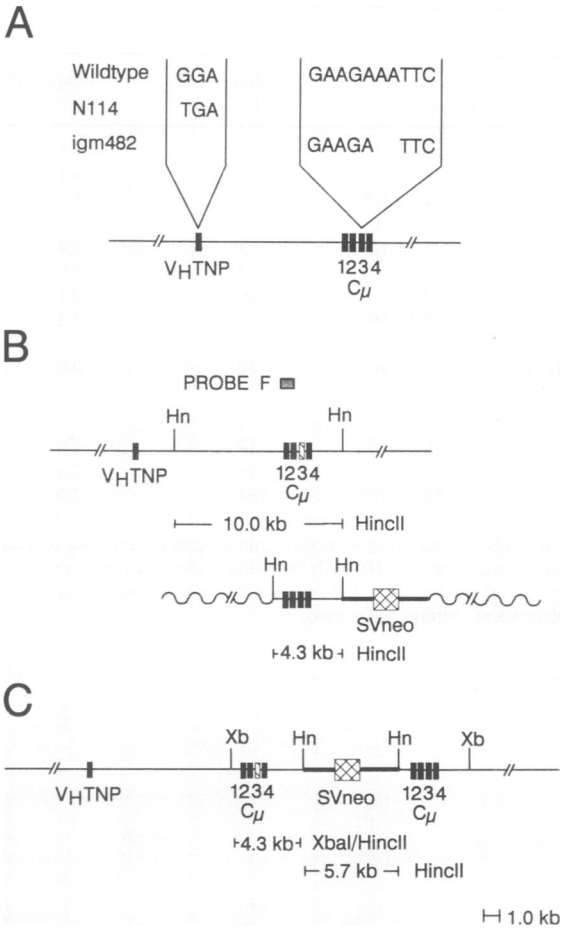

Fig. 1. System for measuring interchromosomal recombination. (**A**) Interchromosomal allelic recombination. This diagram indicates the mutations in μ genes in the N114 and igm482 cell lines. The mutant and normal genes are distinguished by their sensitivity to the enzyme *Xmn*I, which cuts the sequence GAAXXXXTTC. (**B**) Ectopic recombination. This diagram depicts the endogenous μ gene of the igm482 mutant hybridoma cell line and the ectopic wild-type Cμ segment. (**C**) Intrachromosomal recombination. This diagram depicts the recombinant locus in which the wild-type Cμ segment has been inserted into the endogenous μ gene of the igm482 mutant hybridoma cell line. In both (B) and (C) the mutant igm482 Cμ3 exon is denoted by the hatched rectangle. The Cμ specific probe F is an 870 bp *Xba*I–*Bam*HI fragment.

alleles were generated by fusion. The PFC frequencies for H5 and H9 were 12.5×10^{-8}/cell and 16.2×10^{-8}/cell respectively. To estimate the reversion frequencies of the mutant alleles in the hybrid cells, we first measured the frequency of PFC in cultures of each of the parent cell lines. Inasmuch as the hybrid cells contain both mutant alleles, their expected reversion frequency is the sum of the reversion frequencies of N114 and igm482, 4.7×10^{-8}/cell, which is significantly lower than the PFC frequency in the hybrid cell lines ($P < 0.001$, χ^2 test).

Out of concern that the reversion frequency might be higher in the hybrid than in the parental mutant cells, we measured the frequency of PFC in hybrid cell lines which retained only one of the two mutant μ alleles. To obtain such derivatives, we plated the H5 and H9 cell lines at limiting dilution and assayed the genotype of the μ alleles of individual subclones, taking advantage of the fact that the igm482 mutation destroys an *Xmn*I site in the Cμ3

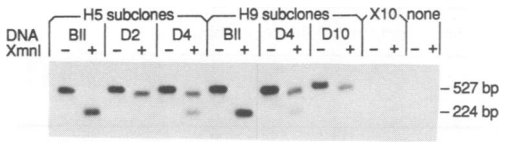

Fig. 2. Analysis of μ alleles in H5 and H9 subclones. Genomic DNA from the indicated subclones was isolated and amplified by PCR using primer oligonucleotides 1 and 2, which flank the igm482 mutation. As indicated, the PCR product was either left intact or digested with *Xmn*I and then fractionated by electrophoresis and probed with an internal oligonucleotide (3) which detects the uncut DNA (527 bp) and one of the digestion products (224 bp). Control lanes using no DNA (none) and DNA from a mutant cell line lacking the μ gene (X10) are indicated.

exon. Subclones which retained either the N114 allele (H5-B11 and H9-B11), the igm482 allele (H5-D2 and H9-D10) or both alleles (H5-D4 and H9-D4) are shown in Figure 2. As indicated in Table I, the PFC frequency for the single allele subclones was comparable with the N114 and igm482 parental cell lines, again approximately one third of the frequency obtained for the subclones which retained both alleles. This table also presents the aggregate of these measurements in which the PFC frequencies for each single allele cell line have been summed and compared with the total PFC frequency for all hybrid cells containing both μ alleles. The excess PFC found in cultures of cells containing both μ alleles occurred at a frequency of 9×10^{-8}/cell [= 12.9×10^{-8}/cell – (2.6×10^{-8}/cell + 1.5×10^{-8}/cell)]. As described below, we isolated plaque forming subclones of H9 cells, which were used in these experiments to measure the plaquing efficiency of typical PFC. Such reconstruction experiments indicated that under these assay conditions the plaquing efficiency was ~30%, implying that the frequency of cells with a wild-type μ gene was ~3-fold higher than the frequency of PFC, i.e. 27×10^{-8}/cell. A total of $\sim10^9$ cells were assayed in these experiments, corresponding to the progeny of ~30 cell generations, thus suggesting an event which occurs at the rate of ~1×10^{-8}/cell generation (see Materials and methods).

Comparison of allelic and ectopic recombination frequencies

Recombination can occur between identical segments on heterologous chromosomes, as well as between alleles, and it was of interest to compare the frequency of such 'ectopic' recombination with the frequency of allelic recombination. Recombination between the natural μ allele of the igm482 cell line and ectopic wild-type Cμ segments has been measured previously for transformants bearing wide ranging numbers of integrated Cμ segments (Baker and Read, 1992). For the purposes of comparison with allelic recombination we have identified transformants of igm482 bearing one or two ectopic copies of the wild-type Cμ segment. The blots shown in Figure 3 present measurements of gene dosage for typical transformants, comparing the intensity of the ectopic (e) and natural (n) Cμ segments. Transformants for which e/n = 1–2 were examined further by Southern blotting of DNA digested with *Sca*I or *Bam*HI, enzymes which cut once in the transfected DNA. In summary (results not shown), the transformants E15 and E29 showed only a single

Table I. Measurement of allelic recombination

	Cell line	Allele	Number of cells assayed	Number of plaques	Plaques/cell ($\times 10^{-8}$)
1.	H5	igm482 + N114	3.2×10^8	40	12.5
2.	H9	igm482 + N114	5.0×10^8	81	16.2
3.	igm482	igm482	4.3×10^8	6	1.4
4.	N114	N114	4.5×10^8	15	3.3
5.	H5-B11	N114	2.2×10^8	3	1.4
6.	H5-D2	igm482	2.2×10^8	2	0.9
7.	H5-D4	igm482 + N114	2.2×10^8	20	9.1
8.	H9-B11	N114	2.2×10^8	7	3.2
9.	H9-D10	igm482	2.2×10^8	5	2.3
10.	H9-D4	igm482 + N114	2.2×10^8	22	10.0
Aggregate results					
igm482 allele only (lines 3, 6 and 9)			8.7×10^8	13	1.5
N114 allele only (lines 4, 5 and 8)			8.9×10^8	25	2.6
Both N114 and igm482 (lines 1, 2, 7 and 10)			12.6×10^8	163	12.9

This table compares recombination frequencies (no. plaques/cell) of the two allele hybrids (H5 and H9) with their mutant parent cell lines and the single allele derivative hybrid cell lines (see text). Aliquots of the indicated cell lines containing ~5×10^4 cells were inoculated into medium in separate flasks, grown to ~10^8 cells/culture and assayed for PFC. For the experiments indicated in lines 1–4 at least six independent cultures were used to measure PFC frequency; for the experiments in lines 5–10 at least two independent cultures were used.

idiosyndetic band with the Cμ or *neo* probes, implying that these transformants have each assimilated a single copy of the transfected DNA at a different site; the transformants E13, E26, E61 and E70 showed a band of unit length at 10 kb, as well as an idiosyndetic band, implying that these transformants contained multiple vectors in tandem, which we judge to be two vectors in tandem from the low e/n value. The transformant E69 yielded some unexpected bands and its structure is still under investigation.

As presented in Table II, the PFC frequency for the low copy number transformants was generally higher than the frequency for igm482: the median value, as well as most values, was ~10×10^{-8}/cell. Previous work showed that the frequency of PFC is not increased by the presence of an ectopic mutant Cμ segment, indicating that the excess PFC derive from recombination (Baker and Read, 1992). The frequency of ectopic recombinants is therefore comparable with the frequency of allelic recombinants, arguing that the extended regions of homology which are potentially available in allelic recombination do not enhance recombination. As noted above, similar results leading to this same conclusion have been reported for both meiotic and mitotic recombination in yeast (Petes and Hill, 1988; Lichten and Haber, 1989; Haber *et al.*, 1991). Inasmuch as intrachromosomal recombination in the mammalian cell lines is much more frequent than ectopic recombination, we have initiated experiments to test whether the ectopic Cμ segment in the transformant E69, which yields PFC at high frequency, has serendipitously inserted very near the IgH locus.

Genetic structure of allelic recombinants

We have searched for evidence of reciprocal exchanges associated with allelic recombination by testing whether the PFC have the igm482 and N114 mutations *in cis*. As indicated in Figure 3, we purified six independent PFC. The cell lines denoted A2, A3 and A4 make the truncated as well as the full-length μ chain, indicating that the igm482 mutation is not *in cis* with the N114 mutation. To further test the PFC, we amplified the 527 bp segment spanning parts of Cμ3 and Cμ4, including the igm482 site. We then tested whether the PCR product was wholly or partially susceptible to *Xmn*I digestion. As indicated in Figure 4, the amplified segment from PFC A1, A5 and A6 was as sensitive as that from the normal allele, implying that none of these PFC contains the igm482 mutation. Thus these PFC provide no evidence for reciprocal recombination. However, this negative result does not exclude reciprocal recombination, i.e. recombination might have occurred after DNA replication such that the reciprocal products segregated to different daughter cells; also the doubly mutant allele might have been lost during the course of culture and subcloning.

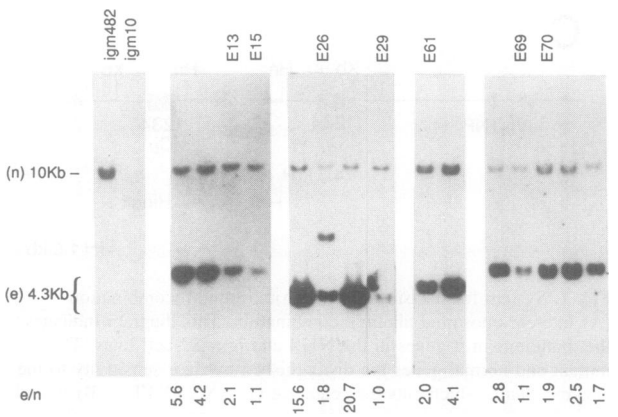

Fig. 3. Analysis of Cμ copy number in ectopic transformants. Ectopic transformants of the igm482 mutant hybridoma cell line were generated as described in Materials and methods. This figure presents the analysis of representative transformants. DNA was digested with *Hin*cII, which yields a 4.3 kb fragment derived from the ectopic wild-type Cμ segment and a 10 kb fragment derived from the natural μ gene of the igm482 mutant hybridoma cell line. The DNA was then examined by Southern blot analysis using Cμ probe F (Figure 1B). The intensities of the bands corresponding to the ectopic (e) and natural (n) Cμ segments were quantified by densitometry or by direct counting of radioactivity. The indicated ratio e/n was used to estimate the number of ectopic copies.

Table II. Measurement of ectopic recombination

Cell line	Cμ copies (ectopic)	Number of cells assayed	Number of plaques	Plaques/cell ($\times 10^{-8}$)
igm482	0	1.6×10^8	2	1.3
E15	1	1.2×10^8	12	10
E29	1	0.8×10^8	7	8.8
E69	1	0.8×10^8	2492	3115.0
E13	2	0.8×10^8	0	<1.3
E26	2	0.8×10^8	19	23.8
E61	2	0.8×10^8	7	8.8
E70	2	0.8×10^8	10	12.5

Transformants of igm482 bearing ectopic copies of the wild-type Cμ segment were generated as described in Materials and methods; analysis of the Cμ copy number is described in the text and Figure 3. Aliquots of the indicated cell lines containing ~5×10^4 cells were inoculated into medium in separate flasks, grown to ~10^8 cells/culture and assayed for PFC.

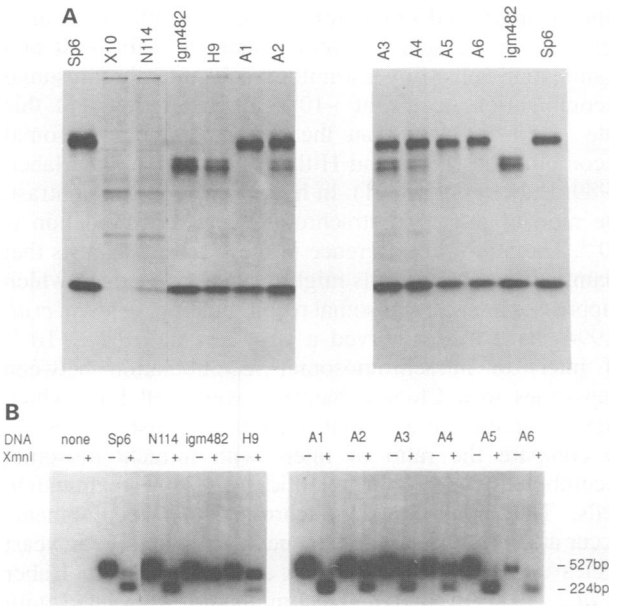

Fig. 4. Analysis of μ genetic structure and IgM production. **(A)** μ production by PFC. Representative PFC (A1–A6) were isolated as described in Materials and methods and the indicated cells were incubated in [^{35}S]methionine-containing medium to label proteins biosynthetically (Baker *et al.*, 1988). IgM was purified from culture supernatants on DNP–Sepharose and eluted with SDS. The disulfide bonds were reduced and the μ and κ chains were fractionated by SDS–PAGE and visualized by fluorography. **(B)** Analysis of DNA structure. Genomic DNA was isolated from the indicated cell lines and amplified by PCR. As indicated, this product was either left intact or digested with *Xmn*I. The DNA was then fractionated by electrophoresis and probed with the internal oligonucleotide 3, as indicated in the legend to Figure 2.

In the course of normal lymphocyte development the IgH locus undergoes two types of rearrangement: VDJ recombination and the heavy chain class switch. Neither of these phenomena is expected to have contributed to the recombination events which we measured with this hybridoma system. The recombination signal sequences with 12 and 23 bp spacers which are the substrates for the VDJ recombinase were not present in the VDJ–Cμ interval in which recombination was selected and VDJ recombinase activity is generally absent in hybridoma cells (Gellert, 1992). Similarly, hybridomas lack the switch recombinase, as indicated by the absence of isotype switching and the absence of either switch-mediated recombination in artificial substrates or in gene targeting (Shulman *et al.*, 1982, 1990; Baker *et al.*, 1988; Ott and Marcu, 1989; Harriman *et al.*, 1993; Oancea and Shulman, 1994). Moreover, the switch region is absent in the Cμ segment used in measuring ectopic and intrachromosomal recombination (Baker and Read, 1992; Bautista and Shulman, 1993).

Discussion

Our measurements of PFC frequency in fused hybridoma cells set an upper limit for the rate of interchromosomal allelic recombination of ~10^{-8}/cell generation, while our previous analyses of intrachromosomal recombination in the Sp6 hybridoma cell line indicated a rate of recombination of 10^{-4}/cell generation (Baker, 1989; Bautista and Shulman, 1993). The ratio of inter- to intrachromosomal recombination in hybridoma cells is therefore ~10^{-4}.

In comparing inter- and intrachromosomal recombination we have sought conditions in which 'accessibility' of the recombining segments does not favour intrachromosomal recombination. Previous work relating recombination and transcription suggests that transcription might be used as a measure of accessibility (Voekel-Meiman *et al.*, 1987; Stewart and Roeder, 1989; Thomas and Rothstein, 1989; White *et al.*, 1991; Nickoloff, 1992). Although the state of the ectopic Cμ segments in the hybridoma cells is unknown, the chromosomal μ genes used in our studies are highly transcribed (Connor *et al.*, 1994). Consequently, we consider that the chromosomal gene segments involved in interchromosomal allelic recombination are at least as accessible as those involved in ectopic and intrachromosomal recombination.

The arrangement which we have used to measure allelic recombination permits the possibility of co-conversion, which in turn could decrease the number of detectable recombinants. In contrast, the DNA segments which were used for ectopic and intrachromosomal recombination are not subject to this effect. If many recombination events were obscured by co-conversion, the real difference between intrachromosomal and allelic recombination would be less than the difference which we have measured. Moreover, such an effect would decrease any excess of allelic over ectopic recombinants. However, co-conversion tracts in mammalian cells are typically <1.5 kb (Walsh, 1987) and in yeast average 1–4 kb (reviewed in Detloff and Petes, 1992). Thus the long (10 kb) interval separating the igm482 and N114 mutations likely obviates this potential problem.

As noted in the Introduction, it is of interest to compare both inter- and intrachromosomal recombination in different cell types and organisms. Ideally this comparison would use the same genetic structures in all cases, an ideal which is not currently obtainable. However, both inter- and intrachromosomal recombination have been measured for each of several different genes and cell types, so it is possible to compare the ratios of inter- and intrachromosomal recombination as a function of cell type and organism. It is of particular interest to compare the

ratio of inter- and intrachromosomal recombination as it depends on the meiotic/mitotic state in both yeast and mammalian cells. In yeast mitotic cells interchromosomal recombination occurs at ~10^{-7}/cell generation and this rate is 20-fold less than the rate of intrachromosomal recombination (Petes and Hill, 1988; Lichten and Haber, 1989; Haber *et al.*, 1991). In hybridoma cells, in contrast, the ratio of inter- to intrachromosomal recombination is 10^{-4}. The 500-fold difference in these ratios suggests that mammalian somatic cells might have a mechanism which suppresses interchromosomal recombination. Godwin *et al.* (1994) have also observed a very low ratio ($<2\times10^{-4}$) of inter- to intrachromosomal recombination between transgenes in a Chinese hamster ovary cell line, which they interpreted in a similar manner. It is also interesting to compare the ratio of inter- with intrachromosomal recombination seen for meiotic yeast and mammalian cells. Thus inter- and intrachromosomal recombinants occur at nearly the same frequency (~2%) in meiotic yeast (Petes and Hill, 1988; Lichten and Haber, 1989; Haber *et al.*, 1991). In studying recombination associated with meiosis during mouse spermatogenesis, Murti *et al.* (1992, 1994) reported that (ectopic) inter- and intrachromosomal recombinants occurred at frequencies of ~0.3 and ~2% respectively, giving a ratio of inter- to intrachromsomal recombination of 0.15. The inter-/intrachromosomal recombination ratio is thus much higher in mouse meiotic cells than observed for mammalian somatic cells (10^{-4} for hybridoma cells, present study; $<2\times10^{-4}$ for CHO cells, Godwin *et al.*, 1994). For linguistic convenience we denote these relationships as 'preferential suppression' of interchromosomal recombination in mammalian somatic cells, without implying anything about mechanism. Thus the preferential suppression of interchromosomal recombination in mouse hybridoma cells compared with mouse germ cells is ~1500-fold ($0.15/10^{-4}$). As noted above, the preferential suppression of interchromosomal recombination in hybridoma cells compared with yeast mitotic cells is ~500-fold.

Several mechanisms might underlie this preferential suppression of interchromosomal recombination in mammalian somatic cells. The most obvious differences between yeast and metazoa and between meiosis and mitosis relate to the larger size of the metazoan nuclei and pairing of chromosomes associated with meiosis. Being a bimolecular reaction, interchromosomal recombination must be more sensitive than intrachromosomal recombination to local chromosome concentration. However, the fact that interchromosomal recombination is preferentially suppressed in mammalian somatic cells compared with meiotic cells argues that the suppression is not due to dilution associated with the larger mammalian nuclei. Suppression might reflect the action of specific biological mechanisms. For example, different chromosomes appear to be restricted to distinct subnuclear sites (Lichter *et al.*, 1988); this localization might be more stringent in mitotic than in meiotic cells and might thus preferentially impede mitotic interchromosomal recombination. Alternatively, interchromosomal recombination might require a factor, e.g. a chromosome pairing factor, which is not needed for intrachromosomal recombination and there might be less of this factor in mitotic than in meiotic cells.

The 'physiological purpose' of suppressing interchromosomal recombination much more than intrachromosomal recombination in somatic cells might relate to the capacity of interchromosomal recombination to alter the phenotype of somatic cells. As noted above, interchromosomal recombination in somatic cells is expected at some frequency to generate deleterious interchanges: recombination between homologous chromosomes can create homozygosity, generating cells in which both copies of some genes are non-functional; recombination involving repetitive sequences on heterologous chromosomes can result in gene disruption or dysregulation. In contrast, intrachromosomal recombination which is restricted to nearby homologous segments, such as in sister chromatid exchange, cannot alter the cellular phenotype, except in the rare or contrived cases of tandemly duplicated segments. Intrachromosomal recombination appears to be the predominant mechanism for repairing DNA damage, at least in yeast (Kadyk and Hartwell, 1992), and this recombination pathway is expected to be of significant benefit in all replicating cells. These considerations lead to the expectations which are confirmed here, namely that interchromosomal recombination should be relatively suppressed in somatic cells while intrachromosomal recombination should be relatively preserved and that this suppression should be stronger in metazoan cells than in microbial organisms.

Materials and methods

Preparation of cell lines

The mutant cell lines igm482, N114 and X10 have been described previously (Baumann *et al.*, 1985; Connor *et al.*, 1993). To fuse these cell lines, pSV2neo (Southern and Berg, 1982) and pSV2his (Hartman and Mulligan, 1988) were transferred to igm482 and N114 respectively, as described (Baker *et al.*, 1988). A G418-resistant igm482 transformant was then fused with a histidinol-resistant N114 transformant (Shulman *et al.*, 1978) and hybrid cells were selected in medium supplemented with G418 and histidinol. To obtain hybrids bearing both alleles we tested whether cells produced truncated μ chain using a Cμ4-specific ELISA (Baumann *et al.*, 1985) and whether transfection of the cells with the TNP-specific VH region generated PFC (Connor *et al.*, 1993). Two independent hybrid cell lines bearing both alleles, H5 and H9, were chosen for further investigation. The isolation of subclones bearing either one or both alleles is described in the text and Figure 2.

To generate ectopic transformants of igm482, this cell line was transfected with the 10 kb vector pTCμ and G418-resistant transformants were selected as described (Baker and Read, 1992). The analysis of the ectopic Cμ copy number is described in the text and Figure 3.

Assay and isolation of plaque forming cells (PFC)

Aliquots of the indicated cell lines containing ~5×10^4 cells were inoculated into medium in separate flasks, grown to ~10^8 cells/culture and assayed for PFC (Baker *et al.*, 1988). Plaque forming cells were recovered from representative plaques and cloned by limiting dilution as described (Baker and Read, 1992).

Analysis of DNA structure and IgM production

Isolation of DNA and protein analysis by SDS–PAGE were conducted as described by Baker *et al.* (1988). The oligonucleotides used for PCR were: (1) 5′-TTCCTCAGCAAGTCCGCTAACCTGAC-3′; (2) 5′-TTGGGGCAAGAGTTGCCCTCTCTGAA-3′; (3) 5′-TAGTGTTTG-TGTGGA-3′.

Calculation of recombination rate

For the sake of comparison we have calculated recombination rate according to the formula $dR = d\tau(\rho N + Nt/\tau)$. N and R are respectively the number of cells and the number of recombinants at time t; ρ is the recombination rate (recombinants per cell and unit time, assumed to be uniform throughout development). It is also assumed that recombination

is irreversible, that recombinants and non-recombinants grow at the same rate and that recombination is linked to cell doubling, e.g. dependent on DNA replication, so t is measured in generations and τ is the generation time. Under these circumstances and where $N = e^{t/\tau}$, $\rho = R/Nt$.

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References

Baker,M.D. (1989) High-frequency homologous recombination between duplicate chromosomal immunoglobulin μ heavy-chain constant regions. *Mol. Cell. Biol.*, **9**, 5500–5507.

Baker,M.D. and Read,L.R. (1992) Ectopic recombination within homologous immunoglobulin μ gene constant regions in a mouse hybridoma cell line. *Mol. Cell. Biol.*, **12**, 4422–4432.

Baker,M.D. and Read,L.R. (1995) High-frequency gene conversion between repeated Cμ sequences integrated at the chromosomal immunoglobulin μ locus in mouse hybridoma cells. *Mol. Cell. Biol.*, **15**, 766–771.

Baker,M.D., Pennell,N., Bosnoyan,L. and Shulman,M.J. (1988) Homologous recombination can restore normal immunoglobulin production in a mutant hybridoma cell line. *Proc. Natl Acad. Sci. USA*, **85**, 6432–6436.

Baumann,B., Potash,M.J. and Köhler,G. (1985) Consequences of frameshift mutations at the immunoglobulin heavy chain locus of the mouse. *EMBO J.*, **4**, 351–359.

Bautista,D. and Shulman,M.J. (1993) A hit-and-run system for introducing mutations into the Ig H locus of hybridoma cells by homologous recombination. *J. Immunol.*, **151**, 1950–1958.

Connor,A., Collins,C., Jiang,L., McMaster,M. and Shulman,M.J. (1993) Isolation of new nonsense and frameshift mutants in the immunoglobulin μ heavy chain gene of hybridoma cells. *Somatic Cell Mol. Genet.*, **19**, 313–320.

Connor,A., Wiersma,E. and Shulman,M.J. (1994) On the linkage between RNA processing and RNA translatability. *J. Biol. Chem.*, **269**, 25178–25184.

Detloff,P. and Petes,T.D. (1992) Measurements of excision repair tracts formed during meiotic recombination in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **12**, 1805–1814.

Gellert,M. (1992) Molecular analysis of V(D)J recombination. *Annu. Rev. Genet.*, **22**, 425–446.

Godwin,A.R., Bollag,R.J., Christie,D. and Liskay,R.M. (1994) Spontaneous and restriction enzyme-induced chromosomal recombination in mammalian cells. *Proc. Natl Acad. Sci. USA*, **91**, 12554–12558.

Haber,J.E., Leung,W., Borts,R.H. and Lichten,M. (1991) The frequency of meiotic recombination in yeast is independent of the number and position of homologous donor sequences: implications for chromosome pairing. *Proc. Natl Acad. Sci. USA*, **88**, 1120–1124.

Harriman,W., Völk,H., Defranoux,N. and Wabl,M.. (1993) Immunoglobulin class switch recombination. *Annu. Rev. Immunol.*, **11**, 361–384.

Hartman,S.C. and Mulligan,R.C. (1988) Two dominant-acting selectable markers for gene transfer studies in mammalian cells. *Proc. Natl Acad. Sci. USA*, **85**, 8047–8051.

Kadyk,L.C. and Hartwell,L.H. (1992) Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. *Genetics*, **132**, 387–402.

Lasko,D., Cavenee,W. and Nordenskjöld,M. (1991) Loss of constitutional heterozygosity in human cancer. *Annu. Rev. Genet.*, **25**, 281–314.

Lichten,M. and Haber,J.E. (1989) Position effects in ectopic and allelic mitotic recombination in *Saccharomyces cerevisiae*. *Genetics*, **123**, 261–268.

Lichter,P., Cremer,T., Borden,J., Manuelidis,L. and Ward,D.C. (1988) Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridization using recombinant DNA libraries. *Hum. Genet.*, **80**, 224–234.

Murti,J.R., Bumbulis,M. and Schimenti,J.C. (1992) High-frequency germ line gene conversion in transgenic mice. *Mol. Cell. Biol.*, **12**, 2545–2552.

Murti,J.R., Bumbulis,M. and Schimenti,J.C. (1994) Gene conversion between unlinked sequences in the germline of mice. *Genetics*, **137**, 837–843.

Nickoloff,J.A. (1992) Transcription enhances intrachromosomal homologous recombination in mammalian cells. *Mol. Cell. Biol.*, **12**, 5311–5318.

Oancea,A.E. and Shulman,M.J. (1994) An improved system of somatic cell molecular genetics for analysing the requirements of Ig synthesis and function. *Int. Immunol.*, **6**, 1161–1168.

Ott,D.E. and Marcu,K.B. (1989) Molecular requirements for immunoglobulin heavy chain constant region gene switch-recombination revealed with switch substrate retroviruses. *Int. Immunol.*, **1**, 582–591.

Petes,T.D. and Hill,C.W. (1988) Recombination between repeated genes in microorganisms. *Annu. Rev. Genet.*, **22**, 147–168.

Petes,T.D., Malone,R.E. and Symington,L.S. (1991) Recombination in yeast. In Broach,J.R., Pringle,J.R. and Jones,E.W. (eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. 1, pp. 407–521.

Shulman,M., Wilde,C.D. and Köhler,G. (1978) A better cell line for making hybridomas secreting specific antibodies. *Nature*, **276**, 269–270.

Shulman,M.J., Heusser,C., Filkin,C. and Köhler,G. (1982) Mutations affecting the structure and function of immunoglobulin M. *Mol. Cell. Biol.*, **26**, 1033–1043.

Shulman,M.J., Nissen,L. and Collins,C. (1990) Homologous recombination in hybridoma cells: dependence on time and fragment length. *Mol. Cell. Biol.*, **10**, 4466–4472.

Southern,P.J. and Berg,P. (1982) Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Mol. Appl. Genet.*, **1**, 327–341.

Stewart,S.E. and Roeder,G.S. (1989) Transcription by RNA polymerase I stimulates mitotic recombination in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **9**, 3464–3472.

Thomas,B.J. and Rothstein,R. (1989) Elevated recombination rates in transcriptionally active DNA. *Cell*, **56**, 619–630.

Voekel-Meiman,K., Keil,R.L. and Roeder,G.S. (1987) Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. *Cell*, **48**, 1071–1079.

Walsh,J.B. (1987) Sequence-dependent gene conversion: can duplicated genes diverge fast enough to escape conversion? *Genetics*, **117**, 543–557.

White,M.A., Wierdl,M., Detloff,P. and Petes,T.D. (1991) DNA-binding protein RAP1 stimulates meiotic recombination at the *His4* locus in yeast. *Proc. Natl Acad. Sci. USA*, **88**, 9755–9759.

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