Age, Vol. 22, 71-88, 1999

RECOMBINATION AND ITS ROLES IN DNA REPAIR, CELLULAR IMMORTALIZATION AND CANCER

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ABSTRACT

Genetic recombination is the creation of new gene combinations in a cell or gamete, which differ from those of progenitor cells or parental gametes. In eukaryotes, recombination may occur at mitosis or meiosis. Mitotic recombination plays an indispensable role in DNA repair, which presumably directed its early evolution; the multiplicity of recombination genes and pathways may be best understood in this context, although they have acquired important additional functions in generating diversity, both somatically (increasing the immune repertoire) and in germ line (facilitating evolution). Chromosomal homologous recombination and *HsRad51* recombinase expression are increased in both immortal and pre-immortal transformed cells, and may favor the occurrence of multiple oncogenic mutations. Tumorigenesis *in vivo* is frequently associated with karyotypic instability, locus-specific gene rearrangements, and loss of heterozygosity at tumor suppressor loci — all of which can be recombinationally mediated. Genetic defects which increase the rate of somatic mutation (several of which feature elevated recombination) are associated with early incidence and high risk for a variety of cancers. Moreover, carcinogenic agents appear to quite consistently stimulate homologous recombination. If cells with high recombination arise, either spontaneously or in response to "recombinogens," and predispose to the development of cancer, what selective advantage could favor these cells *prior to* the occurrence of growth-promoting mutations? We propose that the augmentation of telomere-telomere recombination may provide just such an advantage, to hyper-recombinant cells within a population of telomerase-negative cells nearing their replicative (Hayflick) limit, by extending telomeres in some progeny cells and thus allowing their continued proliferation.

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VARIETIES OF RECOMBINATION

Genetic recombination, most broadly defined, refers to the creation of new gene combinations in a diploid cell or gamete, distinct from those of its progenitors. It thus includes reassortment of chromosomes in eukaryotic gametogenesis, termed *interchromosomal* recombination, but more commonly refers to *intrachromosomal* events involving DNA strand exchange (breakage and reunion of strands), whether occurring in meiosis or mitosis.

There are four main types of recombination: homologous recombination, site-specific recombination, transposition, and illegitimate recombination. ***Homologous recombination*** requires extensive sequence identity between the DNA duplexes to be recombined (Szostak *et al.* 1983; West 1994), and occurs at rates dependent on both the length of "homologous" sequences and the degree to which they are identical (Ayares *et al.* 1986; Liskay *et al.* 1987; Nassif and Engels 1993). ***Site-specific recombination*** occurs between DNA duplexes containing characteristic signal sequences that have little or no homology. Site-specific recombination events include rearrangements that generate immunoglobulin and T-cell receptor diversity (Oettinger *et al.* 1990), and insertion or excision of transposons and viral DNA. Aberrations of these processes would also qualify, such as chromosomal inversions and translocations in human cancers, primarily leukemias, in which immunoglobulin signal sequences may recombine with signal-related sequences in or near oncogenes (Battey *et al.* 1983). ***Transposition***, usually regarded as a subclass of site-specific recombination, is the process by which blocks of DNA sequence move from one place to another in a genome. Transposable elements can cause insertional mutation of interrupted genes, and altered expression of nearby genes, and thus may contribute significantly to genomic evolution (Wichman *et al.* 1992) and somatic mutation (Nikitin and Shmookler Reis, 1997). ***Illegitimate*** or ***nonhomologous recombination*** does not require either a signal sequence or regions of extensive sequence identity. Although its frequency per kilobase of target DNA is much lower than that for homologous recombination, the total over the whole

genome can be substantial (Mansour *et al.*, 1988, 1993). Thus, stable integration of transfected DNA occurs predominantly through illegitimate recombination (Waldman and Waldman 1990; Sweezy and Fishel 1994), even when homologous recombination is greatly enhanced by gene targeting with sequences identical to genomic DNA (Folger *et al.* 1985; Mansour *et al.*, 1988; Sedivy and Sharp 1989).

In eukaryotic organisms, recombination occurs both in mitosis and meiosis. Meiotic recombination — in germ-line cells only — augments the reassortment of gene alleles accomplished by segregation of chromosomes at the first meiotic division (Baker *et al.* 1976). Moreover, meiotic recombination between homologous DNA sequences of multigene families plays a significant role in generating genetic diversity and facilitating the evolution of genes (Arnstutz *et al.* 1985). Mitotic recombination plays a crucial role in repairing DNA damaged by a variety of agents (Friedberg *et al.* 1991), both in somatic tissues and in gametic lineages. Because mitoses greatly exceed meioses in lineages leading to gamete formation, mitotic recombination also contributes greatly to germ-line reassortment and rearrangement of genes (Smith 1974, 1976). Additionally, mitotic homologous recombination is involved in immunoglobulin class switching (Davis *et al.* 1980; Early *et al.* 1980; Sakano *et al.* 1980), and may underlie the loss of heterozygosity which, in regions containing anti-oncogenes, contributes to carcinogenesis (Solomon *et al.* 1991).

MODELS OF HOMOLOGOUS RECOMBINATION AND GENE CONVERSION

Most of our current understanding of the recombination process, expressed through evolving models of the intermediates involved, derives from tetrad and octad analysis of fungal haploid spores segregating for visible spore phenotypes, wherein all four products of meiotic segregation (the tetrad) can be observed. Actually, in some fungi eight spores are generated per meiosis (an octad), since each of the four haploid products of meiosis is replicated in a final mitosis to yield two spores, normally identical, derived from the two strands of each chromatid's DNA duplex. The haploid state of fungal spores allows segregation and recombination to be easily visualized for markers affecting spore appearance, with the frequency and order of alleles (distinguishable variants of a marker) or diallelic combinations providing important clues as to the underlying processes. Aberrant segregations are most readily analyzed in *Neurospora crassa,* a bread mold, because the eight spores remain in an ordered linear array within the *ascus*, or spore pod. Other fungi may produce ordered tetrads (*Ustilago*), unordered tetrads (the mushroom *C. lagopus* and the yeast *S. cerevisiae*), or unordered octads (*Neurospora*) (Raju 1980). In >99% of meioses, distinguishable parental forms of single markers, or *alleles,* segregate in 2:2 ratios (4:4 in octads), as predicted by Mendel and as expected from simple partition of the four parental chromatids. The exceptions (mostly 3:1 or 6:2 ratios) indicate an asymmetric or nonreciprocal process, called "gene conversion" to reflect the apparent transformation of one parental allele to the other. Still more rarely, in octads the paired ascospores representing mitotic products of a single chromatid are not identical, indicating that two strands within a DNA duplex were not quite complementary but comprised a heteroduplex.

A molecular basis for reciprocal and nonreciprocal events in homologous recombination was first proposed by Robin Holliday (1964) based on evidence from *Ustilago* and *Neurospora*, fungi which produce ordered meiotic products. According to the Holliday model, recombination is initiated by introduction of a nick in one strand of each of two homologous chromosomes. Strand exchange proceeds from these nicks to produce a crossed-strand "Holliday junction". Branch migration of a crossover leads to the formation of symmetrical heteroduplex regions which contain one strand from each parental chromosome. Holliday structures can be resolved by cutting the uncrossed strands, to generate recombinant chromatids, in which those alleles which differ between homologs are seen to derive from different parents on either side of the crossover. This exchange of flanking markers, the outcome of half of all crossover events, is the defining criterion of genetic recombination. Alternatively, if the resolution is achieved by cutting the originally crossed strands, it will result in nonrecombinant chromosomes, although each would still contain a heteroduplex region corresponding to the span of branch migration. Asymmetric mismatch repair of heteroduplex segments, whether in recombinant or nonrecombinant products, leads to tracts of gene conversion.

A modification of the Holliday model was presented by Meselson and Radding (1975), in which strand exchange is initiated by a nick in only one of the homologous chromosomes. In 1983, Szostak *et al.* presented a double-strand break model of recombination in yeast, that accounted for marked biases in the direction of gene conversion (*i. e.*, one of the parental alleles prevailed) in some experimental designs. The model proposes that recombination is initiated by a double-strand break in one of the chromosomes, followed by exonuclease action to produce a gap at the break point. One 3' end invades the homologous duplex, resulting in the formation of a D loop. The repair synthesis of gap DNA is followed by branch migration to form a double-crossover version of the Holliday structure, which can then be resolved to generate recombinant or nonrecombinant chromatids. In this model, as in the experimental data it was devised to explain, a broken chromosome is always the recipient of genetic information, whereas in the Holliday and Meselson-Radding models, the nicked duplex was the donor.

It should be noted that the definition of gene conversion has expanded in popular usage, beyond its original

meaning in the context of meiotic segregation distortion. Other nonreciprocal exchanges, even between plasmids or between a chromosomal locus and an identical or related episomal sequence, have been termed gene conversion, as have situations in which the information donor and recipient(s) lie on the same chromatid and the flow of information appears to be unidirectional (Rubnitz and Subramani 1986, 1987; Liu and Wolf 1998; Boissinot *et al.* 1998; Whelden Cho *et al.* 1998; Chen and Jinks-Robertson 1998; Yamaguchi-Iwai *et al.* 1998).

RELATIONSHIP OF HOMOLOGOUS RECOMBINATION TO DNA REPAIR

Intrachromosomal homologous recombination, the generation of new assortments of genetic material by DNA strand exchange (usually detected only if between nonsister chromatids), is a fundamental genetic process which occurs ubiquitously in all living organisms. Mechanisms to facilitate homologous recombination must have arisen very early in evolution, very likely as mediators of DNA repair. As described above, the simplest models of homologous recombination involve an initial nicking of DNA duplexes, invasion of partially identical (often termed homologous) sequences in another duplex, displacement of the crossover point by branch migration, and resolution of the crossover intermediate by a second strand exchange (Holliday 1964; Meselson and Radding 1975). Initiation is now thought to occur primarily with a double-strand break in the "recipient" DNA duplex, followed by exonucleolytic recission of the broken ends and gap repair directed by a homologous donor duplex (Szostak *et al.* 1983). Double-strand break models explain otherwise puzzling features of homologous recombination, account for nonreciprocal information flow — including gene conversion — and place the recombination mechanism firmly in the realm of DNA repair.

Damage to DNA being inevitable, its repair must be regarded among the most fundamental requirements for life. All organisms are exposed to DNA-damaging agents, including exogenous chemicals and radiation, and endogenously-generated oxygen radicals. The resulting lesions (Solomon *et al.* 1991) include missing bases (*e.g.*, nearly 10,000 spontaneous depurinations per mammalian cell per day), modified bases (due to alkylation and radiation ionization), and incorrect bases (due chiefly to deamination). DNA strands may be affected by insertions or deletions, largely spontaneous in origin, and by double- and single-strand breaks — either caused directly by ionizing radiation, or indirectly via DNA repair, elicited by cross-linking agents, UV-induced cyclobutyl dimers, oxidation, and large adducts. In human cells, oxidative damage has been estimated to be found at nearly 10^{-5} of nucleotides in nuclear DNA, and greater than 10^{-4} of bases in mitochondrial DNA (Richter *et al.* 1988; Loft and Poulsen 1996).

DNA lesions are highly mutagenic if unrepaired prior to DNA replication, because they may be bypassed through error-prone (SOS) replication (Kornberg and Baker 1992). Higher fidelity is achieved by an alternative pathway for post-replication repair, recombinational repair, wherein gap filling is achieved by recombination with homologous DNA sequences from the other branch of the replication fork. Either mode of post-replication repair is literally a "stopgap" measure, however, which fails to remove the mutagenic lesion but buys time — another cell cycle — for repair. Double-strand breaks not repaired prior to S phase can have dire consequences because such chromatid breaks may recombine to generate chromosomal rearrangements with the potential for oncogenesis or cell lethality. For example, chromosomal fragments losing or gaining a centromere (acentric or dicentric chromosomes) tend to segregate abnormally. Genetic recombination reduces mutations arising from error-prone repair, and is essential for the repair of double-strand breaks (Resnick *et al.*, 1989; Sweezy and Fishel 1994); its occurrence in mitotic cells may thus be predominantly or entirely repair-related (Roca and Cox 1990). Surprisingly, the repair of DNA damage appears to utilize both homologous and nonhomologous recombination pathways (Sweezy and Fishel 1994), which are described in greater detail below. Moreover, the Rad52 protein, although intimately involved in homologous recombination through interaction with Rad51, is not required for intrachromosomal "gene conversion" or for repair of several types of DNA damage (Yamaguchi-Iwai *et al.* 1998)

GENES MEDIATING HOMOLOGOUS RECOMBINATION

Homologous recombination has been intensively studied in *E. coli*, for which the recombination machinery is at present the best characterized (Sengstag 1994). In this bacterium, the implicated genes encode nucleases (*recB*, *recC*, *recD*, *recE*, *recJ*, and *ruvC*), helicases (*recB*, *recC*, *recD*, and *recQ*), topoisomerases (*gyrA*, *gyrB*, and *topA*), DNA ligase (*lig*), DNA-binding proteins (*ssb*, *recF*, and *recG*), proteins mediating strand migration (*ruvA* and *ruvB*), and *recA* recombinase. The RecA protein plays a central role in mediating the cellular SOS response to DNA damage, as well as homologous recombination, in *E. coli*. This dual role makes sense in the context of DNA repair, since these are precisely the two options available for gap-filling behind the replication fork. The multifunctional RecA protein possesses DNA-dependent ATPase activity, requiring both single-stranded DNA (ssDNA) and ATP, and a protease activity which cleaves LexA and several other DNA-binding repressor proteins. Cleavage of LexA protein triggers the SOS response by relaxing transcriptional repression of DNA-repair genes. RecA is also capable of renaturing ssDNA and pairing homologous DNA sequences, and can mediate the formation of stable triple helices (Hsieh *et al.* 1990). The pairing of homologous DNA sequences, which is an important function of RecA, seems to require only a small peptide containing amino acids 193-212 of the protein (Voloshin *et al.* 1996).

Recombination at double-strand breaks, in *E. coli,* is initiated by the RecB, C, and D components of a multi-enzyme helicase/nuclease complex (Taylor *et al.* 1985). RecBCD binds one of the free ends created by a double-strand break and unwinds the duplex to form paired loops of single-stranded DNA. On encountering a "chi" (χ) cleavage sequence, the RecBCD nuclease cuts one strand, generating a free 3' end to engage in RecA-mediated recombination. The yeast *RAD51* and *DMC1* genes are similar in sequence to the *E. coli recA* gene (West 1994), but no counterpart to *recBCD* has yet been identified in eukaryotic nuclei. A functional equivalent may exist, however, since eukaryotes have hotspots for homologous recombination analogous to (but distinct from) χ sites (Smith 1994), and a few nuclear genes have been noted with segments of sequence identity to *recB, C* or *D.*

DMC1 is a meiosis-specific gene involved in reciprocal recombination and formation of the synaptonemal complex (Bishop *et al.* 1992). *RAD51*, however, is required for both meiotic and mitotic recombinational repair of DNA damage in yeast cells. A human Rad51-like gene (*HsRAD51*) has been isolated (Yoshimura *et al.*, 1993), which encodes a 339-amino-acid protein with a predicted molecular weight of 37 kDa. The deduced amino acid sequence is 83% similar (67% identical) to that of the yeast RAD51 protein and 56% similar (30% identical) to that of *E. coli* RecA protein. HsRAD51 protein has properties much like RecA in *E.coli* and RAD51 in yeast: it is able to bind to double-stranded and single-stranded DNA, exhibits DNA-dependent ATPase activity, and forms helical nucleoprotein filaments with DNA (Benson *et al.* 1994). Immortal human cell lines (Finn *et al.* 1989; Xia *et al.* 1997) and human fibroblasts expressing integrated SV40 large-T antigen (Xia *et al.* 1997) show an average of 4-fold elevation in homologous recombination and in levels of *HsRAD51* mRNA, relative to diploid untransformed cells. Introduction of antisense HsRAD51 oligonucleotides results in complete suppression of T-antigen-induced recombination (Xia *et al.* 1997), implying that HsRAD51 mediates induction of recombination in SV40-transformed cells.

GENES INVOLVED IN NONHOMOLOGOUS RECOMBINATION

The mechanisms of nonhomologous recombination are relatively poorly understood. However, a number of proteins such as topoisomerase I (Ikeda 1994), DNA gyrase (Waldman and Waldman 1990), poly(ADP-ribose)polymerase (Waldman and Waldman 1990), DNA ligase II (Waldman, and Waldman 1990), and nonhomologous recombination (NHR) ligase (Derbyshire *et al.* 1994), have been implicated to play very specific roles in illegitimate (nonhomologous) recombination. NHR ligase has the ability to join DNA duplexes, irrespective of the sequence and structure of their ends (Derbyshire *et al.* 1994). Surprisingly, this enzyme was characterized as a component of a multi-enzyme complex which possesses *homologous* pairing and strand-exchange activity. The complex contains homologous pairing protein (HPP-1, which mediates strand exchange proceeding in a 5' to 3' direction), a 3'-exonuclease, and the human single-strand binding (hSSB) protein, in addition to NHR ligase (Sweezy and Fishel 1994).

These observations led to a model in which nonhomologous recombination is primarily an end-joining ligation reaction between two DNA duplexes; their single-stranded ends, generated by 3'-exonuclease, anneal by base-pairing over short complementary regions and are then ligated (Sweezy and Fishel 1994). Thus, nonhomologous recombination would be mediated through regions of partial sequence identity! This is not altogether surprising, given the much greater efficiency of ligation *in vitro* for cohesive-end joining than for blunt ends; note, however, that the requirement here for congruent sequences is greatly reduced relative to homologous recombination, which proceeds through a triple-stranded intermediate (Hsieh *et al.* 1990) and depends directly on the length and degree of identity of homologous sequences (Ayares *et al.* 1986; Liskay *et al.* 1987; Nassif and Engels 1993). The two processes may share common enzymatic steps, but clearly also have distinctive features. For example, inhibitors of poly(ADP-ribosylation) block random integration of transfected DNA into the host genome (an example of nonhomologous recombination), but not inter- or intra-molecular homologous recombination between plasmid sequences (Waldman and Waldman 1990). Free ends of transfected or broken genomic DNA may induce poly(ADP-ribose) polymerase, which in turn induces double-strand break repair by recruiting and activating DNA ligase II (Creissen and Shall 1982). This mechanism would account for the role of poly(ADP-ribose)polymerase in the stable integration of transfected DNA into the host genome (Waldman and Waldman 1990). However, poly(ADP-ribose) polymerase can also ADP-ribosylate histone H1, which may lead to removal of this histone from nucleosomes to create a relaxed chromatin site, a putative substrate for the integration of transfected DNA (Althaus and Richter 1987).

Plasmids in eukaryotic cells recombine primarily by a nonhomologous route, probably through annealing of cohesive single-strand regions at damaged DNA ends (Wake *et al.* 1984; Anderson and Eliason 1986). Most transfected plasmids cannot replicate in eukaryotic host cells, but may undergo damage and repair synthesis at any phase of the cell cycle, thus favoring their utilization of nonhomologous recombination and/or joining of damaged DNA termini. Nevertheless, plasmid recombination increases along with chromosomal homologous recombination in many "hyper-recombinant" diseases, such as Werner syndrome (Fukuchi *et al.* 1989; Cheng *et al.* 1991) and Ataxia telangiectasia (Meyn 1993; Xia *et al.* 1996), and possibly in cancer (Finn *et al.* 1989; Xia *et al.* 1997), implying either substantial overlap in these pathways, or involvement of homologous recombination

in a subset of plasmid recombination events — which could then become the predominant class when homologous recombination is stimulated.

TO CYCLE OR NOT TO CYCLE...

Post-replication repair in eukaryotic cells should be minimized by p53-mediated cell-cycle arrest, which allows repair to precede S phase, but may nevertheless occur to some extent in rapidly cycling cells, and might be especially prevalent in p53-deficient cancer cells. The p53 protein is a key component of cellular defense against genotoxic agents. Upon damage to DNA, p53 is activated and directs the cell either to apoptosis, or to transient cell-cycle arrest at the G_1/S boundary, by modulation of the transcription of multiple target genes (Kastan *et al.* 1991; Kuerbitz *et al.* 1992; Almasan *et al.* 1995). Apoptosis may be effected by p53-mediated transcriptional activation of "Bax", a member of the Bcl-2 family, which then induces apoptosis (Zhan *et al.* 1994; Larsen 1994; Almasan *et al.* 1995). It has been proposed that different classes of DNA damage could lead to the induction of specific subsets of modifying proteins, which activate p53 both by induction and post-translational modification (Nakamura 1998). For example, DNA damage induced by ionizing radiation results in the activation of ATM (mutated in Ataxia telangiectasia) kinase (Savitsky *et al.* 1995), probably through autophosphorylation (Nakamura 1998), which then activates p53 by phosphorylation at Ser^{15} (Banin *et al.* 1998; Canman *et al.* 1998).

Activated p53 also mediates cell-cycle arrest through *trans* regulation of target gene expression. One target, p21, inhibits cyclin-dependent kinases (Xiong *et al.* 1993), thus preventing phosphorylation of Rb and subsequent release of E2F, which is required for transcriptional activation of S-phase-associated genes. Transcriptional activation of other downstream genes such as GADD45 (Kastan *et al.* 1992), and WAF1/CIP1 (El-Deiry *et al.* 1994) has also been implicated in p53-mediated cell cycle arrest. However, the observation that certain DNA-base-damaging agents, such as methyl methane sulfonate (MMS), can induce GADD45 in the absence of functional p53 (Fornace *et al.* 1989), implies that p53-independent pathways also exist for cell-cycle arrest after DNA damage (Kastan *et al.* 1992). The extent of p53 induction can vary greatly between different DNA damaging agents (Lu and Lane 1993), and/or cell types (Khanna and Lavin 1993), which also supports the hypothesis of multiple signal transduction pathways for cell-cycle arrest.

IMPORTANCE OF "RECOMBINOGENS" IN CARCINOGENESIS

Most carcinogens are mutagenic when assayed in *Salmonella* HIS-reversion assays, but 30-50% are not (Ashby and Tennant 1988; Mason *et al.* 1990). Of those carcinogens for which point-mutagenicity is not commensurate with oncogenicity, some are active in other HIS^- strains requiring small frame-shift insertions or deletions for reversion. However, these assay systems also appear to be inefficient at detecting a subset of carcinogens (Ashby and Tennant 1988; Mason *et al.* 1990), which may act primarily by augmenting recombination. Several carcinogens with low mutagenicity in Ames tests have now been shown to be recombinogenic in bacteria (Luisi-Deluca *et al.* 1984), yeast (Schiestl *et al.* 1989), and transformed mammalian cells (Zhang and Jenssen 1994). Using the HPRT-reversion assay in untransformed human fibroblasts, we find substantial recombinogenic activity for 6/6 carcinogens tested, which increase reversion rates by 2.4- to 12-fold even without addition of a metabolic-activation extract, whereas five noncarcinogenic chemicals altered recombination by no more than 1.7-fold (Li *et al.* 1997). The very high concordance observed between recombinogenicity and carcinogenicity (a perfect 1.0 in this small sample), if confirmed for a larger panel of chemicals and other agents demonstrated to be carcinogens or noncarcinogens, implies that oncogenesis may depend far more on effects stimulating DNA rearrangement than on point mutations. How is this possible, in view of the very well-established although weaker association between carcinogens and point mutagens revealed by Ames tests? Indirect effects could resolve this paradox — *e.g.*, high levels of point mutation, by depleting the cell's capacity for coping with base-pairing mismatches, may impede completion of recombinational repair which involves correction of heteroduplex regions. However, this would be difficult to reconcile with the relative paucity of chromosomal instability in cancer cells deficient in mismatch repair (Lengauer *et al.* 1997, 1998). Alternatively, point mutations might themselves stimulate recombination *via* mismatch repair. In this case, Ames-positive point mutagens should also be recombinogenic, but only in cells with intact mismatch repair pathways. We are currently testing this hypothesis.

ELEVATED RECOMBINATION — AN EXPRESS ROUTE TO IMMORTAL TRANSFORMATION AND CANCER?

Cell immortality

Normal diploid cells decline progressively in cell cycling rate with increasing passage level, effectively limiting their *in vitro* life spans, whereas established cell lines are able to divide indefinitely and are thus termed "immortal". Transformation by a DNA tumor virus, such as SV40, papillomavirus, Epstein-Barr virus, or adenovirus (specific for the cell type affected), facilitates but is not generally sufficient for cell immortalization (Khoobyarian and Marczynska 1993). High levels of chromosomal aberration, characteristic of essentially all immortal cell lines (Hayflick 1977; Solomon *et al.* 1991; Pathak *et al.* 1994), are apparent soon after transformation (Stewart and Bacchetti 1991; Ray *et al.* 1996), although abnormal chromosomes are seen only rarely in mortal cells, despite many generations of growth selection *in vivo* and in

culture (Shmookler Reis and Goldstein 1980; Srivastava *et al.* 1985; Tlsty 1990). Immortally-transformed human cell lines support increased amounts of plasmid homologous recombination (Finn *et al.* 1989; Xia *et al.* 1997) and also express elevated transcript levels for the *HsRAD51* recombinase compared to mortal cell strains (Xia *et al.* 1997). This striking association, between cell immortality and increased levels of both recombination and *HsRAD51* transcripts, implicates homologous recombination in the process leading to immortalization. Chromosomal homologous recombination is also elevated in extended-life-span, *mortal* clones of human fibroblasts upon transformation by, and dependent on expression of, SV40 large T antigen (Cheng *et al.* 1997; Shammas *et al.* 1997). Immortal subclones arise rarely ($<10^{-8}$ per cell generation) from such clones, presumably following subsequent mutations (Cheng *et al.* 1997). Thus, recombinational activation precedes, and may predispose cells toward, escape from cellular senescence.

Carcinogenesis

Tumorigenicity of cancer cell lines is highly correlated with their karyotypic instability, with the most tumorigenic cell lines displaying "new" chromosomes in each cell examined (Wiener *et al.* 1976; Gee and Harris 1979) — suggesting a role of genomic rearrangement in cancer etiology. Since genetic recombination has been implicated in chromosomal translocation (Cheng *et al.*, 1997; Shammas *et al.* 1997; Honma *et al.* 1997), loss of heterozygosity (Honma *et al.* 1997), and gene amplification (Windle *et al.* 1991), it may underlie a variety of chromosomal abnormalities observed with high incidence in many neoplasias. Loss of heterozygosity at loci encoding tumor suppressor genes is associated with many cancers (*e.g.,* Takita *et al.* 1997; Honma *et al.* 1997; Orntoft and Wolf 1998; Lee and Testa 1999), and is believed to occur by somatic gene conversion rendering an intact anti-oncogene identical to its defective homolog. Of course, the converse outcome is equally probable, but would have no phenotypic consequences. Loss of heterozygosity at informative genomic markers occurs at increased rates in cancer cell lines (Vogelstein *et al.* 1989; Phear *et al.* 1996).

Certain types of cancer, usually leukemias, are associated with specific chromosomal translocations affecting the expression or activity of oncogenes and/or anti-oncogenes (Leder *et al.* 1983; Lakshmi and Sherbet 1990; Duesberg *et al.* 1991). Analysis of translocation breakpoints is often consistent with aberrant site-specific recombination, which is fully activated during lymphocyte differentiation, but inferences regarding the frequencies of these rare events are compromised by the highly selective nature of such observations (see below).

Site-specific recombination may occasionally utilize incorrect target sequences, causing activation of oncogenes (Bishop, 1987) or deletion of functional alleles of tumor suppressor genes (Weinberg, 1995). These must be exceptional outcomes, even among the products of errant (not-so-site-specific) recombination, but are brought to our attention by the enhanced cell proliferation they elicit. The sequences at rearrangement junctions in a variety of lymphoblastoid cancers can implicate aberrant site-specific recombination (Bergsagel *et al.*, 1996), but also homologous recombination (Super *et al.*, 1997), and illegitimate recombination not involving site-specific signals (Super *et al.*, 1997; Honma *et al.* 1997; Zucman-Rossi *et al.* 1998), in oncogenesis. The relative frequencies of these processes cannot be inferred, but must reflect the influence of sequences in and around specific oncogenes and tumor-suppressor genes characteristically mutated in each cancer type, and perhaps the ensuing cell-growth advantage conferred.

Recombination can be elevated by a variety of agents — chemical carcinogens, radiation and oncogenic viruses (Rodarte-Ramon 1972; Radman *et al.* 1982; Sengstag 1994; Galli and Schiestl 1995; Cheng *et al.* 1997; Li *et al.* 1997) — which might elicit an increased abundance of substrates, and/or of enzymes mediating recombination. Induction of recombination pathways has not been demonstrated in higher eukaryotes, but is suggested by some data (Xia *et al.* 1997). Such induction could serve to protect chromosomal structure, or may result in an increased number of chromatid breaks due to initial endonuclease activity. Double-strand breaks in DNA are potentially lethal, because if they escape the repair system and persist from G_2 into mitosis, they are very likely to recombine in G_1 and thus produce gene rearrangements. Free ends of DNA can also stimulate illegitimate recombination by an end-joining reaction which is thought to initiate breakage-fusion-bridge cycles and may generate dicentric and other translocated chromosomes (Duesberg *et al.* 1991; Sweezy and Fishel 1994).

Cancer is a multistep process (Farber 1984; Weinberg 1988, 1995) wherein a normal cell acquires, by mutation, a variety of characteristics in progressing to hyperplasia, benign transformation, and malignancy. Most human tumors carry at least four independent mutations (Neiman and Hartwell 1991), and colorectal carcinoma is thought to require seven or more genetic events (reviewed in Tsancheva 1997). In the latter group of cancers, mutations in *c-k-ras, c-myc, APC, MCC, DCC,* and *p53* genes are found very frequently. The most common subclass, hereditary nonpolyposis colorectal cancer, is associated with mutations in multiple DNA mismatch-correction and repair genes, including *hMSH2, hMLH1, hPMS1, hPMS2,* and *hMSH6/GTBP* (Lynch and Lynch 1998). Frequent mutations in *p53* (Yamada *et al.* 1991), *APC* (Horii *et al.* 1992), and *c-K-ras* (Miki *et al.* 1991) have also been found in gastric cancer. Mutations to the *p53* gene are probably the most common in human cancers (Hollstein *et al.* 1991; Suzuki *et al.* 1992), and may themselves be recombinogenic (Mekeel *et al.* 1997; Chang *et al.* 1997; Shammas *et al.* 1997). Multiple mutations also enable tumor progression to increasingly invasive and metastatic phenotypes. To become meta-

static, a neoplasia must acquire abilities which are largely or totally lacking in most normal cells, to breach the extracellular matrix, probably by induction of proteases; to bind and traverse the endothelial surface of blood vessels, by over-expressing receptors for endothelial membrane; to survive in blood circulation; and to escape the blood vessel and establish anchorage and proliferation at new foci.

Such successions of phenotypic changes could occur through a series of mutations and clonal expansions, but are normally impeded by the limited proliferative capacity of diploid somatic cells. In this sense, cellular senescence may serve as an anti-oncogenic defense, by limiting the "window of opportunity" for accrual of mutations jointly required for cancer. Both cellular escape from senescence, and subsequent selective progression of the tumor, would be rendered more likely and more rapid following the acquisition of a "hypermutator" phenotype (Finn *et al.* 1989; Neiman and Hartwell 1991; Xia *et al.* , 1997; Loeb 1997, 1998; Jackson and Loeb 1998). Clinical and molecular studies lend indirect support to this argument. In particular, genetic defects known to increase the rate of mutation are associated with high risk and early incidence of cancer (German 1980; Heddle 1991; Cheng 1991; Digweed 1994). Examples include Xeroderma pigmentosum, comprising defects in seven excision repair genes (reviewed in Bootsma *et al.* 1995); hereditary non-polyposis colorectal cancer, defective in any of several mismatch repair genes (Nicolaides *et al.*1994); Ataxia telangiectasia, in which mutations to a regulatory kinase lead to increases in both homologous and site-specific recombination (Savitsky *et al.* 1995); and Werner (Gray *et al.* 1997), Cockayne (van Gool *et al.* 1994) and Bloom syndromes (Ellis *et al.* 1995; Karow *et al.* 1997), each traced to defects in a putative helicase gene.

In the latter four syndromes, genetic mutability is manifest in high rates of chromosomal aberration. Karyotypic alteration has also been implicated in many specific cancer types, such as gliomas (Kruse *et al.* 1998), retinoblastoma (Cavenee *et al.* 1983) and Burkitt's lymphoma (Leder *et al.* 1983). Genetic instability is seen on a much finer scale in those cancers featuring defects in nucleotide excision repair or mismatch repair, but these defects are not evident in the great majority of cancers which instead display chromosomal rearrangement (Lengauer *et al.* 1998). One interpretation would be that most cancers develop from hypermutable progenitors, which can arise by several alternative mechanisms. Apart from the chromosome-breakage syndromes, there are direct data indicating that cancer cells have increased frequency of karyotypic instability (Weiner *et al.* 1976; Gee and Harris 1979; Lengauer *et al.* 1997) and increased rates of loss for marker heterozygosity (Vogelstein *et al.* 1989; Phear *et al.* 1996). Observations that human cells in culture, soon after SV40-T antigen transformation but prior to immortalization, undergo abrupt increases in recombination (Cheng *et al.* 1997; Xia *et al.* 1997) and karyotypic abnormality (Ray *et al.* 1990; Stewart and Bacchetti 1991), suggest that chromosomal instability may also be a characteristic of precancerous cells which ultimately give rise to chromosomally-unstable tumors.

TELOMERIC RECOMBINATION — ANOTHER MEANS TO JUSTIFY AN END

Telomeres, specialized nucleoprotein structures at the ends of chromosomes, contribute to genomic integrity by protecting genomic DNA from degradation and end-to-end joining of chromosomes (Day *et al.* 1993). The linear DNA duplex of each chromosome terminates at both ends in telomeric DNA — tandem arrays comprising hundreds to thousands of short oligonucleotide repeats (GGGTTA in the vertebrates), with a guanosine (G)-rich strand running 5' to 3' toward the terminus, extending beyond a cytosine (C)-rich complementary strand (Zakian 1989). Telomere shortening may lead to chromosomal loss or telomere fusion (Hastie and Allshire, 1989), either of which can be a cell-lethal event. The regulation and maintenance of telomeric DNA length appears to involve telomere-specific binding proteins such as TRF1 and TRF2 (van Steensel and de Lange 1997, 1998; Broccoli *et al.* 1997; Griffith *et al.* 1998; reviewed in Smith and de Lange 1997). TRF2, for which the gene was cloned recently, may serve to protect chromosomes from end-to-end fusions (van Steensel *et al.* 1998), probably by favoring the formation or stability of D-loops at telomere termini (Griffith *et al.* 1999). Telomeric fusion, which is rarely seen in normal human cells, occurs frequently in cancer cells (Dhaliwal *et al.* 1994; Fitzgerald and Morris 1984; Hastie *et al.* 1990), and in several genetic disorders characterized by hypermutability and/or elevated recombination: Ataxia telangiectasia (Kojis *et al.* 1989; Kojis *et al.* 1991), Fanconi anaemia, Bloom syndrome, and Xeroderma pigmentosum (Digweed 1993).

The G-rich strand of telomere DNA is extended by telomerase, a ribonucleoprotein with reverse transcriptase activity. The RNA component of telomerase serves as the template for the addition of short G-rich repeats at the 3' end of telomeric DNA (Blackburn 1992); when templating is blocked by complementary PNA (peptide nucleic acid) oligomers, immortal cells "revert" to senescent growth arrest (Shammas *et al.* 1999). Human germ-line cells possess telomerase activity and maintain telomere length (Kim *et al.* 1994), whereas somatic tissues can have low levels of telomerase or (more commonly) no detectable activity, and their telomeres undergo a progressive shortening as a function of cumulative cell replication (Harley *et al.* 1990; Hastie *et al.* 1990). Telomerases are re-activated in most cancers (Bacchetti and Counter 1995; Shay and Bacchetti 1997) and immortalized cells (Counter *et al.* 1992; Avilion *et al.* 1996; Bacchetti and Counter 1995; Shay and Bacchetti 1997; Shay and Wright 1996), probably due to somatic mutations or epigenetic changes (*e.g.*, demethylations). However a subset of tumor and immortalized cells lack telomerase activity (Mayne *et al.* 1986; Wright *et al.* 1989; Wright and Shay 1992; Bryan *et al.* 1995), and

typically but not invariably have very long telomeres (Bryan *et al.* 1995; Xia *et al.* 1996; Gollahon *et al.* 1998).

Alternative mechanisms thus must exist for the maintenance, or episodic expansion, of telomere length in telomerase-negative cell lines. Although the process remains to be defined for mammalian cell lines, in yeast the regeneration of telomeric arrays can occur by a recombination mechanism apparently involving gene conversion (Wang and Zakian 1990). That a comparable pathway exists in mammals is suggested by detection of extrachromosomal telomeric DNA fragments in telomerase-negative immortal human cell lines, but not in telomerase-proficient lines or in normal human fibroblasts (Ogino *et al.* 1998). Such fragments provide circumstantial evidence of *intra*-telomere recombination (*e.g.,* excision between inverted telomeric repeats), whereas *inter*-telomere recombination would also have to occur in order to extend telomeres and hence replicative potential.

Immortalization of human fibroblasts, whether telomerase-dependent or independent, is associated with mutation or loss of p53 genes (Gollahon 1998). Since loss of p53 function is also implicated in both cell transformation and in the induction of homologous recombination (Xia *et al.* 1997; Mekeel *et al.* 1997), the higher levels of homologous recombination observed in immortal cells (Finn *et al.* 1989; Xia *et al.* 1997) are not entirely surprising. However, recombination between telomeres remains to be demonstrated as a mechanism for telomere extension in telomerase-negative immortal cell lines.

If telomere-telomere recombination can, like telomerase reactivation, extend the replicative life span of mammalian cells, it may provide an elusive link in the causal chain between elevated recombination and cancer. Although it seems plausible that hypermutability (including recombination) might precede cell transformation to immortality, a process requiring multiple mutations, this is an *a posteriori* argument. *It has not been obvious what the basis would be for selection favoring such hypermutable cells prior to oncogene activation or anti-oncogene inactivation.* Lacking such selection, or facing adverse selection, hypermutable cells might never accumulate in sufficient numbers to support complete transformation. As telomerase-negative diploid cells approach the end of their replicative life spans, however, they could avoid the consequences of telomere shortening either by reactivating telomerase or by inter-telomere recombination (including gene conversion). In the latter case, cell selection would directly favor those cells with the highest recombination rates

INDUCTION OF RECOMBINATION BY SV40 LARGE TUMOR ANTIGEN

Simian virus 40 (SV40) is an oncogenic virus with a 5.3-kbp DNA genome. The virus encodes a multifunctional regulatory protein, the large tumor antigen (T antigen), which plays a variety of roles in controlling viral infection. Both small (t) and large (T) tumor antigens are products of early viral genes, generated by alternative splicing. The names reflect their early detection in tumors arising from the introduction of SV40 virus or SV40-transformed cells. T antigen is a complex protein with multiple substrate-specific binding and catalytic activities (DeCaprio *et al.* 1990; Fanning and Knippers 1992; Ray *et al.* 1992; Ray *et al.* 1996). T antigen monomers or oligomers are known to assemble as hexamers in the presence of ATP, and to then form double hexamers on DNA, binding to the entire SV40 core origin of replication (Borowiec and Hurwitz 1988). Bound T antigen unwinds origin DNA (Borowiec and Hurwitz 1988) and acts as a helicase to expand the replication bubble in both directions (Dodson *et al.* 1987). T antigen also interacts with DNA polymerase α, primase-polymerase α complex, and human single-stranded DNA-binding protein (Dornreiter *et al.* 1990; Murakami and Hurwitz 1993), suggesting that it regulates multiple functions in the process of viral DNA replication. T antigen induces host DNA synthesis (Chou and Martin 1975), through a mechanism thought to involve regulation of $p34^{cdc2}$ and cyclin A levels (Oshima *et al.* 1993), direct or indirect regulation of cyclin-dependent kinases via subunit rearrangement (Xiong *et al.* 1993), and binding to p53, $p105^{Rb}$, cellular phosphoprotein p300, and mammalian enhancer-binding transcriptional activator, TEF-1 (Dickmanns *et al.* 1994).

The integration of the SV40 genome and subsequent expression of T antigen, in semi-permissive (human) and non-permissive (rodent) cells, is necessary and sufficient for the establishment and maintenance of their transformed phenotype by several criteria — although not sufficient for immortalization (Tegtmeyer 1975; Brinster *et al.* 1984; Fanning and Knippers 1992; Lemaire *et al.* 1994; Cheng *et al.* 1997). Expression of T antigen in transgenic mice results in hyperplasia, dysplasia, and tumor development (Brinster *et al.* 1984; Hanahan 1985). In addition, T antigen expression favors the immortalization of human (Shay and Wright 1989) and primary rodent cells (Zhu *et al.* 1991) in culture, although at low frequency. The exact mechanisms of transformation and immortalization by T antigen are still unknown. However, possible candidate functions include T antigen induction of cellular transcription (Khandjian *et al.* 1980) or cellular DNA synthesis (Chou and Martin 1975; Dickmanns *et al.* 1994; Shammas *et al.* 1997), interaction with host proteins including growth suppressor proteins (DeCaprio *et al.* 1990; Ray *et al.* 1996), and induction of chromosomal aberrations (Drize *et al.* 1985; Ray *et al.* 1990).

Although expression of T antigen is necessary, it is not sufficient for tumorigenicity by SV40 (Tegtmeyer 1980), which appears to require subsequent mutational events following T antigen expression. T antigen induces DNA rearrangement and chromosomal aberration in expressing cells (Gurney and Gurney, 1989; Ray *et al.* 1990), suggesting genetic destabilization. The underlying mechanism appears to be homologous recombination,

because chromosomal recombination events are markedly increased in human diploid fibroblasts following stable transformation (Cheng *et al.* 1997) or transient transfection (Shammas *et al.* 1997) with SV40 large T antigen. Nine clones of human diploid fibroblasts generated by stable transfection with SV40 large T antigen had recombination levels 2- to >5-fold higher than those in untransfected fibroblasts, dependent on continuous T antigen expression (Cheng *et al.* 1997). Transient transfection of human diploid cells with SV40 T antigen elicited an average of 7-fold induction in chromosomal homologous recombination (Shammas *et al.* 1997).

Elevation of homologous recombination is strongly correlated to induction of host DNA synthesis, by wild-type T antigen and a series of point mutants (Shammas *et al.* 1997). In T antigen-transformed cells, regulation of both recombination and replication may be primarily mediated by a common molecule, p53. T antigen point mutants unable to bind p53 (Ray *et al.* 1996) lose most of their ability to elevate recombination (Shammas *et al.* 1997; and unpublished data). These and other studies (Mekeel *et al.* 1997) imply that loss of p53 function is associated with elevated levels of homologous recombination. Wild type p53 may regulate levels of recombination through its ability to bind and inactivate HsRAD51 recombinase (Stürzbecher 1996). In SV40 T antigen-transformed cells, levels of homologous recombination and of *HsRAD51* transcripts are approximately 4-fold higher than those in untransformed controls (Xia *et al.* 1997). We proposed that T antigen binding to p53 protein can spare RAD51 recombinase activity, which otherwise would be sequestered by p53, resulting in simultaneous increases in DNA recombination and replication. This is consistent with our observation of a tight coupling between inductions of host DNA synthesis and homologous recombination (see next section).

Other mechanisms are clearly also involved. Protein interactions alone, as described above, cannot account for the increase we observed in *RAD51* transcript levels within immortal cell lines, and in diploid fibroblasts upon T antigen introduction (Xia *et al.* 1997). Because T antigen bound to distal regions of DNA can oligomerize in the presence of magnesium ions, to effect DNA looping (Schiedner *et al.* 1990), the ability to physically associate distant regions of DNA may also contribute to its induction of recombination.

FUNCTIONAL COUPLING BETWEEN DNA REPLICATION AND RECOMBINATION

Coupling of recombination to DNA replication is well documented in bacteria (Leonhardt *et al.* 1991; Asai *et al.* 1993; Kogoma 1996; Kogoma 1997). Replication of bacterial plasmid DNA can proceed through recombination-independent or recombination-dependent pathways (reviewed in Viret *et al.* 1991). The synthesis of linear multimeric plasmid molecules, observed in *E. coli* (Cohen and Clark 1986) and *B. subtilis* (Viret and Alonso 1987), requires recombination as an essential intermediate step (Leonhardt *et al.* 1991).

Functional association between replication and recombination has also been observed for bacterial genomic DNA. Interconversion of DNA intermediates in the two processes (Morgan and Severini 1990) may provide a physical basis for this association. Moreover, *Rec A* is required in one of the early steps during an alternative mode of bacterial genomic DNA replication called "stable DNA replication" (Kogoma 1996). A recent model for homologous recombination proposes that D-loops, formed by invasion of single-stranded DNA into a homologous double-stranded DNA duplex during recombination, serve as sites for primosome assembly (Asai *et al.* 1993). The initiation of DNA replication would thus be coupled to the formation of recombination intermediates. Primosome assembly is initiated by the PriA protein, which is required for both recombination and replication in *E. coli,* and has been implicated in recruitment of DnaB helicase to the replication fork. Evidence that the function of PriA required for homologous recombination is primosome assembly rather than its ATPase or helicase activities (Kuchta and Willhelm 1991; Kogoma *et al.* 1996), suggests the involvement of replication in recombination and *vice versa.*

In mammalian cells, T antigen helicase activity unwinds SV40 origin DNA and also interacts with DNA polymerase α:primase complex at the replication fork, thus participating in lagging-strand synthesis during DNA replication (Murakami and Hurwitz 1993). *T antigen (and presumably its cellular counterpart) may play a role similar to PriA and DnaB in the induction of both DNA synthesis and recombination.* Association between SV40 DNA synthesis and recombination of tandem-repeat arrays has been demonstrated *in vitro* (Kawasaki *et al.* 1994).

Although recombination and replication are closely intertwined processes, induction of recombination in eukaryotic cells may (Mills 1978; Grell 1978; Wong and Capecchi 1987; Kawasaki *et al.* 1994; Shammas *et al.* 1997) or may not (Esposito 1978; Fabre 1978; Galli and Schiestl 1995) depend on DNA synthesis. The frequency with which human cells replicate their nuclear DNA declines with passage in culture by 3- to >7-fold (Razin *et al.* 1977), whereas the level of chromosomal homologous recombination remains constant as human fibroblasts traverse their replicative life span (Cheng *et al.* 1997). Therefore the role of *normal* cell cycling does not seem to affect recombination, although induction of recombination may be associated with *abnormal* entry into S phase (Amlasan *et al.* 1995; Cheng *et al.* 1997).

The relationship between recombination and replication has been explored through comparing the induction of these processes by T-antigen, which can drive even senescent cells to enter S phase (Tsuji *et al.* 1983; Gorman and Cristofalo 1985) and induces DNA recombination by 2- to 7-fold (Cheng *et al.* 1997; Shammas *et al.* 1997). The ability of wild-type and point mutants of T antigen to elevate recombination correlates strongly with their ability to induce host DNA synthesis (Shammas

et al. 1997). Moreover, inhibitors of DNA replication (aphidicolin and hydroxyurea) suppress SV40-induced homologous recombination and DNA synthesis to the same extent. As discussed above, the ability of T antigen to inactivate p53 is implicated in its ability to induce both recombination and replication. We also find that other oncogenes (*c-myc, c-H-ras, c-jun, c-fos,* and *erb-B2*) induce recombination and host DNA synthesis concordantly (unpublished data), although none stimulates either process as strongly as T antigen.

In yeast and in human cells, *RAD51* expression is regulated across the cell cycle, reaching a maximum at the G_1/S boundary (Basile *et al.* 1992; Xia *et al.* 1997), just prior to genomic DNA replication. This would be consistent with co-induction of replication and recombination, which might reflect mutual regulation via p53, or other regulation of shared components catalyzing key steps in the two processes.

IMPLICATIONS FOR CANCER ETIOLOGY

Induction of DNA synthesis is a basic requirement for increased cellular proliferation and has been repeatedly implicated in the overall process of oncogenesis, and specifically in the action of tumor promoters (Busser and Lutz 1987; Yusof and Edwards 1990). A rapidly cycling cell may carry more unrepaired DNA damage through to S phase, which may then activate recombinational repair pathways. However, it is also suggestive that the abilities of T antigen mutants to produce cell transformation (Ray *et al.* 1996) correlate well with their abilities to induce host DNA synthesis and recombination (Shammas *et al.* 1997). These biological properties of T antigen may be linked through a common regulator, p53 (Xia *et al.* 1997), which can bind and inactivate HsRAD51 recombinase (Stürzbecher 1996) to limit the level of recombination. Loss of p53 function results in elevation of homologous recombination, whether it is inactivated by T antigen (Shammas *et al.* 1997; Xia *et al.* 1997) or by other mechanisms (Mekeel *et al.* 1997). Products of the breast cancer-associated genes *BRCA1* and *BRCA2* also interact with the HsRAD51 recombinase (Scully *et al.* 1997; Feunteun 1998), thereby regulating recombinational repair of DNA damage (Feunteun 1998). *BRCA* genes may play a role similar to p53 in this respect, since *BRCA1* and *BRCA2* mutations can elevate the overall mutation rate, predisposing cells to cancer (Feunteun 1998). Regulation of recombination-associated molecules can thus affect genomic integrity and the initiation of oncogenesis. The multiple mutations which must coincide within a cell lineage to achieve full transformation would normally have frequencies, individually, on the order of 10^{-6}. If independent, three such events would coincide in 10^{-18} cells, or in fewer than 0.1% of individuals, since over a lifetime our bodies comprise a total of roughly 10^{15} cells. The much higher prevalence of cancer actually observed could be reconciled by either an initiating elevation of mutation rates, or sequential episodes of mutation and clonal expansion (in which case the mutations are *not* independent). Increased mutation, and especially increased DNA rearrangement, are supported by the marked genetic instability of cancer cells (Lengauer *et al.* 1997, 1998). Since recombination has been implicated in several components of chromosomal instability, including translocation (Honma *et al.* 1997; Lengauer *et al.* 1998), loss of heterozygosity (Honma *et al.* 1997), and gene amplification (Windle *et al.* 1991), overexpression or deregulation of recombination pathways may play a central facilitating role in the process of carcinogenesis.

ACKNOWLEDGMENTS

This work was supported by grants (to RJSR) from the U.S. Dept. of Veterans Affairs and from the National Institutes of Health.

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