REVIEW

Hyper-recombination and Bloom's Syndrome: Microbes Again Provide Clues about Cancer

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Once again, a gene family first identified in microorganisms has been linked to human cancer predisposition. The first in a series of intriguing links between microbial genetics and tumorigenesis in man emerged when the genes responsible for xeroderma pigmentosum were identified as homologs of yeast DNA repair genes (for review, see Cleaver 1994; Tanaka and Wood 1994). Another link surfaced 2 years ago with the identification of human colorectal cancer susceptibility and mutations in a homolog of a bacterial and yeast mismatch repair gene (Fishel et al. 1993; Parsons et al. 1993). Earlier this year, the gene responsible for ataxia-telangiectasia was identified as a member of a kinase gene family that may play a role in DNA damage recognition (Greenwell et al. 1995; Hari et al. 1995; Morrow et al. 1995; Savitsky et al. 1995). The latest link is the identification of the Bloom's syndrome gene, *BLM* (Ellis et al. 1995a). *BLM* encodes a protein homologous to the RecQ/Sgs1 DNA helicase subfamily first identified in bacteria and yeast (Umezumi et al. 1990; Gangloff et al. 1994). Mutations in *BLM* and in the yeast homolog *SGS1* confer the common phenotype of hyper-recombination to cells.

Hyper-recombination collectively refers to any cellular state in which the frequency of genetic exchange is elevated. The exchanges can occur, for example, between sister chromatids, allelic genes, or repetitive DNA sequences scattered throughout the genome. The frequency of recombination can be measured genetically between marked intervals and also cytologically as deletions, inversions, translocations, sister chromatid exchanges, and somatic crossovers. In this review we limit our discussion to mutations in

microbes and man that specifically increase recombination.

Bloom's Syndrome

Bloom's syndrome, originally identified as a distinct erythematous facial rash (Bloom 1954), is inherited as an autosomal recessive trait. In addition, individuals affected with Bloom's syndrome are characterized by growth retardation, abnormal spermatogenesis, and an elevated risk of developing cancer (German 1993). Besides these clinical manifestations, cells from individuals with Bloom's syndrome exhibit elevated levels of chromosome breaks and gaps, somatic crossovers, and sister chromatid exchange (German 1993).

Genetic experiments with Bloom's syndrome cell lines showed that transfer of chromosome 15 could complement increased sister chromatid exchange. Further refinements in the genetic map localized the defective gene to a region near 15q26.1 (Ellis et al. 1994). An important breakthrough came when German's group found that they could exploit an early observation. They noticed that although most cells of individuals with Bloom's syndrome exhibit high sister chromatid exchange, there exists a minor population of lymphocytes with low sister chromatid exchange. Cells with low sister chromatid exchange were predominantly found in individuals whose parental alleles were not identical by descent and therefore contained different mutations in *BLM*. An intragenic crossover between the paternal and maternal alleles generated a wild-type gene on one chromosome and a gene containing both parental mutations on the homologous chromosome. After segregation, half of the recombined cell lines became homozygous for polymorphic markers distal to the *BLM* locus, whereas they remained heterozygous for polymorphic markers

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proximal to the locus (Ellis et al. 1995b). Thus, somatic crossover point mapping helped demarcate the boundaries of the gene and narrow down the chromosomal region. It is interesting to note that the hyper-recombinogenic nature of the *blm* mutation probably led to the high incidence of this somatic event. Then, using direct cDNA selection, a clone was isolated that mapped to the candidate region. Translation of the DNA sequence of this clone revealed motifs homologous to the RecQ/Sgs1 helicase subfamily (Ellis et al. 1995a).

BLM and Sgs1 May Be Homologs

RecQ, the founding member of a subfamily of DNA helicases, is a DNA-dependent ATPase that possesses a 3' → 5' DNA translocation activity. It was first identified as a protein involved in the RecF recombination pathway in *Escherichia coli* (Umezū et al. 1990). The absence of RecQ in *E. coli* does not have any discernible phenotype unless a major recombination pathway (RecBCD, SbcBC) is mutated (Clark 1991). In this situation, loss of RecQ function leads to UV sensitivity and to a decrease in recombination. Recently, the RecQ subfamily of helicases has grown. A human helicase, RECQL, was identified biochemically and was shown to possess the same *in vitro* properties as bacterial RecQ (Puranam and Blackshear 1994). Cloning and sequence analysis revealed

that its protein sequence shared significant homology with bacterial RecQ. In addition, another RecQ homolog has been identified as a result of the *Caenorhabditis elegans* sequencing project (GenBank no. U00052, K02F3.1). To date, no disease mapping to the human *RECQL* gene homolog has been reported. For *C. elegans*, the phenotype of mutants is also unknown. In both of these cases, the homology shared with RecQ is restricted to the seven conserved helicase motifs.

BLM is also homologous to Sgs1, a yeast protein (Gangloff et al. 1994). On the basis of a comparison of their primary structure, four lines of evidence suggest that BLM may be the human homolog of Sgs1 (see Fig. 1). First, unlike what is observed for RecQ and RECQL, the sequence similarity between BLM and Sgs1 extends beyond the helicase domains. Second, the size of the two proteins are nearly identical, more than twice the size of the other RecQ family members. Third, the conserved helicase domain is in the same position in the two proteins, situated ~700 amino acids from the amino terminus. Lastly, the amino-terminal domain of BLM contains two clusters of charged amino acid residues paralleling the Sgs1 organization. In addition to the structural similarities of BLM and Sgs1, *blm* and *sgs1* mutant cells both exhibit increased levels of mitotic recombination. In yeast cells, hyper-recombination is manifested by an increased frequency of marker loss. In Bloom's cells, it is

revealed by increased sister chromatid exchange and symmetrical quadriradials, which probably represent exchange figures between homologous chromosomes in metaphase (German 1964). On the other hand, *recQ* mutants exhibit decreased levels of recombination.

Figure 1 Alignment representing the primary amino acid sequence of four members of the RecQ/Sgs1 DNA helicase subfamily. The four proteins share strong sequence similarities within the region corresponding to the helicase domain. BLM and Sgs1 exhibit regions of homology directly surrounding the helicase domain, which are not found within the other members of the subfamily. BLM and Sgs1 also contain two clusters of acidic residues in their amino-terminal moieties that, in Sgs1, have been proposed to mediate transcriptional activation.

Sgs1 May Provide Clues to BLM Function

First identified as an extragenic suppressor of the slow-growth phenotype of topo III-deficient mutants, the *SGS1* locus was shown to interact physically with both topo II

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and topo III (Gangloff et al. 1994; Watt et al. 1995). As both of these DNA topoisomerases are potentially involved in the resolution of replication intermediates (Wang 1991; Fig. 2), it is tempting to speculate that Sgs1 differentially modulates their activity. For example, in the absence of Sgs1, the failure to resolve the intertwined strands generated at the point where replication forks merge would give rise to recombinogenic lesions (e.g., breaks) in a newly replicated chromatid. Repair of such lesions, using information from the sister chromatid, could lead to a sister chromatid exchange event (Fig. 3A). Alternatively, a cell could repair the damaged chromatid using information on the homologous chromosome resulting in a mitotic exchange (Fig. 3B). In the mitotic division following such an exchange, DNA distal to the

crossover can become homozygous, resulting in loss of heterozygosity.

Hyper-recombination and Cancer

A few human disorders have been associated with increased levels of chromosomal rearrangements (see Table 1). A common facet of these "hyper-recombination" syndromes is their high cancer risk. Table 1 also lists selected genes from bacteria and yeast that show increased recombination (Hartwell and Smith 1985; Barnes et al. 1992; Gangloff et al. 1994; Kato and Ogawa 1994; Morrow et al. 1995; Savitsky et al. 1995; Smith and Rothstein 1995). In bacteria, many of these genes were identified using a simple assay in which recombination between direct repeats leads to a selectable phenotype (Konrad 1977). The fre-

Figure 2 Resolution of merging replicons. Two pathways have been proposed for the resolution of intertwined strands found at the site of merging replication forks (Wang 1991). As the two replicons approach one another, topological constraints block the merging forks leaving a small stretch of unreplicated duplex. In the pathway shown at *left*, DNA synthesis proceeds using the leading strands from both replication forks and the DNA is converted into two intertwined duplexes that are decatenated by topo II. In the pathway at *right*, replication is arrested until the single strands are separated by the combined action of the helicase activity of Sgs1 and the decatenation activity of topo III. In this model the Sgs1 helicase tracks along the unreplicated stretch and the associated topo III decatenates the exposed single strands. DNA synthesis is completed by filling in the single-strand gaps. For the sake of clarity, only a few intertwines are depicted. (Adapted from Wang 1991.)

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Figure 3 Recombinational repair of a DNA lesion. The DNA lesion is depicted simply as a double-strand break for purposes of illustration. In both cases, the repair is shown associated with a crossover. (A) Repair of the lesion by homologous recombination between sister chromatids. (B) Repair of the lesion by homologous recombination between homologs. The letters M and m indicate a distal heterozygous gene. Because mitotic segregation of the replicated chromatids into the daughter cells can produce two outcomes, after a crossover, one-half of the segregations generate a cell in which the distal marker has lost its heterozygosity.

quency of this event can be estimated easily and permits the screening of large numbers of individual mutagenized colonies for an altered frequency of recombination. Similarly, most of the hyper-recombination mutations in yeast were isolated using either natural or artificially created direct repeat recombination assays (for review, see Klein 1995). Recently, a similar strategy has been employed in cell lines derived from patients with ataxia-telangiectasia (Meyn 1993) or Werner syndrome (Monnat 1992).

It has been estimated that most individuals, either as a result of somatic mutation or inheritance, are mosaic for cells that are heterozygous for tumor suppressor genes (Loeb 1991). It is only when loss of heterozygosity occurs for one of these genes that the progression toward tumori-

genesis begins (for review, see Knudson 1991). Thus, any mutation that results in an increase in mitotic recombination can potentially lead to an increase in the frequency of homozygosity of one of these recessive genes.

Increased Incidence of Cancer in *blm* Heterozygotes?

Finally, it is interesting to note that some recessive genes, such as retinoblastoma and *hms2*, give rise to cancer as if they were dominant traits when pedigrees are analyzed. This is owing to loss of heterozygosity at the cellular level. In the case of retinoblastoma, any loss of the wild-type copy by a variety of mechanisms, including recombination between homologs, leads to a cell that has lost growth control. Similarly, in *hms2*-associated colorectal cancers, loss of the unaffected gene causes increased DNA slippage during replication generating subsequent mutational events that lead to cancer. Therefore, individuals heterozygous for *blm* may also show an increased incidence of cancer. This would occur in two steps: loss of the unaffected *BLM* gene followed by loss of heterozygosity of a tumor suppressor gene. In this model, the frequency of the second event would be increased as a direct consequence of the *blm* phenotype in those cells. This hypothesis can be tested by measuring the occurrence of sporadic tumors within Bloom's syndrome families and by analyzing such tumors from heterozygous individuals to detect the homozygosity of *blm*.

Conclusions

The study of a basic biological phenomenon, genetic recombination, in simple microbial systems has led to the identification of many genes involved in DNA metabolism (Table 1). With the ability to rapidly identify the genes underlying human diseases that has been facilitated by the Human Genome Project, the corresponding human genes are starting to be identified. Although many insights will be gained from studying the homologous genes found in simpler organisms, it is clear that many questions remain. For example, the *SGS1* gene was cloned as a result of two-hybrid interactions with both topo II and topo III. Does the amino-terminal region of BLM similarly interact with topoisomerases to form a complex? Do *sgs1* mutants exhibit increased sis-

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Table 1. Hyper-recombination Mutations

Organism	Mutation	Gene product/function
<i>Escherichia coli</i>	<i>lig</i>	DNA ligase
	<i>polA</i>	DNA polymerase
	<i>uvrD</i>	DNA helicase
	<i>topB</i>	DNA topoisomerase
<i>Saccharomyces cerevisiae</i>	<i>cdc9</i>	DNA ligase
	<i>cdc2, cdc17</i>	DNA polymerase
	<i>rad3, srs2, and sgs1</i>	DNA helicase
	<i>top1, top2, and top3</i>	DNA topoisomerase
	<i>rfa1</i>	DNA single-stranded binding protein
	<i>esr1 (mec1)</i>	DNA damage recognition?
	46BR (immunodeficient patient)	DNA ligase
<i>Homo sapiens</i>	Bloom's syndrome	DNA helicase?
	ataxia-telangiectasia	DNA damage recognition?
	Werner syndrome	???
		???

This is not an exhaustive survey of all possible hyper-recombination mutations but only lists some whose gene products may be directly involved with DNA metabolism. The bacterial and yeast genes have been reviewed recently (Kline 1995). The human disorders are referenced in the text. It should be noted that a consensus has not been reached for the hyper-recombination phenotype of 46BR.

ter chromatid exchange? Finally, as shown in Table 1, Werner syndrome is another human disease that may cause predisposition to cancer as a result of hyper-recombination. It will be interesting to see whether this disease is also attributable to a mutation in a gene affecting DNA metabolism.

ACKNOWLEDGMENTS

We thank D. Beach, F. Collins, N. Erdeniz, H. Klein, S. Lovett, S. Meyn, R. Monnat, U. Mortensen, V. Racaniello, J. Smith, L. Symington, and H. Zou for critical reading of the manuscript. We also thank N. Ellis and J. German for helpful suggestions on an earlier version of this manuscript. This work was supported by grants from the National Institutes of Health (GM34587 and GM50237) and National Science Foundation (DMB89191812) to R.R.

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Hyper-recombination and Bloom's syndrome: microbes again provide clues about cancer.

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Genome Res. 1995 5: 421-426

Access the most recent version at doi:[10.1101/gr.5.5.421](https://doi.org/10.1101/gr.5.5.421)

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