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Somatic Intragenic Recombination within the Mutated Locus *BLM* Can Correct the High Sister-Chromatid Exchange Phenotype of Bloom Syndrome Cells

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Summary

Cells from persons with Bloom syndrome feature an elevated rate of sister-chromatid exchange (SCE). However, in some affected persons a minority of blood lymphocytes have a normal SCE rate. Persons who inherit the Bloom syndrome gene *BLM* identical by descent from a common ancestor very rarely exhibit this high-SCE/low-SCE mosaicism; conversely, mosaicism arises predominantly in persons who do not share a common ancestor. These population data suggested that most persons with Bloom syndrome in whom the exceptional low-SCE cells arise are not homozygous for a mutation at *BLM* but instead are compound heterozygotes. Following this clue, we carried out a genotype analysis of loci syntenic with *BLM* in 11 persons who exhibited mosaicism. In five of them, polymorphic loci distal to *BLM* that were heterozygous in their high-SCE cells had become homozygous in their low-SCE cells, whereas heterozygous loci proximal to *BLM* remained heterozygous. These observations are interpreted to mean that intragenic recombination between paternally derived and maternally derived mutated sites within *BLM* can generate a functionally wild-type gene and that low-SCE lymphocytes are progeny of a somatic cell in which such intragenic recombination had occurred.

Introduction

Bloom syndrome (BS) is a rare recessively transmitted monogenic trait, the predominant clinical features of which are small body size, immunodeficiency, and a sun-sensitive facial skin lesion (German 1993). Cells from persons with BS exhibit striking genomic instability: (i) Excessive numbers of microscopically visible gaps, breaks, and structurally abnormal chromosomes are visible at mitosis in cells proliferating in vitro (Ray and German 1983; Kuhn et al. 1985). That the chromosome instability also occurs in vivo is indicated by the increased number of micronuclei observed in interphase cells from urine and buccal mucosa (Rosin and German 1985). (ii) Included among the lesions observable in abundance in cultured BS cells are exchanges both between and within chromosomes, notably homologous chromatid interchanges and sister-chromatid exchanges (SCEs). The interpretation of the homologous chromatid interchanges as cytological evidence that somatic crossing-over can occur in mammalian cells (German 1964) has been supported by virological and immunological studies of BS cells (Young and Fisher 1980; Bubley and Schnipper 1987; Langlois et al. 1989; Kusunoki et al. 1994) and confirmed by molecular evidence (Groden et al. 1990; Kusunoki et al. 1994). (iii) Increased numbers of mutations accumulate at both coding and noncoding loci in BS somatic cells (Gupta and Goldstein 1980; Warren et al. 1981; Vijayalaxmi et al. 1983; Langlois et al. 1989). Because BS cells are hypermutable, persons with the syndrome are enormously predisposed to develop various types of neoplasia at a wide variety of sites (German 1993).

The strikingly elevated SCE rate mentioned above (means of >50 SCEs/metaphase) is uniquely characteristic of BS and is present in all affected individuals. It is elevated in all types of BS cells that have been examined: mitogen-stimulated T and B lymphocytes from the blood in short-term culture; Epstein-Barr virus (EBV)–transformed lymphoblastoid cell lines (LCLs) in long-term culture; cells from the bone marrow in short-term culture; and fibroblasts and SV40-transformed fibroblasts in long-term culture. Nevertheless, in approximately one in five affected individuals, a minor population of circulating blood lymphocytes has been detected that features a low (i.e., a normal) SCE rate (means of <10 SCEs/metaphase) (German et al. 1977; German and Schonberg 1980). These low-SCE T and B lymphocytes are demonstrable in mitogen-stimulated cells drawn from the blood; in addition, many of the LCLs that have been

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established from blood lymphocytes taken from persons with BS exhibit a low-SCE phenotype, even in some cases when low-SCE cells were not detected in short-term blood lymphocyte cultures (Henderson and German 1978; Shiraishi et al. 1983). A cell from such a low-SCE LCL can complement one from a high-SCE LCL when the two are fused in vitro (Weksberg et al. 1988), just as can a cell from a low-SCE LCL derived from a normal person (Bryant et al. 1979). Low-SCE cells in nonlymphoid cultures have never been observed, but very possibly the reason for that is that few cells have been appropriately examined in material other than short-term blood cultures and LCLs.

How can the low-SCE cells in some persons with BS be explained? Because hypermutability is such a striking feature of BS cells, it has seemed reasonable to assume that low-SCE cell populations arise via some somatic mutational event. We report here evidence that a mechanism capable of generating high-SCE/low-SCE mosaicism is *somatic intragenic recombination:* crossing-over between the different mutated sites in the paternally derived and the maternally derived genes in a somatic stem cell can generate a functionally wild-type *BLM* that corrects the high-SCE phenotype of BS cells.

Subjects, Material, and Methods

Subjects and Cells Examined

The Bloom's Syndrome Registry (German and Passarge 1989), the source of the population and cytogenetic data and of the specimens analyzed here, contains information from 179 persons with BS, thought to be most of the persons diagnosed since 1954, when the syndrome first was recognized. Only persons with bona fide clinical BS have been accessioned to the Registry, and in almost all of the registered persons the diagnosis has been confirmed cytogenetically. The Registry has obtained and preserved various biological samples as they have become available from affected families. High-SCE fibroblast cell lines derived from skin biopsies or surgical specimens and both high-SCE and low-SCE LCLs established from blood lymphocytes have been developed and cryopreserved from many of the registered persons and their close relatives. The LCLs have been established using either conventional techniques in which many cells are exposed to EBV, whereby the culture is presumably polyclonal, or a limiting dilution technique (Henderson et al. 1977; Henderson and German 1978), whereby an LCL statistically is derived from a single blood lymphocyte.

We analyzed DNA from one low-SCE cell line derived from each of the 10 persons in the Registry from whom it was possible to obtain for comparison DNA from tissues or cultures in which most cells would have exhibited the characteristic high-SCE phenotype. In addition, from an 11th person low-SCE LCLs were examined, even though a high-SCE source was unavailable; she is identified 12(DeTh) in the Registry and was the sister of 11(IaTh) who was 1 of the 10 just mentioned. Also, 10 additional low-SCE LCLs from 11(IaTh) were examined and 1 additional low-SCE LCL from 12(DeTh), making in all an analysis of DNAs from a total of 22 low-SCE LCLs derived from 11 different affected persons (table 1). The DNAs from eight of the fathers and nine of the mothers in the families also were genotyped. The sources of the DNAs from high-SCE cells were one of the following (table 1): cultured fibroblast cell lines derived from skin biopsies (four samples); uncultured blood leukocytes isolated after density gradient centrifugation through Histopaque-1077 or after centrifugation in LeukoPREP tubes (six samples); or uncultured leukocytes from whole blood (two samples).

Cytogenetics

The technique for estimating the SCE rate has been described (German and Alhadeff 1994). In brief, cells were exposed to BrdU-containing culture medium for two cell-division cycles, and metaphase preparations were made. They were stained with the dye 33258 Hoechst, exposed overnight to an incandescent light bulb, incubated 2 h in 60°C water, stained with Giemsa, and examined by light microscopy. The number of exchanges was scored in metaphases in which the staining of the sister chromatids was well differentiated (light in the bifilarly substituted sister chromatid, dark in the unifilarly).

In order to determine whether two microscopically normal-appearing chromosome 15s were present in selected low-SCE LCLs, 20 or more G-banded metaphases were examined, including at least 2–5 so-called prometaphases.

DNA Isolation and Genotype Analysis

For the present investigation, cryopreserved cells from the Registry supplemented in a few families by fresh blood samples constituted the material from which DNAs were prepared (table 1). Preparation of DNA samples, oligonucleotide primers employed, and conditions for PCR amplification of microsatellite polymorphisms on chromosome 15 have been described elsewhere (German et al. 1994*b;* Ellis et al. 1994).

Results

Population Evidence for Heterogeneity of Mutant Alleles at BLM

Analysis of the population of individuals with BS followed in the Bloom's Syndrome Registry provided an important clue to the understanding of the molecular mechanism underlying the high-SCE/low-SCE mosa-

Table 1

Sources of the Low-SCE and the High-SCE DNAs That Were Genotyped

Individual Genotyped[a]	Cells with Low-SCE Rates[b]	Cells with High-SCE Rates[b]
11(IaTh)	LCLs* (11 lines)	CBL
PF	LCL	
PM	LCL	
12(DeTh)	LCL* (2 lines)	
26(SaTi)	LCL	FBL
PF	LCL	
PM	LCL	
40(DoRoe)	LCL	FBL
PM	LCL	
54(AlTu)	LCL	FCL, FBL
PF	WB	
59(FrFit)	LCL*	FCL, FBL
PF	LCL	
PM	FCL	
65(AnPa)	LCL	WB
PF	WB	
PM	WB	
86(NoKi)	LCL	FCL
PF	LCL	
PM	LCL	
87(AlFra)	LCL	FCL
PF	LCL	
PM	WB	
111(JaKir)	LCL	CWB
PM	LCL	
NR8(KeSol)	LCL	FBL
PF	WB	
PM	LCL	

[a] The persons with BS are identified as in the Bloom's Syndrome Registry. PF = father of the person with BS; and PM = mother of that person.

[b] CBL = mononuclear cells purified by Ficoll density gradient or LeukoPREP-tube centrifugation and cryopreserved with DMSO; CWB = whole blood cryopreserved with DMSO; FBL = blood leukocytes taken from the layer lying on the packed erythrocytes after Histopaque density gradient centrifugation or LeukoPREP-tube centrifugation, then frozen; FCL = fibroblast cell line; LCL = EBV-transformed lymphoblastoid cell line; and WB = fresh whole blood. Those LCLs marked with asterisks (*) were established by a limiting dilution technique and are presumed to be clones. The other LCLs were begun by exposing many lymphocytes to virus and are not presumed to be clones. The LCL from 86(NoKi) was provided to us by K. Tatsumi, the FCL by H. Takebe, both from Kyoto University. The LCL and FCL from 87(AlFra) were obtained from the NIGMS Human Genetic Mutant Cell Repository at the Coriell Institute of Medical Research, their designations there being GM04408A and GM03498D. All other cell lines were established at the New York Blood Center.

icism (German et al., in press). Table 2 classifies the known mosaic and nonmosaic individuals in the Registry with respect to whether the mutant *BLM* was inherited from a common ancestor. The analysis shows that mosaicism very rarely arises in individuals with BS whose parents have inherited their mutant *BLM* genes identical by descent from a common ancestor (3 of 68), i.e., in the subpopulation autozygous at *BLM*. Conversely, mosaicism has been detected in a third of the individuals with BS for whose parents no evidence of common ancestry exists (28 of 83), i.e., in the subpopulation allozygous at *BLM*.

The skewed associations of nonmosaicism with the autozygous subpopulation on the one hand and of mosaicism with the allozygous subpopulation on the other are evidence that individuals with BS in whom a population of low-SCE cells can arise—i.e., some of the persons allozygous at *BLM*—probably carry two different mutations at *BLM*. These data suggested that intragenic recombination between the different mutated sites in the paternally derived and in the maternally derived *BLM* genes in a somatic stem cell could generate a functionally normal gene that would correct the high-SCE phenotype.

Molecular Evidence for Intragenic Recombination

To test this hypothesis we analyzed 12 highly polymorphic markers on chromosome 15 (fig. 1) in DNA derived from high-SCE cells and from low-SCE cells of 11 persons with BS who exhibited mosaicism and in DNA from their parents when available (table 3). The 12 markers consisted of 4 loci, including *FES* (to which *BLM* is tightly linked [German et al. 1994*b;* Ellis et al. 1994]), that cosegregate in CEPH families (referred to here as the *FES* cluster), 2 loci distal to the *FES* cluster, and 6 loci proximal to it. In 5 of these 11 persons, all loci examined distal to and including the *FES* cluster that were heterozygous in their high-SCE cells had become homozygous in their low-SCE cells (the boxed genotypes in table 3; fig. 2), whereas loci proximal to *FES* that were heterozygous in their high-SCE cells remained so in their low-SCE cells. In the remaining six mosaic persons, all the loci examined that were heterozygous in their high-SCE cells remained heterozygous in their low-SCE cells.

In most of the 11 mosaic individuals studied, the genotyping was carried out on a single low-SCE LCL (table 1). However, in one person identified 11(IaTh), 11 low-SCE LCLs were genotyped, each of which had been derived from a different blood lymphocyte. In three of 11(IaTh)'s low-SCE cell lines, constitutionally heterozygous loci at the *FES* cluster and at the one heterozygous locus examined distal to it had become homozygous, whereas constitutionally heterozygous loci proximal to the *FES* cluster remained heterozygous (e.g., the left of his two P^l columns in table 3). In the other eight of 11(IaTh)'s low-SCE cell lines, all heterozygous loci examined remained heterozygous (e.g., the right of his two P^l columns in table 3). These findings indicate that a *minimum* of two circulating low-SCE populations had

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Table 2

Persons with BS Categorized with Respect to their Inheritance of *BLM* from a Common Ancestor and to SCE Rate

Subpopulation	No. of Persons[a]	SCE Rate: High Only	SCE Rate: High and Low[b]
Autozygous at *BLM*[c]:			
Parents are cousins; non–Ashkenazi Jewish	35	33	2[d]
Parents are not cousins; Ashkenazi Jewish	29	28	1[e]
Parents are cousins; Ashkenazi Jewish	4	4	0
Subtotals	68	65 (96%)	3 (4%)
Allozygous at *BLM*[f]:			
Parents are not cousins; non–Ashkenazi Jewish	83	55 (66%)	28 (34%)
Totals	151	120 (79%)	31 (21%)

[a] A total of 179 persons were known to the Bloom's Syndrome Registry at the time of this survey in early 1995. SCE studies had been carried out in the 151 of the 179 tabulated here.

[b] Persons with not only high-SCE cells (>50 SCEs/metaphase), thereby confirming the diagnosis BS, but also some lymphoid cells with a low (normal) number of SCEs (≤10 SCEs/metaphase). Although the number of informatively stained metaphases examined varied both in our laboratory and elsewhere, usually being from a few up to 200, the numbers studied in the two subpopulations were similar, so no bias is apparent.

[c] The autozygous subpopulation is composed of persons with BS for whom evidence exists that they inherited their mutant *BLM* alleles identical by descent. Parental consanguinity was one indicator of autozygosity. The persons with BS referred to here as Ashkenazi Jewish have two Ashkenazi Jewish parents, which is the case in 45 (25%) of the 179 individuals known to the Bloom's Syndrome Registry; they are included in the autozygous subpopulation because a linkage disequilibrium has been demonstrated between *BLM, FES,* and *D15S127* in Ashkenazi Jews with BS (Ellis et al. 1994), which indicates that most of them inherited their two mutant *BLM* alleles from a distant common ancestor. The parents are cousins in two Ashkenazi Jewish families, cytogenetic data being available from only one of them (see pedigree of 79(MeDer) in German et al. [1994*b*]).

[d] These two are Japanese individuals identified in the Bloom's Syndrome Registry 129(MaWat) and 132(HiOka); see text for comments on these exceptional individuals. Low-SCE cell lines derived from them were not available, nor blood for confirmatory cytogenetics here.

[e] Identified in the Registry as 56(JoGr); see text for comments on this exceptional individual. Low-SCE cell lines derived from him were not available.

[f] The allozygous subpopulation is composed of persons with BS from whom no evidence of common ancestry existed, that is, their parents were neither cousins nor both Ashkenazi Jews. Nevertheless, because geographic clustering is known to occur for some cases of BS, the allozygous subpopulation may contain some persons who have inherited *BLM* identical by descent from a common ancestor because of population inbreeding (e.g., see German et al., in press).

been generated independently during development of the lymphoid cell lineages in 11(IaTh).

Exceptions to the Intragenic Recombination Model

We presume that most of the persons allozygous at *BLM* who exhibit high-SCE/low-SCE mosaicism (table 2) probably are compound heterozygotes and arrived at their mosaicism by somatic intragenic recombination. However, three mosaic individuals were observed in the autozygous rather than the allozygous subpopulation (table 2). If these persons are identical by descent at *BLM,* then a mechanism other than intragenic recombination within *BLM* must exist that can generate low-SCE cells in persons with BS.

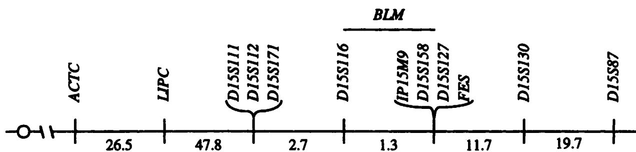

Figure 1 Genetic map of chromosome 15. The array (map distances in cM) shows the 12 polymorphic microsatellites used to genotype DNA samples from high-SCE and low-SCE cells of mosaic individuals with BS and from their parents when available. Loci that so far have failed to segregate by recombinational analysis of selected CEPH families (*curly braces*) are localized to single points on the genetic map (Beckmann et al. 1993). The map position indicated for *BLM* is that deduced from the data obtained in the present study. The *FES* cluster, composed of *FES, D15S127, D15S158,* and *IP15M9,* is contained within a 800-kb YAC and P1 contig; *FES* and *D15S127* themselves are ~30 kb apart (unpublished data obtained jointly with J. Groden and J. Straughen).

To determine whether *BLM* was identical by descent in these exceptional persons, genotype analysis at the polymorphic loci *FES, D15S127, D15S158,* and *IP15M9* was carried out on DNA from high-SCE cells from 129(MaWat), whose parents are cousins and Japanese, and on 56(JoGr) and his parents, both of whom are Ashkenazi Jewish. (DNA from 132(HiOka) was not available.) 129(MaWat) was homozygous at all these loci; we interpret this to mean that he indeed is identical by descent for his mutated *BLM.* Analyses of the DNAs of 56(JoGr) and his parents show the haplotypes of his *BLM*-bearing chromosomes to be *FES**155 D15S127*145 D15S158*95 IP15M9*69* and *FES*155 D15S127*138 D15S158*87 IP15M9*79.*

Table 3

Genotypes at Selected Chromosome 15 Loci of 11 Mosaic Persons with BS and of Their Available Parents

Locus	11(IaTh)[a]					12(DeTh)[b]			25(SaTi)			
	PF	P^l	P^l	P^h	PM	PF	P^l	PM	PF	P^l	P^h	PM
ACTC	88/90	72/90	72/90	72/90	72/92	88/90	72/88	72/92	72/82	72/88	72/88	70/88
LIPC	167/167	165/167	165/167	165/167	165/171	167/167	165/167	165/171	165/167	167/167	167/167	165/167
D15S112									135/149	135/145	135/145	135/145
D15S111	155/155	155/155	155/155	155/155	155/155	155/155	155/155	155/155	145/153	143/153	143/153	143/151
D15S171						117/119	111/119	111/119				
D15S116	A6/A9	A8/A9	A8/A9	A8/A9	A7/A8	A6/A9	A8/A9	A7/A8	A7/A9	A4/A7	A4/A7	A4/A8
FES	151/159	151/151	151/159	151/159[c]	151/155	151/159	151/159	151/155	151/155	151/155	151/155	151/151
D15S127	114/130	134/134	130/134	130/134[c]	134/137	114/130	130/134	134/137	130/147	128/147	128/147	114/128
D15S158	85/95	87/87	87/95	87/95[c]	87/95	85/95	87/95	87/95	85/95	85/87	85/87	87/87
IP15M9	69/83	85/85	69/85	69/85[c]	69/85	69/83	69/85	69/85	69/69	67/69	67/69	67/81
D15S130	219/227	227/227	227/227	227/227	219/227	219/227	227/227	219/227	219/225	219/227	219/227	217/227
D15S87	109/113	111/111	109/111	111/111[c]	111/117	109/113	109/111	111/117	115/117	105/117	105/117	105/111

Locus	40(DoRoe)			54(AlTu)			59(FrFit)			
	P^l	P^h	PM	PF	P^l	P^h	PF	P^l	P^h	PM
ACTC	70/72	70/72	72/86				90/96	92/96	92/96	86/92
LIPC	167/171	167/171	171/171		165/171	165/171	171/171	171/171	171/171	167/171
D15S112							135/147	135/147	135/147	TF
D15S111	143/155	143/155	143/143		143/155	143/155	143/143	143/145	143/145	145/155
D15S171	117/119	117/119	115/119							
D15S116	A7/A8	A7/A8	A7/A7	A2/A7	A7/A11	A7/A11	A3/A5	A3/A10	A3/A10	A10/A11
FES	159/159	159/159	159/159	155/155	155/155	155/159	159/159	159/159	151/159[d]	151/159[d]
D15S127	128/128	128/128	128/135	145/147	147/147	147/147	128/138	138/138	135/138	135/135
D15S158	87/87	87/87	87/97	93/95	93/93	87/93	87/87	87/87	87/89	85/89
IP15M9	81/81	81/81	69/81	69/83	83/83	81/83	69/81	69/69	69/83	69/83
D15S130	219/223	219/223	223/227		217/217	217/225	219/219	219/219	219/225	225/225
D15S87	111/111	111/111	107/111		113/113	113/129	119/121	119/119	113/119	107/113

(*continued*)

Table 3 (continued)

Locus	65 (AnPa)				86(NoKi)			
	PF	P^l	P^h	PM	PF	P^l	P^h	PM
ACTC					72/90	90/92	90/92	90/92
LIPC	167/171	167/167	167/167	167/167	165/171	165/165	165/165	165/171
D15S112					135/147	147/147	147/147	147/147
D15S111	145/151	151/155	151/155	151/155	155/155	155/155	155/155	143/155
D15S171					117/123	119/123	119/123	119/119
D15S116	A9/A10	A7/A10	A7/A10	A7/A12	A5/A10	A5/A6	A5/A6	A6/A9
FES	151/159	151/163	151/163	163/163	155/155	155/155	155/155	151/155
D15S127	116/128	128/130	128/130	128/130	151/153	138/153	138/153	128/138
D15S158	87/93	87/93	87/93	87/87	87/93	87/87	87/87	87/87
IP15M9	69/83	TF	69/83	83/85	67/85	83/85	83/85	83/83
D15S130	217/219	219/227	219/227	217/227	227/227	227/227	227/227	217/227
D15S87	107/115	107/107	107/107	107/107	107/107	107/121	107/121	113/121

Locus	87(AlFra)				111(JaKir)			NR8(KeSol)			
	PF	P^l	P^h	PM	P^l	P^h	PM	PF	P^l	P^h	PM
ACTC	72/84	70/84	70/84	70/84							
LIPC	167/167	167/171	167/171	171/171	171/171	171/171	167/171	167/171	167/171	167/171	167/171
D15S112	TF	135/145	135/145	135/135							
D15S111	143/151	151/155	151/155	155/155	143/143	143/143	143/143	145/155	143/145	143/145	143/143
D15S171	117/119	117/119	117/119	113/119							
D15S116	A8/A9	A7/A9	A7/A9	A7/A7	A10/A11	A10/A11	A10/A11	A7/A8	A8/A8	A8/A8	A8/A11
FES	151/155	155/155	151/155	151/155	151/155	151/155	151/159	151/155	151/151	151/159	159/163
D15S127	134/147	147/147	126/147	126/138	116/130	116/130	116/128	126/134	134/134	130/134	130/130
D15S158	87/93	93/93	85/93	85/93	87/87	87/87	87/87	87/87	87/87	87/95	95/95
IP15M9	81/85	85/85	85/85	71/85	69/83	69/83	69/83	69/83	83/83	69/83	69/69
D15S130	219/229	219/219	219/227	227/227	219/227	219/227	219/227	219/229	229/229	227/229	219/227
D15S87	107/113	107/107	107/117	113/117	113/117	113/117	113/117	107/113	113/113	113/119	107/119

NOTE.—The families are identified by the Bloom's Syndrome Registry designation of the propositi. The table includes all those individuals in the Registry who satisfy the following three criteria: (i) some of their lymphocytes have been shown to have a low rate of SCE; (ii) DNAs were available from their low-SCE cells (P^l); and (iii) DNAs were available from their high-SCE cells (P^h). Genotypes of available fathers (PF) and mothers (PM) also are tabulated. Some genotypes do not appear in the table, some because of technical failure (TF) and others because they were not done (shown as blank spaces). Genotypes in persons with BS in whom allele losses were detected are boxed. The observed allele losses were either from just the paternally derived chromosome (one case) or from just the maternally derived chromosomes (four cases). The homozygosity detected in 40(DoRoe) at all loci in the *FES* cluster could be taken as evidence for identity by descent in this person; however, 40(DoRoe)'s parents are not cousins and do not share common ancestries, the father's kin coming from Bessarabia and the mother's from Germany.

[a] Two genotypes of P^l cells from 11(IaTh) are shown. The first column is a genotype from one of the three LCLs in which allele losses had occurred, and the second is from one of the eight in which allele losses had not occurred. See text.

[b] The two LCLs examined from 12(DeTh) had the same genotypes.

[c] See penultimate sentence in legend to figure 2.

[d] See final sentence in legend to figure 2.

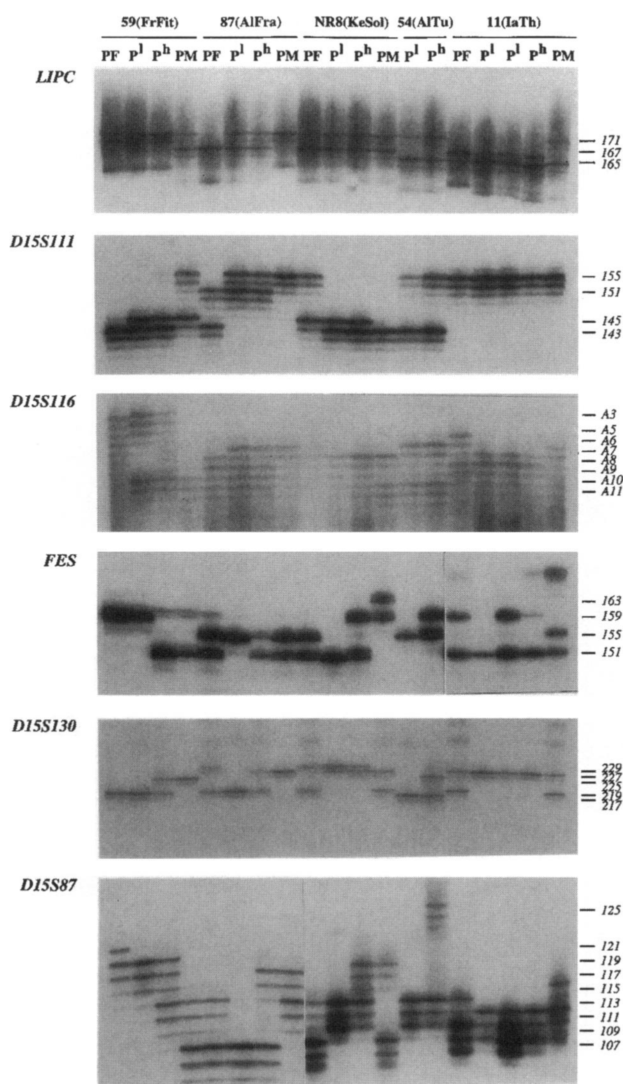

Figure 2 Molecular evidence of allele losses at loci distal but not proximal to *BLM* in 5 of 11 persons examined who exhibit high-SCE/low-SCE mosaicism. Autoradiographic patterns are shown for three polymorphic loci proximal to *BLM—LIPC, D15S111,* and *D15S116*—and three polymorphic loci distal to *BLM—FES, D15S130,* and *D15S87* (see fig. 1). PCRs were carried out using DNAs prepared from both low-SCE cells (P^l) and high-SCE cells (P^h) of the mosaic persons with BS, and using DNAs prepared from their fathers (PF) and mothers (PM) when available. The persons with BS are identified as in the Bloom's Syndrome Registry. Loci and allele names are indicated. For 11(IaTh), patterns are shown employing DNAs from 2 of his 11 cell lines tested that feature a low-SCE rate—one representative cell line in which allele losses were detected and another cell line in which they were not. Each of these 11 low-SCE cell lines had been established from a single blood lymphocyte. In 11(IaTh)'s "high-SCE" DNA, the paternal allele consistently appears weaker in the autoradiographic patterns employing loci distal to *BLM;* because the "high-SCE" DNA was prepared from blood lymphocytes in which 48% of the metaphases exhibited a low-SCE rate after mitogen stimulation (see German et al. 1977), the allele losses are apparent in this DNA sample prepared from lymphocytes taken directly from blood. Finally, the *C3* allele consistently amplified weakly in 59(FrFit) in the genotypes determined at *FES* using DNA from PM and P^h cells, for unknown reasons.

The upper of these two is a haplotype at *FES* and *D15S127* that is commonly found in Ashkenazi Jews with BS and that is thought to have descended from the postulated founder (Ellis et al. 1994; German et al. 1994*a*); the lower has not been detected before in any other Ashkenazi Jew with BS (N. A. Ellis and J. German, unpublished observations). Although analysis of these polymorphic loci failed to reveal evidence for identity by descent from a common ancestor, haplotype analysis at loci immediately proximal to *BLM* suggests that 56(JoGr) in fact is homozygous at *BLM* (N. A. Ellis and J. German, unpublished observations).

Cytogenetic Evidence against Chromosomal Loss or Deletion

The observed allele losses in certain of the low-SCE LCLs examined is not explained by chromosome loss, because heterozygosity proximal to *FES* had been maintained in them. Segmental deletion of one chromosome 15 also would account for the molecular results if it included the entire span of loci from *FES* in 15q26.1 to *D15S87,* the latter a locus near 15qter (Beckmann et al. 1993). Because loss of chromosomal material from 15q26.1 to 15qter would be visible cytogenetically, a microscopic examination was made of the distal segments of 15q in high-resolution G-banded chromosomes: two chromosome 15s with identical and normal-appearing distal segments were present in each of the low-SCE cell lines in which allele losses had been detected in the molecular analysis.

Discussion

Somatic *inter*genic crossing-over was first shown to occur in *Drosophila melanogaster* (Stern 1936) and later in *Aspergillus* (Pontecorvo and Kafer 1958). Evidence that crossing-over occurs in diploid, non-neoplastic human somatic cells consists of (i) the cytological, flow cytometric, and molecular evidence from BS, already cited and (ii) cytogenetic and molecular evidence for crossing-over in various human neoplasms, the first observations having been made in retinoblastoma (Cavanee et al. 1985). *Intra*genic crossing-over first was detected and interpreted as such in diploid yeast cells (Roman 1956). In principle, somatic intragenic recombination could and would occur in mammalian cells, but the frequency of such events presumably is so small that it has not been documented until now.

The Intragenic Recombination Model

Mosaicism rarely arises in the subpopulation autozygous at *BLM;* instead, it arises predominantly in the subpopulation allozygous at *BLM,* indicating that compound heterozygosity is a requisite. This population correlation suggested a model for the origin of the mosaicism: A compound heterozygote for noncomplementing

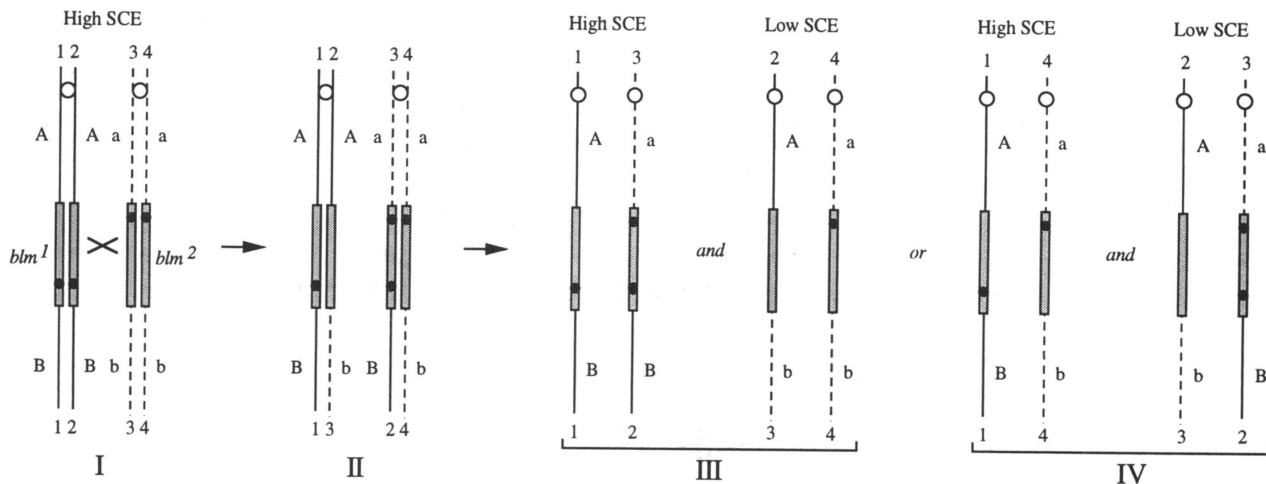

Figure 3 Model to generate a wild-type *BLM* locus via somatic intragenic recombination: I, The two pairs of sister chromatids of the homologous chromosome 15s in a G2 somatic cell of a BS compound heterozygote (*blm*[1]/*blm*[2]) are numbered 1-1 to 4-4. Each of the two mutations in *BLM* (*hatched rectangle*), represented by blackened dots, one inherited from each parent, is at a different site in the gene. Flanking markers proximal to and distal to the mutated loci are heterozygous *A/a* and *B/b*. II, After homologous interchange between chromatids 2-2 and 3-3 at a point between the sites of mutation within *BLM* (the × in I), a wild-type gene is reconstituted on chromatid 2-3 that corrects to normal the high-SCE phenotype of BS cells. Simultaneously, the distal marker *b* becomes associated with the wild-type gene on chromatid 2-3. III and IV, By segregational events at mitosis, two pairs of daughter cells are possible. If chromatids 2-3 and 4-4 cosegregate to the same daughter cell, the distal marker becomes homozygous *b/b* (the diagram on the right side of III). On the other hand, if chromatids 2-3 and 3-2 cosegregate, the distal marker remains heterozygous *b/B* (the diagram on the right side of IV). The proximal marker remains heterozygous *A/a* in both cases. In the sister cells, segregation of chromatids 1-1 and 4-4 (the diagram on the left side of IV) or of chromatids 1-1 and 3-2 (the diagram on the left side of III) do not give rise to a low-SCE phenotype. (Note that cells of heterozygous carriers of a mutation at *BLM*, namely, *blm*/+ parents of persons with BS, display a low-SCE rate.)

mutations at *BLM* has clinical BS (fig. 3, I)—the same phenotype exhibited by homozygotes for any one of the postulated mutations. Homologous recombination in somatic cells can and occasionally will occur between the sites of the blm^1 and blm^2 mutations themselves (the × in fig. 3, I), and it generates a wild-type gene at *BLM* (fig. 3, II, sister chromatid 2-3). One of such a recombinant cell's two daughters then will inherit the newly generated, functionally normal gene, and the frequency of SCEs in that daughter cell and its progeny will be low (fig. 3, diagrams on the right sides of III and IV). The chromatid that contains the functionally normal copy of *BLM* that was generated by recombination can segregate with either the recombinant or the nonrecombinant chromatid of the homologous chromosome (cf. the diagrams on the right sides of IV and III, respectively, in fig. 3). Consequently, in half of the cases of high-SCE/low-SCE mosaicism, polymorphic loci distal to *BLM* that are heterozygous in high-SCE cells will be homozygous in low-SCE cells, whereas heterozygous loci proximal to *BLM* will remain heterozygous. In the other half of the cases, polymorphic loci both distal and proximal to *BLM* that are heterozygous in high-SCE cells will remain heterozygous in low-SCE cells.

Evidence for the Intragenic Recombination Model

The molecular genetic analysis presented here provides powerful support for the somatic intragenic recombination model (presented in fig. 3). In nearly half of the individuals examined—the proportion predicted by the model—reduction to homozygosity had taken place at the *FES* cluster and at informative loci distal to it but not at loci proximal to it (table 3; fig. 2). Because heterozygous loci proximal to the *FES* cluster remained heterozygous in the low-SCE cells, diparental diploidy of chromosome 15 was retained, and cytogenetic observation confirmed the presence of two normal-appearing chromosome 15s. Consequently, neither deletion nor monosomy of chromosome 15 explains the reduction to homozygosity that was observed in low-SCE cell lines from the 5 of 11 mosaic persons studied. These cytogenetic, population, and molecular observations indicate that somatic intragenic recombination is the explanation for most cases of high-SCE/low-SCE mosaicism in BS.

Because loci distal to and including the *FES* cluster had become homozygous, whereas loci proximal to it had not, the putative points of intragenic exchange are situated in a 1.3-cM interval bounded by the *FES* cluster and *D15S116* (see fig. 1). In a meiotic analysis of consanguineous families genotyped at polymorphic loci in the *FES* region (N. A. Ellis and J. German, unpublished data), we identified six recombinational events between *BLM* and *D15S116* and one between *BLM* and *IP15M9*, a member of the *FES* cluster. Therefore, the study of mosaic persons here has as-

signed *BLM* to the same 1.3-cM interval defined by the molecular genetic analysis of BS families.

Pinpointing *BLM* to a genetic interval of 1.3 cM is a major step forward in the positional cloning of *BLM*. More important, the cloning effort now is facilitated greatly by the availability of low-SCE cell lines in which, with the appropriate set of closely spaced polymorphic loci, a shift from heterozygosity to homozygosity that occurs within *BLM* itself will signal that gene's position exactly.

Evidence for an Alternative Mechanism That Generates Mosaicism

The persons with BS in the autozygous subpopulation are presumed to be homozygous at *BLM;* thus, intragenic recombination at *BLM* in these persons' somatic stem cells should not generate high-SCE/low-SCE mosaicism. However, three exceptional persons autozygous at *BLM* were detected in whom high-SCE/low-SCE mosaicism had arisen (table 2). Genotyping in one of those whose parents are cousins, 129(MaWat), disclosed that he is homozygous at loci tightly linked to *BLM,* indicating that he also must be homozygous for a mutation at *BLM*. Haplotype analysis in the one whose parents are Ashkenazi Jewish, 56(JoGr), initially failed to uncover evidence for his having inherited this region of his genome identical by descent; however, haplotype analysis employing polymorphic loci immediately proximal to the *FES* cluster indicates that he is identical by descent at *BLM*. These observations of high-SCE/low-SCE mosaicism in persons judged to be homozygous at *BLM* suggests that occasionally a mechanism other than intragenic recombination operates to generate mosaicism.

Conclusion

Cytogenetic, population, and molecular analyses of the high-SCE/low-SCE mosaicism that arises in some persons with BS has revealed the existence of a previously unrecognized genetic phenomenon in mammalian somatic cells, namely, somatic intragenic recombination. Crossing-over between different mutated sites within *BLM* is the most plausible explanation for the reduction to homozygosity observed at loci distal to *BLM* in LCLs that exhibit a normal SCE rate. Thus, intragenic recombination becomes the most recently recognized mechanism for the generation of genetic diversity among mammalian somatic cells.

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References

Beckmann JS, Tomfohrde J, Barnes RI, Broux O, Richard I, Weissenbach J, Williams M, et al (1993) A linkage map of human chromosome 15 with an average resolution of 2 cM and containing 55 polymorphic microsatellites. Hum Mol Genet 2:2019–2030

Bryant EM, Hoehn H, Martin GM (1979) Normalisation of sister chromatid exchange frequencies in Bloom's syndrome by euploid cell hybridisation. Nature 279:795–796

Bubley G, Schnipper L (1986) The effect of Bloom's syndrome fibroblasts on genetic recombination and mutation. Proc Annu Meet Am Assoc Cancer Res 27:101

Cavanee WK, Hansen MF, Nordenskjold M, Kock E, Maumenee I, Squire JA, Phillips RA, et al (1985) Genetic origin of mutations predisposing to retinoblastoma. Science 228:501–503

Ellis NA, Roe AM, Kozloski J, Proytcheva M, Falk C, German J (1994) Linkage disequilibrium between the *FES, D15S127,* and *BLM* loci in Ashkenazi Jews with Bloom syndrome. Am J Hum Genet 55:453–460

German J (1964) Cytological evidence for crossing-over *in vitro* in human lymphoid cells. Science 144:298–301

——— (1993) Bloom syndrome: a mendelian prototype of somatic mutational disease. Medicine 72:393–406

German J, Alhadeff B (1994) Sister-chromatid exchange (SCE) analysis. In: Dracopoli NC, Haines JL, Korf BR, Moir DT, Morton CC, Seidman CE, Seidman JG, et al (eds) Current protocols in human genetics, John Wiley & Sons, New York, pp 8.6.1–8.6.10

German J, Ellis NA, Proytcheva M. Bloom's syndrome. XIX. Cytogenetic and population evidence for genetic heterogeneity. Clin Genet (in press)

German J, Lennon D, Proytcheva M, Falk C, Ellis N (1994*a*) Linkage disequilibrium between *BLM, FES, D15S127,* and *IP15M9* in Ashkenazi Jews with Bloom's syndrome. Am J Hum Genet Suppl 55:A152

German J, Passarge E (1989) Bloom syndrome. XII. Report from the Registry for 1987. Clin Genet 35:57–69

German J, Roe AM, Leppert M, Ellis NA (1994*b*) Bloom syndrome: an analysis of consanguineous families assigns the locus mutated to chromosome band 15q26.1. Proc Natl Acad Sci USA 91:6669–6673

German J, Schonberg S (1980) Bloom's syndrome. IX. Review of cytological and biochemical aspects. In: Gelboin HV, MacMahon B, Matsushima T, Sugimura T, Takayama S, Takebe H (eds) Genetic and environmental factors in experimental and human cancer. Japan Scientific Societies, Tokyo, pp 175–186

German J, Schonberg S, Louie E, Chaganti RSK (1977) Bloom's syndrome. IV. Sister-chromatid exchanges in lymphocytes. Am J Hum Genet 29:248–255

Groden J, Nakamura Y, German J (1990) Molecular evidence that homologous recombination occurs in proliferating human somatic cells. Proc Natl Acad Sci USA 87:4315–4319

Gupta RS, Goldstein S (1980) Diphtheria toxin resistance in human fibroblast cell strains from normal and cancer-prone individuals. Mutat Res 73:331–338

Henderson E, German J (1978) Development and characterization of lymphoblastoid cell lines (LCLs) from "chromo-

some breakage syndromes" and related genetic disorders. J Supramol Structure Suppl 2:83

Henderson E, Miller G, Robinson J, Heston L (1977) Efficiency of transformation of lymphocytes by Epstein-Barr virus. Virology 76:152–163

Kuhn EM, Therman E, Denniston C (1985) Mitotic chiasmata, gene density, and oncogenes. Hum Genet 70:1–5

Kusunoki Y, Hayashi T, Hirai Y, Kushiro J, Tatsumi K, Kurihara T, Zghal M, et al (1994) Increased rate of spontaneous mitotic recombination in T lymphocytes from a Bloom's syndrome patient using a flow-cytometric assay at *HLA-A* locus. Jpn J Cancer Res 85:610–618

Langlois RG, Bigbee WL, Jensen RH, German J (1989) Evidence for increased *in vivo* mutation and somatic recombination in Bloom's syndrome. Proc Natl Acad Sci USA 86:670–674

Pontecorvo G, Kafer E (1958) Genetic analysis by means of mitotic recombination. Adv Genet 9:71–104

Ray JH, German J (1983) The cytogenetics of the "chromosome-breakage syndromes." In: German J (ed) Chromosome mutation and neoplasia. Alan R. Liss, New York, pp 135–167

Rosin MP, German J (1985) Evidence for chromosome instability *in vivo* in Bloom syndrome: increased numbers of micronuclei in exfoliated cells. Hum Genet 71:187–191

Roman H (1956) Studies of gene mutation in *Saccharomyces*. Cold Spring Harbor Symp Quant Biol 21:175–183

Shiraishi Y, Yoshimoto S, Miyoshi I, Kondo N, Orii T, Sandberg AA (1983) Dimorphism of sister chromatid exchange in Bloom's syndrome B- and T-cell lines transformed with Epstein-Barr and adult T-cell leukemia viruses. Cancer Res 43:3836–3840

Stern C (1936) Somatic recombination within the white locus of *Drosophila* melanogaster. Genetics 21:625–730

Vijayalaxmi, Evans HJ, Ray JH, German J (1983) Bloom's syndrome: evidence for an increased mutation frequency in vivo. Science 221:851–853

Warren ST, Schultz RA, Chang CC, Wade MH, Trosko JE (1981) Elevated spontaneous mutation rate in Bloom syndrome fibroblasts. Proc Natl Acad Sci USA 78:3133–3137

Weksberg R, Smith C, Anson-Cartwright L, Maloney K (1988) Bloom syndrome: a single complementation group defines patients of diverse ethnic origin. Am J Hum Genet 42:816–824