Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7968–7972, September 1992
Genetics

Elevated sister chromatid exchange phenotype of Bloom syndrome cells is complemented by human chromosome 15

(genetic complementation/chromosome mapping/DNA repair)

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Communicated by Evelyn M. Witkin, May 4, 1992

ABSTRACT **Bloom syndrome (BSx) is a rare autosomal-recessive chromosome-instability disorder manifested by a constellation of clinical features including a significant predisposition to early onset of neoplasia. BSx cells display cytogenetic abnormalities, the pathognomonic feature being an increased rate of spontaneous sister chromatid exchanges (SCEs), 10- to 15-fold more frequent than SCEs seen in control cells. Identification of the primary biochemical defect in BSx and its relationship to SCE frequency and neoplasia have been complicated by reports that BSx cell lines exhibit defects in the structure and/or activity of a number of different enzymes. The rare occurrence of the disorder and lack of informative families have precluded mapping of the primary defect by standard linkage analysis. We have utilized BSx cells as recipients for microcell-mediated chromosome transfer to map a locus that renders complementation of the elevated SCE phenotype. Studies with the BSx cell line GM08505 demonstrated a stable frequency of SCEs 10-fold higher than control values, offering a phenotype suitable for complementation studies. Transfer of different independent human chromosomes from somatic cell hybrids into BSx cells permitted identification of a single chromosome that dramatically reduced the SCE frequency to a level near that seen in control cells. Detailed characterization revealed this complementing element to be human chromosome 15.**

Bloom syndrome (BSx) is a rare autosomal-recessive disorder, recognized clinically by proportional dwarfing, characteristic erythema, chronic infections (1), and subtle craniofacial abnormalities (2). A variety of neoplasms, most notably carcinomas and leukemias, are more frequent in BSx patients with onset usually before the age of 30 (3). BSx cells in culture exhibit a number of cytogenetic abnormalities including increases in chromosome breakage and multiradial formation. However, the pathognomonic feature of this disorder is an increased frequency of spontaneous sister chromatid exchanges (SCEs) that is 10- to 15-fold higher than SCEs seen in control cells (4). Although much remains to be learned about chromosome breakage, SCE formation, and factors that influence these events, the elevated occurrence of neoplasms observed in BSx patients is presumed to reflect the biological consequences of the high rate of chromosome aberrations, including SCEs (3, 5–7). Consistent with this hypothesis, BSx exhibits both *in vivo* and *in vitro* spontaneous mutation rates significantly greater than those seen in controls (7–9).

Biochemical approaches to an understanding of BSx have resulted in a number of controversies. Although most enzymatic activities associated with DNA replication, recombination, and repair appear normal in BSx cells, subtle alterations in the activity and/or structure of DNA ligase I (10–12), uracil DNA glycosylase (13), topoisomerase II (14), thymidylate synthetase (15), and superoxide dismutase (16) have been reported. Efforts by others have failed to reproduce several of these protein defects (17, 18) and recent examination of DNA ligase I cDNA isolated from BSx cells has failed to reveal mutations (19, 20). The observation that individual BSx cell lines each exhibit alterations in all of the aforementioned proteins for which they have been examined is consistent with the proposal that this disorder is represented by a single gene defect. Furthermore, a lack of genetic heterogeneity in BSx is supported by the observations that cell fusions between control and BSx cells yield complementation of the SCE phenotype, whereas fusions between various BSx cell lines representing unique ethnic origin fail to result in any change in SCE frequency (21). Collectively, these data suggest that the primary BSx defect leads to a cascade of events that has the potential to influence numerous proteins.

The rare occurrence of many autosomal-recessive disorders, including BSx, results in a lack of informative families precluding efforts to map gene defects by classic genetic linkage analysis. This laboratory (22–25) and others (26, 27) have shown that phenotypic correction after microcell-mediated chromosome transfer (MMCT) can be used to map gene defects in disorders that offer stable recessive cellular phenotypes. Data presented here demonstrate that the elevated SCE phenotype in the BSx cell line GM08505 is sufficiently stable to permit gene mapping by MMCT complementation. Correction of the elevated SCE phenotype is shown to correlate with introduction of normal human chromosome 15.

MATERIALS AND METHODS

Cell Culture. Experiments utilized simian virus 40-transformed human fibroblast cell lines representing BSx (GM08505), Lesch–Nyhan syndrome (GM00847), xeroderma pigmentosum complementation group D (GM08207), and normal control cells (VA-13, subline 2RA). Additional controls included the fibrosarcoma cell line HT1080 and a subclone of the osteogenic sarcoma cell line 143 (28), which had been transfected with both the neomycin-resistance and herpes simplex thymidine kinase genes (143neoTK). Mouse A9 cells and mouse–human hybrids constructed with mouse A9 cells (22–25) were also used. MMCT (22–25) and SCE analyses (29) were performed as described.

PCR. PCR amplifications were performed in an Ericomp TwinBlock thermal cycler using *Taq* polymerase and the supplier's recommended reaction conditions (Cetus). Sequences for primers representing the Prader-Willi region (PWCR, D15S10), obtained from the on-line Genome Data Base (Johns Hopkins University), were 5′-GTAACACTAT-

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Abbreviations: BSx, Bloom syndrome; SCE, sister chromatid exchange; MMCT, microcell-mediated chromosome transfer; IRS, interspersed repetitive sequence.

GAATTGTTAGTG and 5'-GACAGCTGAACGTAGTTAAAG. Primers representing exon 2 of the human limb deformity/formin (*LD*) gene (5'-TTGAGATGAAGAACAAACTCGAG and 5'-GACTCCGTTTTTCAGGAAGGGGA) and the isovaleryl-coenzyme A dehydrogenase cDNA (5'-AGTTACGGTGCCCACTCCAAC and 5'-TCGGGAGATACTTCTCTTTCT) were selected from the published sequences (refs. 30 and 31, respectively). Primers for the *CYP19* locus were 5'-GCAGGTACTTAGTTAGCTAC and 5'-AGTGAGCCAAGGTCGTGAG (A. Bowcock, personal communication).

Human interspersed repetitive sequence (IRS) primers *alu* (32) and LINE (33) were used to amplify the human sequences retained in the rodent–human hybrids. Resulting IRS-PCR products were biotinylated and used for fluorescence *in situ* hybridization (34) or electrophoresed on 1.3% agarose gels to permit "PCR-karyotyping" of somatic cell hybrids as described by Ledbetter *et al.* (35).

Fluorescence *in Situ* Hybridization. Fluorescence *in situ* hybridization was performed by the methods of Lichter *et al.* (34, 36). Biotinylated IRS-PCR products generated from the A15 and A15-1 chromosome 15 hybrids were preannealed with human Cot-1 fraction DNA (GIBCO/BRL) at 37°C for 30 min before hybridization.

RESULTS

Quantitation of the SCE Phenotype. To study correction of the elevated SCE BSx phenotype, we selected the simian virus 40-transformed BSx cell line GM08505. Previous characterization indicated that this cell line displays an elevated SCE frequency, pseudotetraploid karyotype, and reduced DNA ligase I activity (12). Examination of the GM08505 culture, as well as clonal isolates, demonstrated limited variability and a consistent SCE frequency that averaged 2.12 SCEs per chromosome (Table 1). Five control cell lines and individual subclones isolated from the control cell line GM00847 also demonstrated consistent SCE frequencies. However, these averaged 0.24 or ≈10% of that seen in the BSx cells (Table 1). The ranges of SCEs observed in BSx and in control cultures were never observed to overlap (data not shown), suggesting that a reduction in SCE frequency after chromosome transfer should be readily detected.

To evaluate the level of correction that might be expected after introduction of the complementing locus, GM08505 cells were fused to two control cell lines that had been characterized in this study. Correction of the elevated SCE phenotype was observed after whole-cell fusion between GM08505 and both of these control cell lines, with resulting hybrids demonstrating SCE frequencies near those of the control cultures (Table 1). Complete correction to the levels seen in control cells was never observed.

MMCT Complementation. After a detailed evaluation of the SCE phenotype and demonstration of correction by whole-cell fusion, MMCT experiments were initiated to identify a human chromosome capable of complementing the GM08505 elevated SCE phenotype. Chromosomes were transferred to BSx cells from somatic cell hybrids bearing human chromosomes "tagged" with the dominant selectable marker for neomycin resistance (22–25). Identification of the human chromosome carried in these hybrids was based on previous detailed cytogenetic characterization or on preliminary G-banded analysis (refs. 22–25 and unpublished observations). The transfer of chromosomes from such hybrids resulted in a variable number of G418-resistant BSx clones. As shown in Table 2, changes in the SCE phenotype were not observed after the transfer of most chromosomes. However, transfer from a mouse–human hybrid designated A15 resulted in the reduction of SCE frequency in nearly all recipient GM08505 clones recovered to levels similar to those seen with whole cell hybrids (Table 2). The recovery of multiple SCE-complemented BSx colonies resulting from the transfer of this chromosome provided confirmation for correction as opposed to a rare reversion event. Nine of 11 clones resulting from four transfer experiments exhibited correction. Two clones retained the elevated SCE phenotype associated with BSx (Table 2) and likely resulted from transfer of an incomplete chromosome, an event associated with MMCT (22–25). As described in greater detail below, subcloning of the A15 hybrid permitted isolation of the clonal cell line A15-1, bearing a truncated version of the human chromosome present in A15. This hybrid lost the ability to render correction of the elevated SCE phenotype by chromosome transfer to BSx cells (Table 2).

Table 1. SCE frequency for parental cell lines and whole-cell hybrids

Cells	Metaphases counted, no.	Chromosome number	No. SCEs per chromosome
Control cell lines			
GM00847	30	76.9	0.18 ± 0.04
GM00847 neo (6 clones)*	180	80.7	0.22 ± 0.10
143neoTk	30	65.5	0.40 ± 0.16
VAneo	90	83.3	0.20 ± 0.09
HT1080	30	67.5	0.24 ± 0.05
GM08207	30	43.0	0.37 ± 0.10
Average	390	75.9	0.24 ± 0.12
Bloom cells			
GM08505	92	77.2	2.15 ± 0.45
GM08505 (7 clones)	211	73.3	2.10 ± 0.41
Average	303	74.5	2.12 ± 0.42
Whole-cell fusions			
Bloom × 143neo (8 clones)	229	137.9	0.43 ± 0.11
Bloom × GM00847neo (4 clones)	60	128.3	0.43 ± 0.09

Data for the number of SECs per chromosome are the average ± SD.
*GM00847 cells were electroporated with plasmid pSV2neo and six G418-resistant clones were isolated and screened for SCE frequency.

Transfer of human chromosomes from rodent–human hybrids to human recipient cells rarely results in the transfer of rodent DNA sequences (22–25). Southern blot hybridization of DNA from complemented clones, using radiolabeled rodent DNA as probe, offers a sensitive means to detect rodent sequences (>200 kilobases) that have been transferred. Probing DNA from complemented BSx clones with total mouse DNA confirmed the absence of any detectable mouse sequences. Additionally, metaphase spreads from complemented BSx clones were probed with biotinylated mouse genomic DNA. No fluorescence signal could be detected with fluoresceinated avidin, even after three rounds of amplification with biotinylated anti-avidin antibody. These results indicate that the observed complementation is rendered by human sequences present in the A15 hybrid. Consistent with this proposal, the transfer of mouse sequences to GM08505 cells by whole-cell fusion or MMCT has failed to yield complementation (unpublished data).

Table 2. SCE frequency after MMCT of human chromosomes from independent mouse–human hybrid donors

Donor	*n*	Metaphases counted, no.	Chromosome number	No. SCEs per chromosome
A(11/X)-1 (human 11/X translocation)	12	180	73.9	2.38 ± 0.43
A17 (human chromosome 17)	3	55	69.8	1.92 ± 0.38
25B (human chromosome unknown)	8	120	80.0	1.90 ± 0.37
Y31A (human chromosome unknown)	1	15	71.1	1.94 ± 0.36
X31 (human chromosome 11/18 translocation)	7	105	72.0	2.21 ± 0.52
A14-4 (human chromosome 14)	6	91	66.6	2.04 ± 0.32
A15 (human chromosome 15)	9	135	70.0	0.50 ± 0.12
A15 (human chromosome 15, uncomplemented)	2	30	79.3	1.66 ± 0.32
A15-1 (human chromosome 15, deletion)	4	60	71.6	2.26 ± 0.33

Data for the chromosome number are the average. Data for the number of SCEs per chromosome are the average ± SD. *n*, Number of clones.

Classical and Molecular Cytogenetic Analysis of the A15 Hybrid. The karyotype of GM08505 cells was found to be both aneuploid and unstable. This dynamic state precluded identification of a newly introduced chromosome in BSx metaphases. Therefore, characterization of the complementing chromosome focused on the hybrid A15. Preliminary cytogenetic characterization of the A15 hybrid by G-banding revealed an apparently normal chromosome 15 (data not shown). Therefore, definitive assignment of SCE complementation was dependent on demonstrating that chromosome 15 was the only human material present in A15 hybrid cells.

Fluorescent *in situ* hybridization using biotinylated total human DNA as probe to A15 metaphases identified only one human chromosome, acrocentric in structure (Fig. 1*A*). Probing A15 metaphases with plasmid pSV2neo revealed hybridization near the centromere of a single acrocentric chromosome (Fig. 1*B*). Simultaneous hybridization with a labeled probe specific for the human chromosome 15 satellite region and plasmid pSV2neo produced signal on the same single chromosome in the A15 hybrid (Fig. 1*C*), suggesting that chromosome 15 is present in the A15 hybrid and is "tagged" near the centromere facilitating selection in BSx cells.

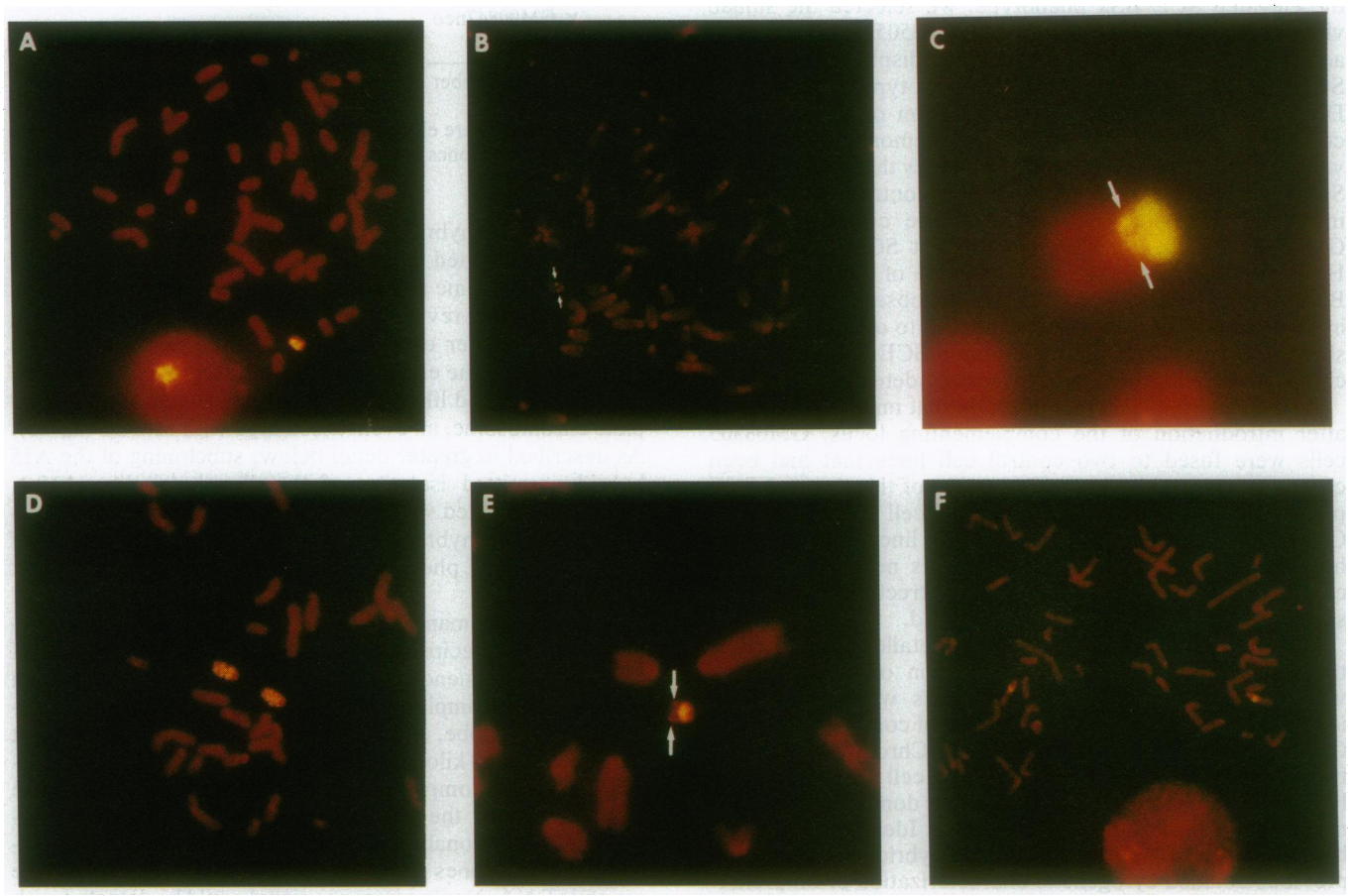

FIG. 1. Characterization of the human chromosome present in hybrids A15 and A15-1 by using fluorescence *in situ* hybridization. (*A–C*) A15 metaphase spreads hybridized with biotinylated total human DNA (*A*), which revealed the presence of a single acrocentric human chromosome, with labeled plasmid pSV2neo (*B*), and with pSV2neo plasmid plus the chromosome 15-specific β-satellite probe D15Z1 (*C*), which indicates that a single chromosome (enlarged image) bears the satellite sequences and a plasmid integration site near the centromere on the q arm. (*D*) Amplified human sequences from A15 hybrid DNA were biotinylated and hybridized back to normal human metaphases, producing signal exclusively on one pair of acrocentric chromosomes. (*E*) Hybridization of metaphases from the hybrid A15-1 with pSV2neo plasmid and the chromosome 15-specific β-satellite probe gave results similar to those in *C*, but on a chromosome apparently deleted of distal q-arm sequences. (*F*) Amplified human sequences from A15-1 DNA hybridize only to the proximal q-arm of chromosome 15 in normal human metaphases.

Amplification of the human DNA sequences in interspecific hybrids is possible using human-specific interspersed repetitive sequences (*alu* and LINE) as primers in an IRS-PCR (32, 33). Use of IRS-PCR products as probes on human metaphases provides a sensitive means to identify the normal chromosomal source of the human DNA present in somatic cell hybrids (33, 34). Use of IRS-PCR products generated from A15 DNA as probes on normal human metaphases resulted in hybridization to a single pair of acrocentric chromosomes (Fig. 1*D*). These data demonstrate that the acrocentric chromosome present in A15 is representative of a single chromosome from the normal human karyotype. As indicated by the chromosome-specific satellite probe (Fig. 1*C*), that chromosome is number 15.

Routine cytogenetic analysis of metaphases from the A15 hybrid permitted detection of the appearance of a rare subpopulation of cells bearing a substantially smaller human chromosome than that originally identified. Subcloning of A15 cells and analysis by *in situ* hybridization led to the establishment of a subclone, designated A15-1, carrying only the smaller chromosome. This chromosome was also found to hybridize with chromosome 15 satellite and pSV2neo sequences (Fig. 1*E*), in a manner consistent with a spontaneous deletion from the distal q-arm of the chromosome 15 originally identified in the A15 hybrid. Consistent with this proposal, IRS-PCR products generated from A15-1 DNA, when hybridized to normal human metaphases, produced signal only on the proximal portion of a single pair of acrocentric human chromosomes (Fig. 1*F*). As reported above, the deleted human chromosome in A15-1 fails to complement the elevated SCE phenotype of BSx cells (Table 2).

Molecular Analysis of A15 and A15-1. Southern blot hybridizations were performed with hybrid DNA and five probes spanning chromosome 15. Additionally, four PCR primer sets that produce human-specific products were used to analyze A15 and A15-1 DNA. These results are summarized in Fig. 2. All nine markers produced positive results with A15 DNA, but only the most proximal probes and primers yielded positive results with A15-1 DNA. Probes

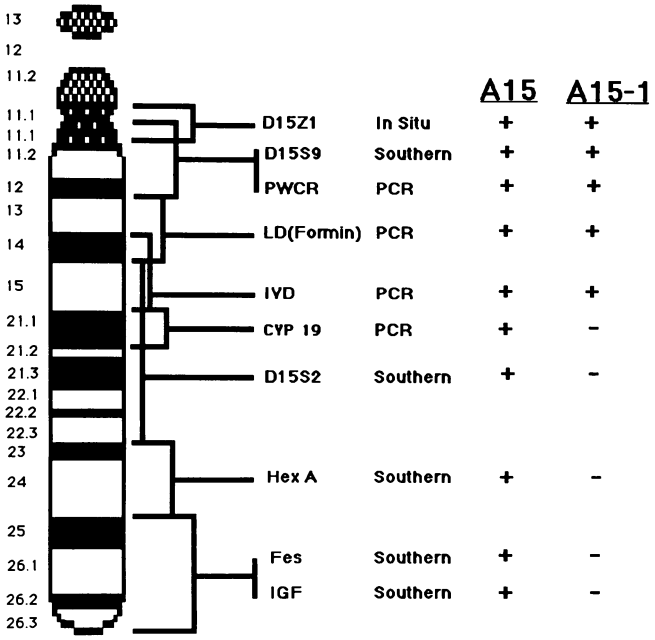

FIG. 2. Summary of results obtained by *in situ* hybridization (In Situ), Southern blot analysis (Southern), or PCR analysis using previously mapped chromosome 15 markers and DNA from the hybrids A15 and A15-1.

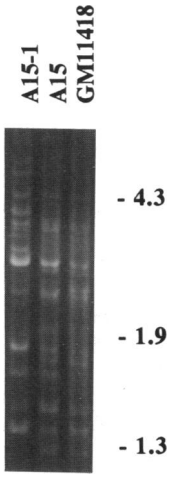

FIG. 3. PCR-karyotype for the hybrids GM11418, A15, and A15-1 generated with the human-specific *alu* primer 559 (32).

specific for the acrocentric human chromosomes 13 and 14 all failed to detect human sequences present in A15 DNA (data not shown).

In addition to their utility for *in situ* hybridizations, IRS-PCR products produced from somatic cell hybrids offer band patterns in agarose gels that are characteristic for each human chromosome (35). Such an analysis with DNA from hybrid A15 yielded results shown in Fig. 3. Fragments produced were nearly identical to those observed using DNA from a well-characterized CHO hybrid, GM11418, previously shown to bear a human chromosome 15 (37). The slight differences seen would be consistent with the minor variation previously reported for chromosomes derived from different sources (35). As expected, DNA from the A15-1 deletion hybrid produced a pattern lacking several major bands representative of chromosome 15 (Fig. 3).

DISCUSSION

MMCT offers an alternative approach for mapping human disease genes. This technique also provides a means to quantitatively assess the biological consequences of introducing a single copy of a normal allele into a mutant cell. Data presented here demonstrate substantial correction of the elevated SCE phenotype in BSx cells after transfer of chromosome 15. The specificity of this response is illustrated by the failure of other chromosomes tested to produce an effect in BSx cells. Although the level of BSx correction by chromosome 15 was found to be significant, the SCE frequency in complemented cells remained greater than that seen in normal control cultures. This incomplete correction is consistent with the results observed in whole-cell fusions between GM08505 and two control cell lines (Table 1). Therefore, chromosome 15 correction of BSx is unlikely to represent a spurious effect rendered by a gene product not directly related to BSx or to mechanism(s) responsible for SCE formation. The specific cause for the partial correction remains unclear. The aneuploid karyotype of the BSx cell line GM08505 might restrain complementation by gene dosage effects. Imprinting or deleterious contributions from other loci on chromosome 15 could also contribute to the results observed. It is relevant to note that in studies addressing MMCT correction of the UV sensitivity and DNA repair defect in xeroderma pigmentosum complementation group A (XP-A) cells, chromosome 9q was found to render substantial, yet incomplete, correction of the associated phenotypes (22, 38). Subsequently, the XP-A gene was cloned and

mapped to chromosome 9q, confirming the accuracy of the MMCT mapping data (39).

Detailed characterization of a somatic cell hybrid used in MMCT complementation is critical for the assignment of a complementing locus to a given human chromosome. Such efforts have been used to identify both normal and rearranged structures in previous MMCT complementation experiments (22–27). Although small rearrangements and translocations may be difficult to detect, recent advances in molecular cytogenetic techniques have dramatically improved the ability to characterize human sequences retained in somatic cell hybrids (32–35), offering greater confidence in the integrity of a human chromosome present. In the present study, all analyses of the hybrid A15 support the conclusion that the elevated SCE frequency in BSx cells has been corrected by a normal chromosome 15. Additionally, characterization of the hybrid subclone A15-1, which retains a terminal q-arm deletion, appears to have lost only distal 15q sequences. These data are consistent with the existence of a normal intact structure for the chromosome 15 present in the original A15 hybrid and suggest that the BSx complementing locus maps to 15q14–qter.

In addition to the elevation in SCE frequency, minor alterations in the activity or structure of DNA ligase I (10–12), uracil DNA glycosylase (13), topoisomerase II (14), thymidylate synthetase (15), and superoxide dismutase (16) have been implicated in BSx. The genes encoding each of these proteins map to chromosomes 19, 12, 17, 18, and 21, respectively [McKusick, V. A., Online Mendelian Inheritance in Man (machine-readable data file), Update May 24, 1991]. Data presented here demonstrate correction of the elevated SCE phenotype in BSx cells, coincident with the introduction of a single copy of a well-characterized structurally normal chromosome 15. Thus these observations suggest that the primary defect in BSx resides in a gene on chromosome 15 that influences a number of substrates either directly through mechanisms related to protein modification (e.g., phosphorylation) or indirectly through more global cellular pathways (e.g., cell cycle or nucleotide metabolism).

We thank Drs. Errol Friedberg, Alan Tomkinson, and Stephen Warren for comments and/or critical review of the manuscript. This work was supported by grants to R.A.S. from both the American Cancer Society (PDT386) and the National Institutes of Health (CA52121).

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