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Biochemistry

A wild-type DNA ligase I gene is expressed in Bloom's syndrome cells

(chromosomal breakage syndrome/degenerate polymerase chain reaction/human genetic disorders)

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ABSTRACT **Alteration of DNA ligase I activity is a consistent biochemical feature of Bloom's syndrome (BS) cells. DNA ligase I activity in BS cells either is reduced and abnormally thermolabile or is present in an anamolously dimeric form. To assess the role of DNA ligase function in the etiology of BS, we have cloned the DNA ligase I cDNA from normal human cells by a PCR strategy using degenerate oligonucleotide primers based on conserved regions of the *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* DNA ligase genes. Human DNA ligase I cDNAs from normal and BS cells complemented a *S. cerevisiae* DNA ligase mutation, and protein extracts prepared from *S. cerevisiae* transformants expressing normal and BS cDNA contained comparable levels of DNA ligase I activity. DNA sequencing and Northern blot analysis of DNA ligase I expression in two BS human fibroblast lines representing each of the two aberrant DNA ligase I molecular phenotypes demonstrated that this gene was unchanged in BS cells. Thus, another factor may be responsible for the observed reduction in DNA ligase I activity associated with this chromosomal breakage syndrome.**

Bloom's syndrome (BS) is an autosomal recessive disorder that is characterized by a high incidence of cancer, variable combined immunodeficiency, and a markedly increased mutational rate (1–3). Cells from BS patients exhibit pronounced genomic instability, having an increased frequency of sister chromatid exchange, a high rate of chromosomal breakage, and gross cytologic abnormalities such as quadriradial chromosomes (4, 5). Complementation analysis using BS cells from patients of diverse ethnicity suggests that the defect in BS is attributable to a single gene (6).

Biochemical analysis reveals that DNA ligase I displays one of two aberrant molecular phenotypes in BS cells. In most BS cells, DNA ligase I activity is reduced and thermolabile, whereas certain BS cells contain an anomalously dimeric form of DNA ligase I (7–10). Thus, it has been proposed that the BS defect is attributable to a mutation of the gene encoding DNA ligase I (8, 10).

A second biochemical defect has been observed in BS cells. The activities of the DNA-repair enzymes uracil DNA glycosylase and hypoxanthine DNA glycosylase are induced late in the G_1 phase of the cell cycle in normal cells, attaining maximal levels early in S phase. In BS cells this induction is delayed, and maximal levels of uracil and hypoxanthine DNA glycosylases are not attained until late in S phase, although the maximal activity of these enzymes is not reduced in BS cells. In addition, a monoclonal antibody that recognizes uracil DNA glycosylase from normal cells is unreactive with BS uracil DNA glycosylase, indicating that the enzyme is structurally altered or modified in BS cells (11–14).

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To assess the molecular basis of this disease, we have cloned the cDNA corresponding to the human DNA ligase I gene expressed in normal cells and from two BS fibroblast lines, GM8505 and GM5289. The DNA ligase I gene was isolated by a PCR strategy employing degenerate oligonucleotides based on conserved regions of the DNA ligase genes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (15). A complete DNA sequence analysis from two BS cell lines revealed that each BS DNA ligase I gene was indistinguishable from the wild-type gene. A single 3.1-kilobase (kb) DNA ligase I mRNA was detected at normal steady-state levels in BS cells by Northern blot analysis. Also, the GM8505 DNA ligase I gene was able to complement the *S. cerevisiae cdc9* DNA ligase mutation. Given the reduction in DNA ligase I activity in BS cells, these results suggest that a factor which modulates DNA ligase I activity in normal cells is defective in BS. Alternatively, a defect in a modifying activity that acts upon DNA ligase I and some DNA-repair enzymes may account for the altered repair phenotypes in BS cells.

MATERIALS AND METHODS

Cell Lines. BS fibroblast lines GM8505 and GM5289 were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ.

cDNA Synthesis. RNA was prepared as described (16). cDNA reactions were carried out in 50 mM KCl/10 mM Tris·HCl, pH 8.3/3 mM $MgCl_2$, 0.01% (wt/vol) gelatin as follows. Either 5 μg of total RNA or 1 μg of poly(A)$^+$ RNA was incubated with 150 pmol of random hexanucleotides (Pharmacia) at 70°C for 5 min and allowed to cool to room temperature. In the initial experiments with mouse and human RNA, synthesis was primed with 100 pmol of oligo(dT) or antisense degenerate oligonucleotide 193 (sequence given below). A mixture containing 200 μM dNTPs, 13 units of RNase inhibitor (Boehringer Mannheim), and 10 units of Moloney murine leukemia virus reverse transcriptase (BRL) was added in a final volume of 50 μl. After incubation at 42°C for 2 hr, reactions were heat-inactivated at 65°C for 15 min. Mock reactions were carried out with heat-inactivated reverse transcriptase.

PCR. PCRs (17) were carried out in 50 μl of 50 mM KCl/10 mM Tris·HCl, pH 8.3/3 mM $MgCl_2$/0.01% (wt/vol) gelatin containing 200 μM dNTPs and 50 pmol of each oligonucleotide primer with 6% of the reverse transcriptase reaction mixture. Negative control reactions contained 6% of a mock reverse transcriptase reaction mixture. The PCR cycling program consisted of 1 min at 94°C, 1.5 min at 52°C (or 55°C with degenerate oligonucleotides), 2 min at 72°C for 40 cycles, followed by 10 min at 72°C. Degenerate oligonucleotides were based on the boxed amino acid sequences of the *S. cerevisiae* and *Sch. pombe* ligases in Fig. 1: 193 (an-

Abbreviations: BS, Bloom's syndrome; PHA, phytohemagglutinin.

tisense), IAC-ICC-GSW-CAG-GTA-RTC-YTT-YTT-IAS-YTT; 195 (sense), AAI-SAY-WGI-TGY-GAR-GGI-CTG-ATG-ITS-AA (where I is inosine; R is G or A, Y is C or T, S is G or C, and W is A or T. Template DNA consisted of random hexamer-primed cDNA or cDNA from mouse and human cDNA libraries. In the latter case, only one of the degenerate oligonucleotide primers was used in conjunction with a primer specific for the λgt11 cDNA cloning vector.

Southern Blots. Ten percent of each degenerate PCR mixture was fractionated in a 1.5% agarose gel and transferred to nitrocellulose. The rest of the heterogeneous PCR products were fractionated in 6% polyacrylamide gels for isolation of hybridization probes. The 75- to 200-base-pair (bp) size range was excised, eluted in 5–10 volumes of 300 mM NaOAc/0.1% SDS at 37°C overnight, and precipitated by addition of 2.5 volumes of 95% EtOH. Heterogeneous PCR size fractions were cloned as described below or radiolabeled according to Feinberg and Vogelstein (18) for use as hybridization probes. Hybridizations were carried out at 42°C in 0.90 M NaCl/0.06 M sodium phosphate, pH 7.4/6 mM EDTA/50% formamide/2× Denhardt's solution/0.2% SDS containing sonicated herring sperm DNA (50 μg/ml). Filters were washed three times at 68°C for 30 min in 2× standard saline citrate/0.1% SDS.

Cloning and DNA Sequencing of DNA Ligase I PCR Fragments. The DNA ligase I genes were isolated in four overlapping PCR segments. In most reactions, DNA ligase I PCR primers contained, or were adjacent to, restriction sites to facilitate cloning into pBluescript (Stratagene) and subsequent assembly of expression constructs after appropriate restriction digestion. Where restriction sites were not used, PCR-derived DNA was treated with polynucleotide kinase in 50 mM Tris·HCl, pH 8/10 mM $MgCl_2$, 1.5 mM spermidine/10 mM ATP before cloning into phosphatase-treated, *Sma* I-digested pBluescript. DNA ligase I PCR primers were based on the DNA sequence of 32B2 (see *Results*). PCR amplification of BS DNA ligase I fragments from single-stranded cDNA was effected with the following primer pairs (+, sense strand; −, antisense strand).

L22+	CAAGGAGCAGCTGACAGA
L9−	CTGCAGTTCCTGCTTCG
L20+	GGGAATTCAGAGCCTGAGGTG
L21−	CGTTCCGCGGCCACTGCCTTGAG
L14+	CGGGATCCGGATGGTGGAGA
L1−	CCAAGCTTGAGCCAGTTGTGCGATCTC
RL4+	CGGGATCCTTCATCCTGGAC
L10−	GCGGATCCGTCCAACTCATG

Sequence analysis of double-stranded DNA was carried out by standard procedures with modified T7 DNA polymerase (United States Biochemical).

DNA Ligase Assays. Transformation of the *cdc9* mutant of *S. cerevisiae* was carried out as described by Ito *et al.* (19) except that all incubations were at room temperature. Crude extracts were prepared from *cdc9* transformants by physical disruption (20). Two micrograms of extract (protein) was diluted into 28 μl of assay buffer (60 mM Tris·HCl, pH 8/10 mM $MgCl_2$/5 mM dithiothreitol/1 mM ATP with bovine serum albumin at 50 μg/ml) containing 100 units of T4 DNA ligase (New England Biolabs) where noted and 5′-^{32}P-labeled $(dT)_{25-30}$ annealed to either poly(dA) or poly(rA) (Pharmacia) in a 1:1 molar ratio as described (21). After incubation at 37°C for 1 min, reactions were stopped by heating at 68°C for 30 min and then treated with 3 units of alkaline phosphatase (Boehringer Mannheim) for 3 hr at 55°C. After precipitation with 10% trichloroacetic acid, products were assayed for radioactivity in a scintillation counter. One unit of DNA ligase activity corresponds to the conversion of 1 nmol of ^{32}P to an alkaline phosphatase-resistant form per microgram of protein per minute. The assay was linear over 10 min for the assay conditions described.

RESULTS

Cloning of Human DNA Ligase Genes by Use of Degenerate Oligonucleotides. The products of the DNA ligase genes from *S. cerevisiae* and *Sch. pombe* share modest overall homology (53% amino acid identity) but are >95% identical over a 47-amino acid segment at their carboxyl termini (22). Given the conservation of DNA ligase function, we assumed that the high degree of amino acid sequence conservation observed in this portion of the known ligase genes might extend to mammalian DNA ligases. Such conservation would allow for isolation of the mouse and human DNA ligase genes by a PCR strategy utilizing degenerate oligonucleotide primers. The degenerate oligonucleotides employed in this strategy are based on the boxed amino acid sequences in Fig. 1, corresponding to the sense and antisense strands encoding the indicated amino acids. The two oligonucleotides, 195 and 193, were highly degenerate (64- and 128-fold, respectively) and contained inosine residues at 7 of 59 positions (see *Materials and Methods*). cDNA synthesis from mouse and human poly(A)$^+$ RNA was primed with oligo(dT) or the antisense degenerate oligonucleotide 193. The resulting cDNA populations were then subjected to PCR with the sense and antisense degenerate oligonucleotide pair.

If the degenerate oligonucleotides were sufficiently homologous to both the mouse and human DNA ligases, the complex PCR products obtained from each species would include DNA ligase sequences. This product could then be used as a hybridization probe to detect the DNA ligase sequence from the other species. Since the conserved sequences in the yeast genes corresponding to the degenerate PCR primers are separated by 117 bp, products in the 75- to 200-bp size range were isolated from the mouse and human PCR mixtures after fractionation in a 6% polyacrylamide gel and used as hybridization probes in Southern blot analysis. PCR products were hybridized with the size-selected heterogeneous mouse (Fig. 2, lanes 1–4) or human (lanes 5–8) PCR products as radiolabeled probes. Whereas each probe detected a complex pattern of bands in the species from which it was derived, a single 114-bp band was detected in the heterologous PCR. This result suggested that the degenerate oligonucleotides amplified an analogous gene fragment from the mouse and human cDNA populations. The complexity of the products detected by the heterogeneous probes in PCR

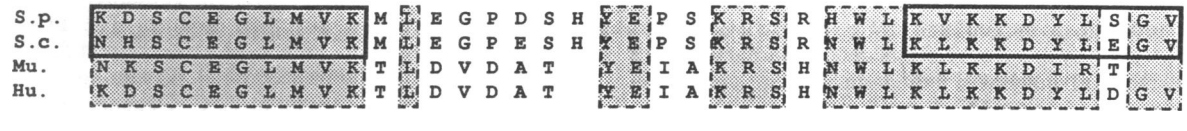

FIG. 1. Human and mouse sequences from degenerate PCR are homologous to yeast DNA ligases. The amino acid sequences at the carboxyl termini of the *S. cerevisiae* (residues 566–604; ref 15) and *Sch. pombe* (residues 570–608; ref. 22) DNA ligases are compared with the deduced amino acid sequences of the mouse and human PCR fragments obtained as described in the text. Shaded areas indicate amino acid identity between the mouse or human segment and one of the yeast segments. S. p., *Sch. pombe*; S. c., *S. cerevisiae*; Mu., mouse; Hu., human.

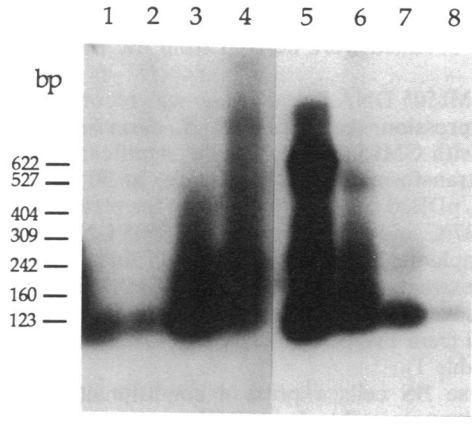

FIG. 2. Complex degenerate PCR products contain conserved sequences. Degenerate PCR reactions were analyzed by Southern blotting using heterogeneous size-selected probes as described in the text. Lanes 1–4, hybridization with the mouse probe; lanes 5–8, rehybridization of the same filter with the human probe. Template cDNA was synthesized using antisense primer 193 (lanes 1 and 5, human; lanes 3 and 7, mouse), or oligo(dT) primer (lanes 2 and 6, human; lanes 4 and 8, mouse).

mixtures from the same species indicates that the single band detected by the heterologous probe represents a relatively small fraction of the total amplified population.

Mouse and human size-selected PCR products were cloned into a plasmid vector and bacterial colonies were screened by hybridization to the appropriate heterologous probe. The DNA sequences of positive mouse and human clones were determined and their deduced amino acid sequences are shown in Fig. 1. The mouse and human DNA sequences are 87% homologous, with 86% amino acid identity over the 114-bp region amplified by the degenerate oligonucleotides. The sequences of mouse gene segments corresponding to the degenerate primers were obtained by PCR experiments with cDNA library DNA as the template. In such experiments, one of the degenerate oligonucleotides was used in conjunction with a PCR primer specific for λgt11 sequences near the cloning site. Significantly, these amplified segments exhibit striking homology to the yeast ligase genes; 28 of 38 amino acids are identical to one of the yeast ligase genes, as indicated by the shaded boxes in Fig. 1.

The 114-bp human gene segment was used to screen a human tonsillar cDNA λgt11 library (kindly provided by L. Klickstein, Center for Blood Research, Boston). Nineteen positive bacteriophage clones were isolated, and the nucleotide sequence of the largest clone, 32B2, was determined. The 32B2 insert is 3.0 kb in length, containing an open reading frame of 2.757 kb. The nucleotide sequence of 32B2 is identical to lig.2, a DNA ligase I cDNA sequence previously reported (23), with the exception of two silent nucleotide substitutions; at residues 1083 and 2650 of the published sequence, GGT (Gly) and CCG (Pro) are GGA (Gly) and CCA (Pro) in 32B2. Amino acid residues 7–36 of lig.2 are absent from 32B2, suggesting that 32B2 represents an alternatively spliced DNA ligase I mRNA. PCR analysis showed that the major form of DNA ligase I expressed in fibroblasts corresponds to lig.2 (data not shown). We constructed a cDNA, 139, that contains the 90-bp sequence found in the lig.2 cDNA.

Human cDNAs Encode DNA Ligase I Activity. We placed the 32B2 and 139 inserts into a *URA3*-containing yeast expression vector (pDB20) under the control of the yeast alcohol dehydrogenase promoter (24) and introduced the plasmids into *cdc9*, a *S. cerevisiae* DNA ligase mutant (*cdc9, ura3*) (25). At a permissive temperature, 30°C, both gene constructs conferred a significant growth advantage relative to *cdc9* cells transformed with pDB20 alone (data not shown). At a nonpermissive temperature, 37°C, both cDNAs complemented the *cdc9* mutation (Table 1); 45 of 45 32B2/pDB20 and 76 of 76 139/pDB20 colonies grown at 30°C were able to grow at 37°C when retested.

Mammalian cells contain two DNA ligase activities, I and II (26). These two activities are distinguishable by their substrate specificity. DNA ligase II, but not DNA ligase I, is able to mediate ligation of DNA ends in DNA·RNA heteroduplexes [oligo(dT)·poly(rA)], whereas both are able to mediate joining of DNA·DNA duplexes [oligo(dT)·poly(dA)] (21). We tested the ability of crude protein extracts prepared from 32B2/pDB20 and 139/DB20 *cdc9* transformants to mediate joining of the two ligase substrates. DNA ligase activity obtained from the *cdc9* transformants was at least 80-fold higher with the oligo(dT)·poly(dA) substrate than the activity obtained with extracts prepared from control transformants (Table 1). Mock reaction mixtures with heat-inactivated extracts contained essentially equivalent amounts of acid-precipitable material as the control extracts (data not shown). Crude extracts from 32B2/pDB20 were consistently less active than extracts from 139/pDB20 in this assay, indicating that the absence of the 90-bp sequence had a deleterious effect on human DNA ligase I when expressed in yeast. Similarly, a 5′-truncated form of human DNA ligase I was only 25% as active as the full-length molecule in *S. cerevisiae* extracts (23). Neither of these extracts exhibited any DNA ligase II activity with the oligo(dT)·poly(rA) substrate, although it was efficiently ligated when purified T4 DNA ligase (100 units) was added to each of the crude extracts before incubation (Table 1). The substrate specificity of crude protein extracts indicated that the cloned cDNAs encoded DNA ligase I.

In addition to substrate specificity, DNA ligases I and II are distinguishable by their pattern of expression. DNA ligase I activity is highest in proliferating cells and is thus ascribed a role in DNA replication, whereas DNA ligase II activity is present constitutively at low levels and is thought to play a role in DNA repair (26, 27). We tested whether the DNA ligase I gene was transcribed in a cell cycle-specific fashion. Peripheral blood T cells are an extremely homogeneous population of resting cells that are induced to enter S phase within 15–24 hr of stimulation with phytohemagglutinin (PHA). Total RNA was isolated from resting T cells at 12-hr intervals following PHA stimulation (28). The steady-state

Table 1. Complementation of *cdc9* DNA ligase activity

cdc9 transformant	Growth at 37°C,* %	Ligase activity of crude extracts,† units × 10^6			
		dA·dT	dA·dT + T4 lig.	rA·dT	rA·dT + T4 lig.
139/pDB20	100	1.46 ± 0.27	3.32 ± 0.63	0.05 ± 0.09	1.16 ± 0.65
32B2/pDB20	100	0.24 ± 0.10	3.05 ± 1.08	0.07 ± 0.10	1.04 ± 0.56
GM8505/pDB20	100	1.17 ± 0.43	2.43 ± 0.22	0.04 ± 0.03	1.07 ± 0.81
pDB20	<0.9	0.003 ± 0.003	2.04 ± 0.37	0.01 ± 0.01	0.89 ± 0.47

*Colonies from plates grown at 30°C were tested for the ability to grow at 37°C.
†Substrate was poly(dA)·oligo(dT) (dA·dT) or poly(rA)·oligo(dT) (rA·dT) with or without added phage T4 DNA ligase (T4 lig.). Activity units are defined in *Materials and Methods*. Values represent four independent experiments.

level of DNA ligase I mRNA in PHA-stimulated cells was determined by Northern blot analysis using the 32B2 cDNA as a probe. DNA ligase mRNA was barely detectable in resting cells (Fig. 3*A*) but was significantly induced, reaching maximal levels at 24–36 hr after PHA stimulation. The DNA content of cells at each time point was determined by propidium iodide binding and was used to estimate the cell cycle stage (28). This analysis indicated that the maximal expression of DNA ligase mRNA coincided with the onset of DNA synthesis. We obtained similar results with stimulated human B cells and observed that the abundance of DNA ligase I mRNA was diminished when the cells left S phase (data not shown). Inhibition of DNA synthesis by treatment with 2 mM hydroxyurea did not affect the induction of DNA ligase I mRNA accumulation (Fig. 3*A*), indicating that the transcriptional activation of the DNA ligase I gene occurred before the initiation DNA synthesis.

The DNA Ligase I Gene from BS Cells Is Normal. We undertook a molecular analysis of the DNA ligase I gene from two BS cell lines. GM8505 is a simian virus 40-transformed fibroblast line in which DNA ligase I activity is abnormally thermolabile and reduced by a factor of 3 (9). GM5289 is an untransformed BS fibroblast line that contains an aberrantly high molecular weight form of DNA ligase I but does not have diminished levels of DNA ligase I activity (29). One microgram of total RNA from these two BS fibroblast lines was converted to single-stranded cDNA and the DNA ligase I gene from each was isolated in four overlapping segments by PCR amplification. In all cases, PCR was also carried out on mock reverse transcriptase reactions to ensure that the PCR products obtained were derived from the BS DNA ligase I genes.

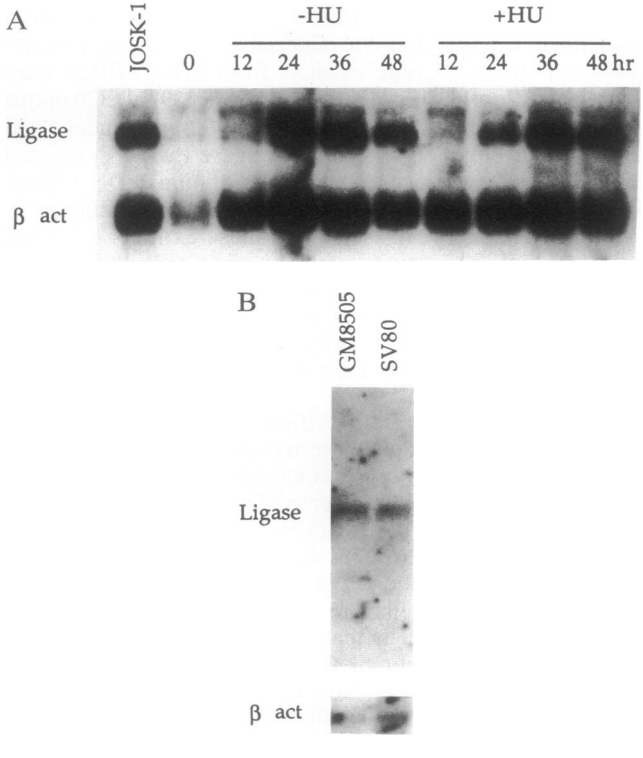

FIG. 3. (*A*) DNA ligase I transcription is induced during G_1 to S transition. Total cellular RNA was isolated from peripheral blood T cells at the indicated times following PHA stimulation in the presence or absence of 2 mM hydroxyurea (HU). RNA (15 μg per lane) was fractionated in 1.2% agarose/formaldehyde gels and transferred to nitrocellulose (16). The filter was hybridized simultaneously with 32B2 (Ligase) and β-actin (β act) probes. JOSK-1 is a human monocytic leukemia cell line included as an unsynchronized control (28). (*B*) DNA ligase I is normally transcribed in GM8505. Poly(A)$^+$ RNA (0.5 μg per lane) from SV80 and GM8505 cells was fractionated as above and hybridized separately with 32B2 and β-actin probes. Blots were exposed to film for 6 days after each hybridization.

The GM8505 DNA ligase I gene was reconstructed in the yeast expression vector pDB20. *S. cerevisiae cdc9* transformed with GM8505/pDB20 grew significantly better than control transformants (pDB20 alone) at 30°C. Each of 57 GM8505/pDB20 colonies tested from the plate grown at 30°C grew at 37°C, indicating that the GM8505 DNA ligase I gene fully complements the *cdc9* mutation (Table 1). Additionally, protein extracts prepared from the GM8505/pDB20 transformants were as active for DNA ligase I activity as extracts prepared from 139/pDB20 or 32B2/pDB20 transformants of *cdc9* (Table 1).

Because BS cells express a conditionally active DNA ligase I, it was possible that mutations exerting a subtle effect on DNA ligase I activity would not be detected by expression in a heterologous system. Therefore, we determined the complete nucleotide sequence of the DNA ligase I cDNAs from GM8505 and GM5289. The DNA sequences of the DNA ligase I genes were derived from subcloning and DNA sequencing of the four overlapping PCR-amplified segments described above. Both BS cell lines were found to contain DNA ligase I genes that were indistinguishable from the wild-type gene (23). To confirm the identity of GM8505 (obtained from the Human Genetic Mutant Cell Repository, Camden, NJ) as a BS fibroblast, the sister chromatid exchange frequency in this cell line was determined and found to be extraordinarily high (data not shown), indicative of the BS phenotype (5).

Transcription of the DNA Ligase I Gene in BS Cells Is Normal. GM8505 may have reduced DNA ligase I levels due to changes in the levels of transcription. We determined the steady-state levels of DNA ligase I mRNA in the BS fibroblast line GM8505 and in a wild-type control cell line, SV80, by Northern blot analysis using 32B2 as the hybridization probe. SV80 cells are normal human fibroblasts transformed by simian virus 40 in an analogous manner to GM8505. The levels of DNA ligase I mRNA in GM8505 and the SV80 control were essentially identical (Fig. 3*B*). The constitutively expressed β-actin gene was used to control for the abundance of RNA in each preparation. DNA ligase I activity is not reduced in GM5289 (29), but it is possible that the aberrant molecular weight of DNA ligase I activity in this BS cell results from changes in the DNA ligase I gene or in processing of the mRNA. We detected a single 3.1-kb DNA ligase I mRNA in GM5289, indicating that the DNA ligase I gene in GM5289 is normally transcribed (data not shown). Thus, the alteration of DNA ligase I activity observed in these BS cells does not result from alteration in the synthesis or stability of the DNA ligase I mRNA.

DISCUSSION

An alteration in DNA ligase I enzyme activity in BS cells has been established by extensive biochemical characterization (7–10). We demonstrate here that a normal DNA ligase I gene is present and transcribed normally in cells representing both BS ligase mutant phenotypes.

The alteration of DNA ligase I activity in BS may result from a defect in a factor that modulates the activity of DNA ligase I. The existence of a heat-resistant factor that is associated with DNA ligases and promotes ligase activity on linear DNA has been observed in human fibroblasts as well as *Xenopus laevis* ovaries (30, 31). In addition, it has been proposed that the ligation of nonhomologous DNA ends is facilitated by proteins that align the ends (32) and may interact with DNA ligase. It has been suggested that the amino-terminal portion of the DNA ligase I protein may be involved in protein–protein interactions (26). This portion of

the molecule is not required for DNA ligase I activity in protein extracts (26, 33), and an incomplete cDNA encoding the 3′ half of DNA ligase I is sufficient for complementation of *cdc9* (23). However, these experimental settings do not necessarily reflect the activity of this enzyme on its *in vivo* substrate(s), and so the importance of the DNA ligase I amino-terminal domain is unclear. The structure of the 32B2-encoded protein, which differs from that of the DNA ligase I cDNA previously reported (23) at the amino-terminal end of the molecule, raises the possibility that alternative forms of DNA ligase I may play a role in the regulation of such interactions. The availability of the DNA ligase I molecular clone will now allow functionally relevant domains of the molecule to be tested.

Many of the molecular features of BS are consistent with a reduction in the activity of DNA ligase I. The reduced rate of DNA replication, the elevated frequency of sister chromatid exchange, and the reduced ligation efficiency of exogenous plasmid DNA all appear to reflect an impairment of DNA ligase activity (4, 34, 35). Thus, changes in DNA ligase I activity may still dictate the molecular phenotypes and clinical features of this disease, even though we have demonstrated that the DNA ligase I gene in BS is normal.

Some aspects of the BS phenotype may not be a consequence of altered DNA ligase I activity. Sirover and coworkers (11–14) have identified an alteration in the structure and expression of uracil DNA glycosylase and hypoxanthine DNA glycosylase in BS cells. These enzymes are unlikely to affect the levels of DNA ligase I activity directly. Since these enzymes are necessary in the base-excision repair pathway, it is possible that an alteration in their levels of expression would directly affect the rate of mutation in BS cells. The aberrant expression of these DNA-repair enzymes coincident with the alteration in DNA ligase I activity may indicate that a protein-modifying pathway that acts upon enzymes involved in DNA-repair processes is defective in BS cells. This hypothesis suggests that the biochemical alterations thus far described may represent a subset of the perturbations in DNA metabolic pathways of BS cells.

The human cDNA encoding DNA ligase I was isolated by a PCR strategy using degenerate oligonucleotide primers based on a highly conserved region at the carboxyl termini of the proteins encoded by the yeast DNA ligase genes. Amino acid sequence conservation between the two yeast ligases is higher in this region than at the segment that most likely comprises the enzyme's active site (33). We failed to detect sequences encoding DNA ligase II by this approach, perhaps suggesting that human DNA ligase I is more closely related to the yeast DNA ligases than is DNA ligase II. Proteolytic analysis of the AMP-binding domains of human DNA ligases I and II suggests that these enzymes share significant homology at the active site (36). Since our approach did not rely upon sequences at the active site, the possibility remains that DNA ligase II sequences could be isolated by a similar strategy employing degenerate oligonucleotides spanning this region. Low-stringency Northern blot hybridization using 32B2 as a hybridization probe revealed a single RNA species corresponding to DNA ligase I. Similarly, we were unable to detect cDNA clones corresponding to DNA ligase II upon low-stringency screening of a human cDNA library, suggesting limited overall DNA sequence homology between the two human ligases. This is interesting in light of the fact that the two mammalian ligases, which appear to be distinct gene products, exhibit some functional similarity (21, 26, 36).

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