The Cockayne Syndrome Group B Gene Product Is Involved in General Genome Base Excision Repair of 8-Hydroxyguanine in DNA*

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Cockayne Syndrome (CS) is a human genetic disorder with two complementation groups, CS-A and CS-B. The CSB gene product is involved in transcription-coupled repair of DNA damage but may participate in other pathways of DNA metabolism. The present study investigated the role of different conserved helicase motifs of CSB in base excision repair. Stably transformed human cell lines with site-directed CSB mutations in different motifs within its putative helicase domain were established. We find that CSB null and helicase motif V and VI mutants had greater sensitivity than wild type cells to γ-radiation. Whole cell extracts from CSB null and motif V/VI mutants had lower activity of 8-hydroxyguanine incision in DNA than wild type cells. Also, 8-hydroxyguanine accumulated more in CSB null and motif VI mutant cells than in wild type cells after exposure to γ-radiation. We conclude that a deficiency in general genome base excision repair of selective modified DNA base(s) might contribute to CS pathogenesis. Furthermore, whereas the disruption of helicase motifs V or VI results in a CSB phenotype, mutations in other helicase motifs do not cause this effect. The biological functions of CSB in different DNA repair pathways may be mediated by distinct functional motifs of the protein.

CSB genes have been cloned, and the basic structure and function of the genes have been characterized (3–5). Studies using skin-originated CS-B fibroblast cells demonstrated an increased sensitivity to UV radiation, accompanied by a delay in RNA synthesis recovery (1, 6). The hypersensitivity of CS to UV radiation is attributed to a pronounced defect in the repair of DNA damage triggered by UV radiation (7). The predominant DNA damage introduced by UV radiation is the generation of cyclobutane pyrimidine dimers and 6-4 photoproduct adducts. These lesions are bulky and helix-distorting and can be removed by nucleotide excision repair (NER) in a transcription-coupled repair (TCR) pathway (8–12). It is generally believed that the inherited defects in TCR of NER constitute the molecular basis of CS (13, 14). However, the UV sensitivity alone may not explain the progressive neurodegeneration and other clinical appearances in CS because short wavelength radiation like UV cannot reach the inner organs and tissues. Thus, TCR of NER is unlikely to be the only pathway defective in CS. There are indications that CSB also plays a role in transcription and chromatin remodeling (9, 15).

It has been proposed that oxidative damage to DNA plays a vital role in the development of progeroid syndromes (16). Living organisms are constantly exposed to oxidative stress from environmental agents, including ionizing radiation, and from endogenous metabolic processes. A significant consequence of oxidative stress is DNA base modifications, which can result in mutations and other types of genomic instability. 8-Hydroxyguanine (8-OH-Gua) is one of the principal modified bases in DNA resulting from oxidative damage, and it is repaired mainly by the process of base excision repair (BER) (17, 18). Although TCR is responsible for processing some types of oxidative DNA damage in studies using CSB as a model (19–22), alternative pathways for the function of CSB in the repair of DNA base modifications remain to be further elucidated. This laboratory reported that whole cell extract (WCE) from primary CSB cells incised 8-OH-dGuo at a reduced level in comparison with normal cell lines and that this BER deficiency in primary CS-B cells is associated with a down-regulated transcription of the human 8-hydroxyguanine glycosylase/apurinic site lyase (hOgg1) gene (18). This deficiency can be complemented by transfection of the cells with the normal CSB gene (18).

The human CSB gene encodes a protein of 1493 amino acids with a molecular mass of 168 kDa (5, 23). By sequence homology, the CSB gene product belongs to the SWI2/SNF2 family of proteins. All proteins in this family contain seven putative consecutive DNA and RNA helicase motifs (24) (see Fig. 1). In addition to the helicase motifs, CSB contains an acidic amino
acid stretch, a glycine-rich region, and two putative nuclear localization signal sequences (5) (see Fig. 1). Despite the presence of the conserved motifs, CSB has not been demonstrated to possess helicase activity as defined experimentally by an ability to unwind double-stranded DNA in a classic strand displacement assay (25). As are other members of the SWI/SNF subfamily, CSB is likely to be involved in a wide variety of cellular functions, including DNA repair, transcription regulation, maintenance of chromosome stability, and chromatin remodeling (9, 26). However, limited information is available that addresses the functional significance of the individual motifs. The characterization of motif II of CSB in DNA repair has been performed by the construction of a point mutation in motif II in hamster cells (27). The motif II mutant lost the ability to confer cellular resistance to 4-nitroquinoline-1-oxide (4-NQO) and UV exposure. Because motif II functions in ATP hydrolysis, the cellular data suggested that ATP hydrolysis by CSB was essential to certain types of DNA repair, for example the TCR of NER (27). Recent studies in this laboratory have shown that the BER of 8-OH-Gua was deficient in CSB null cells. However, we have found no evidence for a function of motif II in BER.3

To gain further understanding of the importance of CSB protein in BER, site-specific mutations were introduced in various helicase motifs of the CSB protein in human cells. Stably transfected CS-B cell lines with altered CSB genes were established by transfecting plasmids containing a site-directed CSB mutation into CS1AN cells, a human CS-B fibroblast. The cell lines were tested for their sensitivity to oxidative stress, the ability of whole cell extract to repair modified DNA bases in vitro, and the accumulation of modified bases in genomic DNA after oxidative stress. We report here that CSB plays a role in lesion-specific BER and that helicase motifs V and VI of CSB are crucial in this function. We also find that mutations in certain motifs of CSB in human fibroblast lines lead to the accumulation of oxidative DNA base lesions in cells that have been exposed to oxidative stress.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions**—All cell lines were derived from CS1AN.S3.G2, a SV40-transformed human fibroblast cell line belonging to CS complementation group B. The characteristics of the cells have been previously described (28). The sensitivity of each cell line to UV was tested before use. CS1AN.S3.G2 transfected with the mammalian expression vector pcDNA3.1 alone (Invitrogen; abbreviated as pc3.1) or pcDNA3.1 containing intact or constructed mutated CSB gene are designated in Fig. 1. A neomycin resistance gene in the pc3.1 plasmid was used for the selection. All cell lines were routinely grown in minimal essential medium supplemented with 15% fetal bovine serum, 0.3 mg/ml l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 400 μg/ml geneticin (G418, Life Technologies, Inc.).

**Establishment of Stable Transfected Cell Lines with Site-directed Mutations in CSB**—Site-directed mutagenesis of CSB was performed by a uracil containing DNA base protocol (27, 29). The plasmid pcBluesE6 with the entire human CSB cDNA was kindly provided by Dr. Jan Hoeijmakers. Mutations in CSB were constructed to replace highly conserved residues in motifs Ia, III, IV, and VI of the putative helicase domain and in the second putative nucleotide-binding (NTB) domain. The design of most of the constructs is presented elsewhere.3 The newly made CSBR946A was constructed by replacing arginine with alanine using primer 5′-CTCATGCTGCTCCGCCC-3′. After verification by sequencing, the wild type or mutated CSB genes were cloned into the mammalian expression vector pc3.1 and transfected into CS1AN.S3.G2 using SuperFect Transfection Reagent (Qiagen, Santa Clarita, CA). The cells were selected with complete medium containing geneticin (400 μg/ml). Individual colonies were screened for expression of the CSB transcript.

**Analysis of CSB Expression in the CSB Transfectants**—The entire CSB transcript in the transfectant clones was verified by PCR. RNA was extracted using RNA STAT-60 (Tel-Test Inc., Friendswood, TX) followed by treatment with RNase-free DNase I (Roche Molecular Biochemicals). cDNA was synthesized by using a reverse transcription kit (Retroscript, Ambion, Austin, TX) with equal amounts of total RNA (5 μg) from various cell lines according to the manufacturer’s recommendation. A high fidelity PCR master kit (Roche Molecular Biochemicals) was used for PCR amplification of cDNA products. The CSB (4.7 kb) cDNA was amplified as six overlapping fragments using primers and annealing conditions as previously described (27, 30). PCR products were detected by electrophoresis on agarose gels with ethidium bromide staining (Fig. 2A).

Relative quantitative RT-PCR was processed for comparing relative transcript abundance standardized by normalisation of CSB fragment and highly conserved fragments of 18 S rRNA from the transfected cell lines. The RNA isolation and cDNA synthesis were as described above. The primers 5′-GGTGGATGTTGGATGGATGGAAT-3′ and 5′-GTATCCTGTAAGACACACATGCA-3′ were used to produce a 671-base pair product from CSB mRNA (27, 30). The amplification using primers

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Fig. 1. Structure of CSB protein and the location of the designed mutants. The protein contains the seven conserved helicase motifs, a highly acidic region, and two nuclear localization signals (NLS).
for 18 S ribosomal RNA produced a 488-bp base-pair fragment. The linear range of PCR cycles for CSB was determined by titrating synthesized cDNA. For PCR amplification, 0.5 unit of AmpliTaq Gold (Applied Biosystems) was used according to the manufacturer’s procedures. In addition to standard reaction components, PCR mixtures contained 0.2 μM of CSB primers, 0.2 μM of 18 S RNA primer:competemprimer (4:6) (QuantumRNA Classic 18S, Ambion, Austin, TX) in a total volume of 50 μl. PCR hot start was 5 min at 80 °C, 5 min at 49 °C, 1 min at 94 °C, followed by 30 cycles consisting of 30 s at 94 °C, 45 s at 67 °C, 1 min at 72 °C, and one final extension cycle for 7 min at 72 °C. The ratio of CSB transcript and 18 S rRNA was determined by running 1% agarose gel and scanning with the ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

WCEs were used to evaluate the CSB protein expression. These WCEs were prepared as previously described (31). Equal amounts of protein (40 μg) were loaded on 3–8% polyacrylamide gels (NuPAGE) and resolved at 200 V for 1.5 h in NuPAGE Tris-acetate SDS running buffer (Invitrogen). The proteins were transferred onto a polyvinylidene difluoride membrane at 100 V for 1 h in 20 mM HEPES-KOH, pH 7.8, 100 mM KCl, 5 mM dithiothreitol, 5 mM CaCl2, and 2 mM EDTA. The reaction products were visualized by autoradiography and quantified on a phosphorimager (Molecular Dynamics) using ImageQuant software.

Characterization of the CSB Transfected Cell Lines—The validity of the CSB transfected cell lines was evaluated by sequencing and RT-PCR. All six overlapping segments of CSB were detected from cDNAs of CSB transfected cell lines (Fig. 2A, from CSBR946A). The efficiency of CSB expression was examined by quantitative RT-PCR. The ratios of CSB transcript to 18 S internal standard were similar in all CSB transfected cell lines (data not shown). Similar levels of the CSB protein were detected by immunoblot analysis in extracts from the CSB transfected cell lines (Fig. 2B). No band corresponding to the CSB protein was detected in the CSB null cell line (Fig. 2B). These data showed that the intact CSB protein was expressed in all CSB transfected cell lines. Because UV sensitivity is a typical characteristic of CSB null cell lines and because we have previously reported that CSB null cell lines are also sensitive to 4-NQO (27), all CSB transfected cell lines were tested for sensitivity to those two agents by clonogenic assay. The typical survival curves are presented in Fig. 3. These data are from the CSB transfected cell lines containing a point mutation in motif VI, CSBR946A. Cells with mutations in motifs V and VI of the putative helicase domain were unable to complement the UV and 4-NQO sensitivity in comparison with wild type CSB. The helicase motif Ia and III mutants demonstrated an intermediary level of UV and 4-NQO sensitivity.
The second putative NTB domain mutant showed UV and 4-NQO resistances equivalent to that of wild type CS-B cell line.3

Sensitivity to Oxidative Stress of the Transfected CSB Cell Lines—The colony forming ability of CSB transfected cell lines after γ-radiation is shown in Fig. 4. CSB null cells were the most sensitive to γ-radiation. The AUC of CSB null cells was 62% of that of wild type cell. CSBQ942E and CSBRC3.1 (motif VI mutants) were unable to complement the γ-radiation sensitivity of CSB null cells. AUCs of motif VI mutants were ~68% of that of wild type cells and were similar to that of CSB null cells. CSBT912V and CSBT912/913V (motif V mutants) partially complemented the γ-radiation sensitivity of CSB null cells. AUCs of motif V mutants were 84% of the wild type cell. The motif Ia mutant, CSBP573A, the motif III mutant, CSBQ678E, and the second putative NTB mutant, CSBK1137Q, demonstrated γ-radiation resistance equivalent to that of wild type cells. A higher dose of γ-radiation (3 Gy) led to larger survival differences between wild type cells and motif VI mutants.

Glycosylase/Apurinic Lyase Activities (Incision) on Oligonucleotides Containing a Single Base Modification in WCE from the CSB Transfected Cell Lines—The 8-OH-Gua incision activities in WCEs from various transfected CS-B cell lines are shown in Fig. 5A, and the results from 4 independent experiments are summarized in Fig. 5B. We found reduced 8-OH-Gua incision activity in WCEs from CSB null cells (~33% of that of CSBWT, \(p < 0.05\)), from motif VI mutants (CSBQ942E and CSBRC3.1, ~50% of that of CSBWT, \(p < 0.05\)), and from motif V mutants (CSBT912V and CSBT912/913V, ~76% of that of CSBWT, \(p < 0.05\)). The 8-OH-Gua incision activity in WCEs from the motif Ia mutant (CSBP573A), motif III mutant (CSBQ678E), and the second putative NTB mutant (CSBK1137Q) were equivalent to that of wild type CSB. A time course experiment further substantiates this difference. The relative incision activities of 8-OH-Gua among the transfected cell lines (CSB WT>mutants>CSB PC3.1) were similar at all time points. The time course curves of the most 8-OH-Gua incision-deficient cell lines are presented in Fig. 5C. Reduced 8-OH-dGuo incision in CSBQ942E and CSBPC3.1 was observed at all time points analyzed. The largest differences were at 3 h when the reaction was close to saturation.

To determine whether the BER defect was general or limited to the removal of 8-OH-Gua, we assayed for the incision of two lesions recognized by other glycosylases. There were no apparent differences among the cell lines in incision activities of either 5-OH-Cyt or uracil in oligonucleotide duplex (Figs. 7A and 8A). The cell lines with the most 8-OH-Gua incision deficiency (CSBQ942E and CSBPC3.1) appeared to have normal 5-OH-Cyt and uracil incision at all tested time points (Figs. 7B and 8B). Incision activities of both 5-OH-Cyt and uracil were close to saturation at 60 min. Thus, the BER deficiency in the transfected CS cell extracts appeared to be specific for certain damage(s). Incidentally, the incision activities of 5-OH-Cyt and uracil in various cellular extracts suggested that the cell extracts all functioned proficiently and similarly with regards to DNA repair incision.

Accumulation of 8-OH-Gua in Genomic DNA from Transfected CSB Cell Lines after γ-Radiation—Quantification of 8-OH-Gua in genomic DNA as its nucleoside form 8-OH-dGuo was performed by liquid chromatography/isotope-dilution mass spectrometry with selected ion mode. Fig. 9A illustrates the typical ion current profiles at \(m/z 168\) (8-OH-dGuo) and \(m/z 170\) (8-hydroxy-2′-deoxyguanosine-8,16O). There was no difference between the levels of 8-OH-dGuo from DNA before exposure to γ-radiation (0 Gy) of all tested cell lines (Fig. 9B). However, significantly greater (50–80%) levels of 8-OH-dGuo were observed at all time points analyzed. The largest differences were at 3 h when the reaction was close to saturation.
observed 30 min after exposure to \( \gamma \)-radiation at a dose of 2 Gy \( (p < 0.05) \), thus demonstrating that 8-OH-dGuo accumulated in these mutants relative to wild type.

**DISCUSSION**

In the present study, human fibroblast cell lines with site-directed mutations of the CSB gene in various motifs of its helicase signature were generated by replacing the highly conserved amino acids in specific motifs of CSB. The aim was to elucidate the biological importance of the intact CSB gene and of the specific motifs within this gene in BER. The results demonstrate that the integrity of the CSB gene is important for cellular resistance to \( \gamma \)-radiation. Amino acid substitutions in helicase motifs V and VI diminished the cellular resistance to \( \gamma \)-radiation. In the *in vitro* assay of BER of certain lesions by WCE, the absence of an intact CSB gene resulted in deficient glycosylase/apurinic lyase activity of 8-OH-Gua in DNA but did not affect the incision of 5-OH-Cyt and uracil in DNA. Mutations in helicase motifs V and VI of CSB resulted in reduced

**FIG. 5.** Glycosylase/apurinic lyase activities (incision) of 8-OH-Gua in WCEs from various CSB transfected cell lines. A, denaturing polyacrylamide gel graph; the 29-mer band was the substrate, whereas the 11-mer band was the product. The reaction was carried out for 3 h. B, the average of the conversion rate of 29-mer to 11-mer from four independent experiments. An asterisk denotes a value significantly different from CSBWT \( (p < 0.05) \). C, time course of 8-OH-Gua incision activities in WCEs from the most deficient CSB transfected cell lines.

**FIG. 6.** Correlation of *in vitro* 8-OH-Gua incision activities of WCEs and *in vivo* \( \gamma \)-radiation survival of various CSB transfected cell lines.

**FIG. 7.** The glycosylase/apurinic lyase activities (incision) of 5-OH-Cyt in WCEs from various CSB transfected cell lines. A, denaturing polyacrylamide gel graph; the 29-mer band was the substrate, whereas the 11-mer band was the product. The reaction was carried out for 60 min. B, time course of 5-OH-Cyt incision activities in WCEs from the most 8-OH-Gua incision-deficient CSB transfected cell lines.
8-OH-Gua glycosylase/apurinic lyase activity. Furthermore, CSB null or helicase motif VI mutant cells showed a higher accumulation of 8-OH-Gua in genomic DNA after exposure of the cells to \(^{60}\)Cr- or \(^{60}\)Co-radiation. The resistance of the CSBWT cell line to diverse types of DNA damaging agents that are repaired by TCR or general genome repair suggests that this gene is involved in more pathways than TCR of active genes. Whereas UV radiation-induced photoproducts are removed by TCR of NER, both 4-NQO and \(^{60}\)Co-radiation generate modified DNA bases that are repaired without strand bias (37, 55), e.g., the pathway of global genome repair (38). Recently, TCR of 8-OH-Gua was observed using an 8-OH-Gua-containing extrachromosomal plasmid model, and the process was associated with multiple proteins (20, 21, 39).

Although the integrity of CSB is important for cell survival, different conserved helicase motifs did not contribute equally to the function. We have previously reported differences in the functional importance of motif II and the acidic region of CSB in the resistances to UV and other DNA damaging agents (40). We have now established seven transfected human cell lines with mutations distributed in the four conserved helicase motifs and in a second putative NTB domain in the C terminus of CSB. A single or double amino acid change in the highly conserved residues of motifs Ia, III, V, and VI abolished the function of the CSB protein in survival, RNA synthesis recovery, and apoptosis after UV and 4-NQO exposure. In contrast, a point mutation in the putative second NTB of CSB protein showed full complementation in its ability to repair DNA damage induced by UV light or 4-NQO. The pattern of resistance of different CSB mutants to \(^{60}\)Co-radiation was similar to those of UV light and 4-NQO but more deficient in motif V and VI mutants than in motif Ia and III mutants.

The \textit{in vitro} deficits in 8-OH-Gua incision observed for some mutant cell line extracts were reflected by the hypersensitivity of the cells to \(^{60}\)Co-radiation and in the observed accumulation of 8-OH-Gua in genomic DNA after exposure of the DNA to \(^{60}\)Co-radiation. The resistance of the CSBWT cell line to diverse types of DNA damaging agents that are repaired by TCR or general genome repair suggests that this gene is involved in more pathways than TCR of active genes. Whereas UV radiation-induced photoproducts are removed by TCR of NER, both 4-NQO and \(^{60}\)Co-radiation generate modified DNA bases that are repaired without strand bias (37, 55), e.g., the pathway of global genome repair (38). Recently, TCR of 8-OH-Gua was observed using an 8-OH-Gua-containing extrachromosomal plasmid model, and the process was associated with multiple proteins (20, 21, 39).

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The \textit{in vitro} deficits in 8-OH-Gua incision observed for some mutant cell line extracts were reflected by the hypersensitivity of the cells to \(^{60}\)Co-radiation and in the observed accumulation of 8-OH-Gua lesions in the DNA of the respective cell lines. The helicase motif contribution to 8-OH-Gua glycosylase/apurinic lyase activities paralleled the \(^{60}\)Co-radiation resistance. Thus, the functional assays support the observation that the disruption of specific helicase motifs of CSB causes a BER defect. These results suggest that different motifs of the CSB helicase domain may function in different metabolic pathways.

Helicase superfamilies (SF1 and SF2) are composed of proteins that share similar structures and participate in processes including replication, recombination, DNA repair, transcription, and chromatin assembly (41, 42). Because there has been no previous functional analysis of the putative helicase motifs I, III, IV, V, and VI in CSB, speculation on the biochemical function of these conserved regions is largely based on information from structural and functional studies of other helicases.

Crystal structure of \textit{Escherichia coli} Rep helicase revealed that motif Ia acts directly with single stranded DNA and potentially functions in the energy transduction from ATPase to
DNA (43). Mutation in motif II of eIF-4A, a RNA helicase, resulted in a large decrease in the rate of ATP hydrolysis and a loss of helicase activity (44). Biochemical characterization of PerA helicase showed that mutation of motif III affects ATP hydrolysis (45). Our study on the function of motif II of CSB in human cells using a base incision assay uncovered that this motif was not involved in BER of 8-OH-Gua and suggests that ATPase function is not essential in this process. Thus, BER of 8-OH-Gua may not be an energy-consuming process, or it may require energy from alternative sources. This notion is supported by the data in the current work showing that motif Ia and III mutants display normal survival after γ-radiation, a treatment that causes oxidative DNA base damage.

Crystal structure analysis of motif V of E. coli Rep helicase and of motif V of E. coli UvrB protein suggests that this motif is involved in single-stranded DNA binding (43, 46). E. coli UvrB and eIF-4A exhibit nucleic acid binding defects when conserved motif VI residues are altered (44, 46). Based on crystal structure data, helicase motif VI is not directly involved in DNA or nucleotide binding (46, 47). However, by virtue of its close proximity to both the NTP-binding site and the DNA-binding site, it may act to communicate between these sites by mediating conformational changes associated with helicase function (41). We find that mutation in the conserved motifs V and VI of CSB caused a severe compromise of both the in vivo and in vitro end points examined. It was previously reported that helicase motif V and VI of E. coli UvrB were responsible for the enhanced catalytic activity in the presence of damaged DNA (46). Further study on the interaction of CSB and damaged DNA with our mutant cell lines might add more information.

Hydroxyl radical-mediated base modifications are among the most deleterious processes induced in DNA by ionizing radiation (48, 49). Hydroxyl radicals are produced in the hydration layer of DNA in γ-radiated cells and induce the generation of 8-OH-Gua (50). To determine whether the deficiency in BER leads to the accumulation of 8-OH-Gua in genomic DNA, the level of the nucleoside form of 8-OH-Gua, i.e. 8-OH-dGuo in DNA was measured by liquid chromatography/mass spectrometry after exposure of cells to a low dose of γ-radiation. The results showed an enhanced level of 8-OH-dGuo in genomic DNA after exposure of CSB null cells and two motif VI CS-B mutants to 2 Gy of γ-radiation as compared with CS-B cells transfected with the wild type CSB gene. These data demonstrate that CSB null and motif VI mutants had compromised resistance, further supporting the conclusion that CSB plays a role in the removal of 8-OH-Gua from DNA and the importance of motif VI in this function. Recently, Le Page et al. (20) demonstrated that thymine glycol and 8-OH-Gua were removed by a TCR process that did not involve NER. It was also concluded in those studies that the repair defect in CS-B cells was due to deficient TCR alone without any defect in general genome BER (21). Our results in CSB null and certain CSB mutants suggest a deficiency in removal of 8-OH-Gua in the general genome BER because we have used a DNA oligomer containing base damage in nontranscribed DNA. This repair reflects the general genome rather than TCR. It is possible that CSB directly interacts with other proteins involved in BER, and/or it may regulate the expression of certain BER proteins via its role as transcriptional activator and its effect on chromatin assembly. γ-Radiation introduces a variety of lesions in DNA. Many of these are base modifications, but some are direct single- or double-stranded breaks. At the doses that we have used here there are relatively few single strand breaks and almost no double-stranded breaks (51). However, we cannot exclude a contribution of the CSB protein to the repair of strand breaks via other pathways than BER.

A basal transcription defect has been observed in human CS-B lymphoblastoid cells and fibroblasts in the absence of exposure to DNA damaging agents (8). A recent study showed that CSB binding to DNA caused an alteration of the DNA conformation, a remodeling of chromatin structure, and an interaction with core histones (52), all of which occur during basal transcription (1, 53, 54). Thus, the CS phenotype may arise from a combined deficiency in DNA repair and transcription. The down-regulation of certain repair related genes in CSB null or mutant cells could indirectly lead to the incapability of BER.

We suggest that the biological functions of CSB in different DNA repair pathways may be mediated by distinct functional motifs of the protein. Our observation of increased accumulation of oxidative DNA base lesions in DNA in CS-B cells supports the notion that these lesions accumulate in CS patients and may contribute significantly to the phenotype of the disease.

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