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Multiple interaction partners for Cockayne syndrome proteins: implications for genome and transcriptome maintenance

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Abstract

Cockayne syndrome (CS) is characterized by progressive multisystem degeneration and is classified as a segmental premature aging syndrome. The majority of CS cases are caused by defects in the CS complementation group B (CSB) protein and the rest are mainly caused by defects in the CS complementation group A (CSA) protein. Cells from CS patients are sensitive to UV light and a number of other DNA damaging agents including various types of oxidative stress. The cells also display transcription deficiencies, abnormal apoptotic response to DNA damage, and DNA repair deficiencies. Herein we have critically reviewed the current knowledge about known protein interactions of the CS proteins. The review focuses on the participation of the CSB and CSA proteins in many different protein interactions and complexes, and how these interactions inform us about pathways that are defective in the disease.

Keywords

Cockayne syndrome; protein interactions; DNA repair deficiency; transcription deficiency; mitochondria

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¹APE-1, Apurinic/apyrimidinic endonuclease 1; BER, Base excision repair; CMP, Chromatin immunoprecipitation; CSA, Cockayne syndrome group A; CSB, Cockayne syndrome group B; CSN, COP9 signalosome; EMSA, Electric mobility shift assays; Fapy Gua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; Fapy Ade, 4,6-diamino-5-formamidopyrimidine; HMG1, High mobility group nucleosome binding domain 1 protein; IP, Immunoprecipitation; mtDNA, Mitochondrial DNA; NEIL1, endonuclease VIII-like 1 glycosylase; NER, Nucleotide excision repair; NuRD, Nucleosome remodeling and deacetylation complex; OGG1, 7,8-dihydro-8-oxoguanine DNA glycosylase 1; PTM, post translational modification; RNA pol I, RNA polymerase I; RNA pol II, RNA polymerase II; SSBs, Single strand breaks; TC-NER Transcription coupled nucleotide excision repair; TIP5, TTF-1 interacting protein 5; TTF-1, Transcription termination factor 1; UVsS, UV sensitive syndrome; UVSSA, UVsS complementation group A; XAB2, Tetratricopeptide (TRP) protein; 8-oxoG, 7,8-dihydro-8-oxoguanine

1 Introduction

Cockayne syndrome (CS)¹ is a rare human hereditary autosomal disorder with complex and variable characteristics. In general, the syndrome is characterized by severe postnatal growth failure, progressive neurological degeneration, cutaneous photosensitivity and segmental premature aging, including sensorineural hearing loss, retinal degeneration, cataracts, dental caries, muscle weakness and loss of subcutaneous fat (Frontini and Proietti-De-Santis, 2012; Laugel et al., 2010; Stevnsner et al., 2008). Phenotypically, the syndrome also resembles some mitochondrial diseases. Most patients die during childhood with a mean lifespan of 12.5 years. There are two complementation groups for CS, *CSA* (*ERCC8*) and *CSB* (*ERCC6*). Approximately 80% of CS patients carry a mutation in *CSB*, with the majority of the remainder carrying a mutation in *CSA*. A few CS patients have mutations in *XPB*, *XPD*, and *XPG*. Mutations in the *CSB* gene have also been found in de Sanctis-Cacchione syndrome (Colella et al., 2000), Cerebro-oculo-facio-skeletal syndrome (Meira et al., 2000) and UV-sensitive syndrome (Horibata et al., 2004).

Cellular and molecular features for CS include sensitivity to UV-light and some types of oxidative stress, DNA repair deficiencies, transcription deficiencies, and augmented p53 up-regulation and apoptosis after different types of stress (Fousteri and Mullenders, 2008; Latini et al., 2011; Licht et al., 2003; Stevnsner et al., 2008).

2 CSB protein

The human *CSB* gene (*ERCC6*), located on chromosome 10q11, was cloned in 1992 (Troelstra et al., 1992a; Troelstra et al., 1992b). The *CSB* gene product (CSB) encodes a 168 kDa protein of 1493 amino acids belonging to the SWI2/SNF2 protein family. CSB harbors an ATPase domain, which includes seven helicase-like motifs like other members of this family. Additionally, CSB includes an acidic region, a glycine rich region, two putative nuclear localization signal (NLS) sequences and a nucleotide binding domain (NTB). An ubiquitin binding domain was recently identified in the C-terminal of CSB (Anindya et al., 2010; Troelstra et al., 1993; Troelstra et al., 1992a; Troelstra et al., 1992b) (Fig. 1A). The crystal structure of CSB has not yet been determined. However, mutant and wild-type recombinant CSB proteins, and recombinant CSB fragments were used to address the functional significance of the individual motifs and to map the direct interaction region of CSB with other proteins (Fig. 1A) (Muftuoglu et al., 2009a; Muftuoglu et al., 2002; Selzer et al., 2002; Thorslund et al., 2005). Importantly, an alternatively spliced mRNA from *CSB* encodes a 120kDa CSB-PiggyBac fusion protein, CSB-PGBD3, with the 465 N-terminal amino acids of CSB followed by PGBD3. The fusion protein thus includes the N-terminal and acidic domain of CSB but not the helicase motifs. The CSB-PGBD3 protein is expressed in several wild type and CS cell lines (Newman et al., 2008). The importance of the CSB-PGBD3 fusion protein in development of the CS phenotype is still not clarified (see Weiner *et al.* this special issue).

CSB recruits transcription coupled nucleotide excision repair (TC-NER) pathway proteins to the site of a stalled RNA polymerase, potentially as part of a more general role in transcription. CSB also participates in the repair of oxidative DNA damage in the base excision repair (BER) pathway (Licht et al., 2003; Stevnsner et al., 2008). CSB motif II, V and VI are important for the function of the protein in TC-NER whereas motif V and VI, but not II are required for its function in the BER pathway (discussed below) (Muftuoglu et al., 2002; Selzer et al., 2002). CSB has a DNA-dependent ATPase activity (Citterio et al., 2000; Selby and Sancar, 1997), which is linked to CSB functions in TC-NER and chromatin remodeling, but not in BER (Selzer et al., 2002; Stevnsner et al., 2002). Single stranded DNA annealing and exchange activities of CSB do not require ATP hydrolysis (Muftuoglu

et al., 2006). The biological relevance of these activities is not yet clear, but are presumably associated with DNA repair.

2.1 Physical and functional interaction of CSB protein with BER proteins

We and others have recently characterized the interaction of CSB with other proteins to determine the molecular pathways in which it functions. Novel physical and/or functional interactions of CSB with several proteins involved in the BER pathway were detected. These include 7,8-dihydro-8-oxoguanine DNA glycosylase 1 (OGG1) (Stevnsner et al., 2002; Tuo et al., 2002), poly(ADP-ribose)polymerase 1 (PARP-1) (Thorslund et al., 2005), apurinic/apyrimidinic endonuclease 1 (APE1) (Wong et al., 2007), and endonuclease VIII-like 1 (NEIL1) (Muftuoglu et al., 2009b) (Table 1; Fig. 1B and Fig. 2). The functional consequences of the physical interaction of CSB with each of these BER proteins are discussed below. In general, CSB participates in nuclear BER by stimulating the function of specific DNA glycosylases and modulating PARP-1 in the BER pathway whereas in mitochondria, CSB stimulates specific DNA glycosylases and is a component of the nucleoid complex. In addition, CSB may also be involved in the recruitment of BER proteins to the repair complexes associated with the inner mitochondrial membrane.

2.1.1 Physical and functional interactions of CSB with nuclear BER proteins

2.1.1.1 CSB interacts with DNA glycosylases

CSB is in a protein complex with nuclear OGG1: Nuclear OGG1 was the first BER protein demonstrated to be in complex with CSB. While there is no direct physical interaction between CSB and nuclear OGG1, CSB stimulates the incision activity of nuclear OGG1 (Dianov et al., 1999; Tuo et al., 2002). The increased incision activity correlates with increased *OGG1* gene expression (Dianov et al., 1999; Tuo et al., 2002). OGG1 is the main glycosylase responsible for recognition and removal of 7,8-dihydro-8-oxoguanine (8-oxoG) lesions. Increased 8-oxoG accumulation can be detected in nuclear DNA of CSB-deficient cells (Tuo et al., 2003), and in brain and kidney of *csb*^{-/-} mice (Muftuoglu et al., 2009b). This suggests that CSB functions in the repair of 8-oxoG in nuclear DNA. In the processing of 8-oxoG lesions, helicase motif V and VI of the CSB protein are important, whereas the helicase motif II is dispensable. Thus, the ATPase activity of CSB is not required in BER (Selzer et al., 2002; Stevnsner et al., 2002; Tuo et al., 2001). However, the precise mechanism by which CSB participates in the repair of 8-oxoG in nuclear DNA is not yet understood.

CSB interacts with NEIL1: The oxidatively induced DNA lesions 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde) are the main substrates for NEIL1 and are detected in genomic DNA at higher levels than 8-oxoG (Hu et al., 2005). CSB interacts physically with NEIL1 and the two proteins exist in the same protein complex in human cells (Muftuoglu et al., 2009a). CSB stimulates both incision and AP lyase activities of NEIL1, and the N-terminal part of CSB (CSB₂₋₃₄₁) is responsible for the stimulation of NEIL1 incision activity, thus reflecting a direct stimulation of a BER protein by a specific domain of CSB. The role of CSB in the repair of FapyAde and FapyGua lesions is supported by the endogenous accumulation of FapyGua and FapyAde in the nuclear DNA in brain and kidney of *csb*^{-/-} mice. Thus, CSB plays a role in the repair of formamidopyrimidines by interacting with and stimulating NEIL1, and the accumulation of such modifications may have a causal role in the pathogenesis of CS (Muftuoglu et al., 2009a).

2.1.1.2 CSB interacts with apurinic/apyrimidinic endonuclease 1 (APE1): Studies on elucidating the molecular involvement of CSB in BER have also revealed another BER

protein partner for CSB, APE1. In the mammalian BER pathway, APE1 is the major apurinic/apyrimidinic (AP) endonuclease operating to initiate repair of mutagenic and cytotoxic AP sites by incising the DNA backbone immediately adjacent to the lesion. AP sites are formed by spontaneous base loss as well as by increased base release due to chemical modification (e.g. alkylation or oxidation) or through the action of DNA repair glycosylases that excise specific base damages (Wilson and Barsky, 2001). CSB physically interacts with APE1 and the two proteins exist in a protein complex in human cells (Wong et al., 2007). In addition, CSB stimulates the AP site incision activity of APE1 in an ATP independent manner (Wong et al., 2007). Since the stimulatory effect of CSB on APE1 endonuclease activity is more profound in DNA bubble substrates, mimicking a DNA transcription intermediate, than with the fully paired AP duplex (classical abasic site BER substrate), it is possible that CSB facilitates APE1 dependent repair within actively transcribed genomic regions (Wong et al., 2007). At least, CSB-APE1 interaction is likely most critical to regions of the genome where complex DNA structures are formed, such as during transcription or replication, or at sites of recombination (e.g. telomeres).

2.1.1.3 CSB interacts with poly(ADP-ribose)polymerase-1 (PARP-1): PARP-1 binds to single strand breaks (SSBs) in DNA and is thereby activated. When activated, PARP-1 adds polymers of ADP-ribose to various proteins as well as to itself (reviewed in (Herceg and Wang, 2001)). CSB binds to both unmodified and activated poly(ADP-ribosyl)ated PARP-1 (Thorslund et al., 2005). This direct interaction of CSB and PARP-1 is mediated by the N-terminal of CSB (CSB₂₋₃₄₁), the same part of CSB that stimulates the NEIL1 incision activity (Muftuoglu et al., 2009b), as described above. The kinetics of the interaction between CSB and the unmodified or activated form of poly(ADP-ribosyl)ated PARP-1, respectively, are very similar, indicating that CSB binds unmodified and activated PARP-1 with the same affinity. CSB is poly(ADP-ribosyl)ated by PARP-1 after oxidative stress through its N-terminal (CSB₂₋₃₄₁), implicating CSB in the PARP-1 poly(ADP-ribosyl)ation response to SSBs. The poly(ADP-ribosyl)ation of CSB reduces its ATPase activity (Thorslund et al., 2005). While the ATPase activity of CSB is not essential for the function of CSB in BER it seems necessary for the DNA unwrapping function of CSB (Beerens et al., 2005).

In vivo studies have demonstrated that the CSB/PARP-1 complex relocates to DNA damage in the nucleus after oxidative stress, and that CSB is present at the sites of active PARP-1 (Thorslund et al., 2005). Moreover, the presence of CSB is vital for PARP-1 stimulation of 8-oxoG repair (Flohr et al., 2003). Thus, it has been suggested that PARP-1 stimulation of BER depends on CSB, more specifically in the repair of 8-oxoG (Flohr et al., 2003; Thorslund et al., 2005). The biological function of the poly(ADP-ribosyl)ation of CSB is not yet clear. However, since both CSB and PARP-1 are involved in chromatin remodeling, transcription and DNA repair (De Vos et al., 2012; Stevnsner et al., 2008), it may be speculated that CSB and PARP-1 function together in chromatin remodeling to regulate transcription of DNA repair factors or to repair DNA.

Interestingly, PARP-1 has been shown to bind to AP sites and to compete with APE1 binding. Also, the activity of PARP-1 is greatly stimulated by APE1 incision of AP sites (Khodyreva et al., 2010). Recent publications also show that NEIL1 and OGG1 both interact with PARP1 (Hooten et al., 2012; Noren Hooten et al., 2011). Hence, the involvement of CSB with PARP-1 could be coordinated through the involvement of CSB with OGG1, NEIL1 and APE1.

In summary, CSB interacts physically and functionally with several proteins known to have important roles at different steps in the nuclear BER pathway (Fig. 1B and Fig. 2). Based on the multiple interactions of CSB with core BER proteins it is likely that at least part of the

phenotypical traits seen for CS patients are due to the lack of one or more of these interactions.

2.1.2 Physical and functional interaction of CSB with mitochondrial BER

proteins—Mitochondria have an independent BER pathway to protect the integrity of mitochondrial DNA (mtDNA). All components of this BER pathway are encoded by nuclear genes and the pathway resembles nuclear BER. Mitochondria contain APE1 and the glycosylases mitochondrial OGG1 (mtOGG1) and NEIL1, and these enzymes are involved in repair of oxidative mtDNA damage (de Souza-Pinto et al., 2001; Hu et al., 2005; Nishioka et al., 1999). Recently, it was demonstrated that CSB is also present in mitochondria, and translocates to the mitochondria after oxidative stress. This suggests that CSB is associated with mtDNA repair (Kamenisch et al., 2010; Aamann et al., 2010). In support, CSB-deficient cells have decreased levels of mitochondrial 8-oxoG, and uracil and 5-OH-U incision activities as well as AP endonuclease activity are decreased (Stevnsner et al., 2002; Aamann et al., 2010). Another oxidative lesion, FapyAde, accumulates in liver mtDNA of *csb*^{-/-} mice (Muftuoglu et al., 2009a). Since NEIL1 is the only DNA glycosylase known to repair FapyA in human cells CSB may also be involved in mtDNA repair together with NEIL1.

The interaction of CSB with mtDNA takes place in nucleoids indicating that CSB does not operate only by interacting with and stimulating specific glycosylases, but that the protein is part of an organizational complex connecting the BER process to the nucleoid (Kamenisch et al., 2010). Although its exact role in the mtBER pathway remains to be elucidated, CSB may be required to recruit and stabilize BER proteins to repair complexes associated with the inner mitochondrial membrane (Aamann et al., 2010).

In summary CSB interacts with and affects the function of numerous proteins involved in nuclear and mitochondrial BER, and it seems conceivable that some (if not all) of these interactions may be important for efficient genome maintenance

2.2 Interactions between CSB and proteins involved in transcription and transcription coupled nucleotide excision repair

CS proteins have been found to physically and functionally interact with several proteins involved in nuclear transcription and transcription coupled nucleotide excision repair (TC-NER) (Fousteri and Mullenders, 2008). Recently, a physical and functional interaction between CSB and the mitochondrial transcription apparatus has been identified as well. An overview of transcription proteins interacting with CSB is presented in Fig. 3.

2.2.1 Interactions between CSB and proteins involved in nuclear transcription and TC-NER

CSB deficient cells have reduced general RNA synthesis (Balajee et al., 1997; Bradsher et al., 2002), reduced ability to recover RNA synthesis after exposure to e.g. UV-light (Troelstra et al., 1992b; van der Horst et al., 1997) and deficient TC-NER. There is a significant overlap between proteins involved in transcription and in TC-NER, but this is discussed elsewhere in this special issue of Mechanisms of Ageing and Development.

2.2.1.1 CSB is in complex with RNA polymerase I: By immunoprecipitating (IP) CSB from cellular extracts, it has been shown that it is in complex with RNA polymerase I (RNA pol I) (Bradsher et al., 2002; Yuan et al., 2007). The immunoprecipitated complex could substitute for RNA pol I in a reconstituted transcription reaction supporting that the RNA pol I in complex with CSB is transcriptionally competent (Bradsher et al., 2002). By anion exchange chromatography, CSB and RNA pol I were found in the same fraction. The co-elution of TFIIF with RNA pol I required CSB protein indicating that CSB mediates a link

between TFIIH and RNA pol I (Bradsher et al., 2002). However, a direct interaction between CSB and RNA pol I still remains to be shown.

2.2.1.2 Interaction between RNA polymerase II and CSB: Several reports support an interaction between CSB and RNA polymerase II (RNA pol II). Thus, CSB was found to bind RNA Pol II stalled at the DNA template in electrophoretic mobility shift assays (EMSA) but only in the presence of hydrolysable ATP (Tantin et al., 1997). The binding of CSB to a stalled RNA pol II was confirmed in studies using either a cisplatin induced lesion or nucleotide deprivation to stall the RNA pol II (Sarker et al., 2005).

Using HeLa cell extracts, CSB and RNA pol II were found in the same fraction upon separation according to size or charge. Co-immunoprecipitation (Co-IP) of RNA pol II and CSB was stable at high salt concentrations, and the interaction was not disturbed by ethidium bromide, supporting a strong and DNA independent interaction. In the same report CSB did not interact with numerous other NER proteins (van Gool et al., 1997). In contrast, another study demonstrated that RNA Pol II and CSB could be found in a complex when using 50mM salt but not using physiological salt concentrations (Bradsher et al., 2002). Incidentally, this illustrates the complexity of comparing protein-protein interactions, which may vary due to small experimental differences. The CS1AN cell line is established from a CS patient with mutation in the *CSB* gene. In an *in vitro* transcription assay it was shown that RNA pol II co-purify with CSB from CS1AN SV40 transformed cells expressing HA and His₆ double tagged CSB. In contrast, the CSB eluate did not contain TFIIH, TFIIIB, TFIIIF, TFIIIE or TBP, supporting that a specific interaction exists between CSB and RNA pol II (van den Boom et al., 2004). Using crosslinked chromatin, pull down of CSB contained elongating RNA pol II, and this interaction increased upon UV irradiation. DNase treatment did not affect the co-IP. This suggests that CSB is present in the same complex as the elongating RNA pol II when the polymerase is stalled at UV lesions, and that DNA is not mediating the interaction (Fousteri et al., 2006).

2.2.1.3 Interaction between CSB, TFIIH and TFIIIE: A complex immunoprecipitated with CSB antibody from HeLa cell extract has been shown to contain XPB (alias p89) and XPD, both subunits of TFIIH (Bradsher et al., 2002). Functionally, the immunoprecipitated complex was able to replace TFIIH in a reconstituted nucleotide excision repair assay and in a reconstituted RNA pol II transcription assay (Bradsher et al., 2002). By chromatin immunoprecipitation (ChIP) of crosslinked cellular extracts from UV irradiated cells, CSB and XPB were pulled down with RNA pol II. The pull down of XPB together with RNA pol II on the UV damaged DNA was dependent on CSB (Fousteri et al., 2006). The interaction was not dependent on DNA as DNase treatment did not disrupt the CSB and XPB co-IP when using cells expressing HA and His₆-tagged CSB (Fousteri et al., 2006). EMSA with purified CSB from insect cells has shown that CSB and TFIIH bind to a stalled RNA pol II. This binding of TFIIH to RNA Pol II was dependent on CSB, suggesting that CSB mediates the interaction and thereby directly interacts with TFIIH (Tantin, 1998). However, in whole cell extracts, TFIIH did not co-IP with HA and His₆ tagged CSB under conditions where an interaction with RNA pol II was observed, suggesting that the interaction with TFIIH is less stable than the interaction with RNA pol II (van den Boom et al., 2004; van Gool et al., 1997) or that it does not take place under the same conditions.

A single study has suggested that CSB also interacts physically with TFIIIE and XPA (Selby and Sancar, 1997). TFIIIE facilitates initiation of RNA synthesis in conjunction with TFIIH, and pull down assays performed on HeLa cell free extracts showed that CSB was pulled down when antibodies against GST was used to pull down the GST-tagged p34 subunit of TFIIIE. In the same experiments it was shown that CSB could also be pulled down when GST-tagged XPA was in the cell free extract (Selby and Sancar, 1997).

2.2.1.4 UVSSA stabilizes CSB in TC-NER: UV-sensitive syndrome (UV^SS) is an autosomal recessive disorder characterized by photosensitivity and deficiency in transcription coupled repair. In contrast to CS patients UVSS patients mainly exhibit sun sensitivity, without any clear additional complications. UV^SS comprises three groups, UV^SS/CS-A, UV^SS/CS-B and UV^SS-A, caused by mutations in *CSA*, *CSB* and *UVSSA*, respectively. As will be described below, CSB is ubiquitinated in response to UV irradiation, and UVSSA (formerly known as KIAA1530) was recently shown to be part of a UV-induced ubiquitinated protein complex and to be involved in TC-NER (Nakazawa et al., 2012; Schwertman et al., 2012; Zhang et al., 2012). In an affinity purification experiment Zhang and co-workers found CSB, CSA and RNA Pol II in the chromatin fraction after UV irradiation. In addition, Nakazawa and co-workers showed in a co-IP experiment with extracts from HEK293T cells that UVSSA was pulled down with CSB, but it was not clarified whether the interaction was mediated by DNA or other protein factors (Nakazawa et al., 2012). Knockdown of UVSSA decreased the CSB level in a proteasome- and UV-dependent manner and attenuated the assembly of GFP-tagged CSB protein at locally induced UV-damage. UVSSA forms a complex with the deubiquitinating enzyme USP7 and UVSSA was shown to deliver USP7 to the TC-NER complex thereby protecting CSB against proteasomal degradation (Nakazawa et al., 2012; Schwertman et al., 2012; Zhang et al., 2012).

It is likely that the phenotypic difference between CS and UV^SS is due to the restricted role of UVSSA in TC-NER whereas CSB deficiency also affects repair of oxidative damage, which may be causative in neurodegeneration.

2.2.1.5 CSB interacts with factors involved in chromatin remodeling: For damage signaling and repair, chromatin remodeling is required to provide accessibility to DNA repair enzymes. Chromatin remodeling occurs by at least two mechanisms: 1) covalent histone modifications by means of post-translational modifications (PTMs) and 2) displacement of histones or entire nucleosomes, either by sliding along the DNA or by removal. *In vitro* studies have documented the ability of large multiprotein complexes, including several SWI2/SNF2-related proteins, to be involved in remodeling chromatin structure in an ATP-dependent manner (Kingston and Narlikar, 1999; Kwon et al., 1994), and a link between CSB activity and chromatin remodeling has been proposed (Beerens et al., 2005; Christiansen et al., 2003; Citterio et al., 2000; Newman et al., 2006). In addition, multiple proteins known to be involved in chromatin remodeling have been found to interact or be in complex with CS proteins as described below.

CSB interacts with histone proteins: Histones involved in forming the chromatin structure, have been found to directly interact with CSB protein. The interaction was supported by Far Western experiments showing that the histone tails were essential for the interaction (Citterio et al., 2000).

CSB interacts with Transcription Termination Factor 1: Transcription termination factor 1 (TTF-1) is involved in regulation of RNA pol I transcription by binding to the promoter-proximal terminator site and is involved in recruiting chromatin remodeling factors to facilitate RNA pol I transcription. CSB has been shown to interact with TTF-1. Thus, using reciprocal co-IP of TTF-1 and CSB from partially fractionated nuclear extracts it was shown that TTF-1, CSB and RNA pol I are in the same complex. A direct physical interaction between CSB and TTF-1 was shown using tagged and immobilized CSB and *in vitro* translated TTF-1. The interaction was mapped to the N-terminal part of TTF-1 (Yuan et al., 2007). TTF-1 interacting protein 5 (TIP5) is part of the NoRC chromatin remodeling complex, which induces ATP dependent nucleosome sliding (Strohner et al., 2001). The interaction between CSB and TTF-1 competes with the binding between TTF-1 and TIP5.

Since TIP5 is involved in repression of rDNA transcription, it has been suggested that recruitment of either CSB or NoRC, including TIP5, to TTF-1 determines whether rDNA is transcribed or silenced (Yuan et al., 2007) (Strohner et al., 2001).

CSB interacts with G9a methyl transferase: A link between G9a methyl transferase, capable of mono- and dimethylating histone 3 lysine 9 (H3K9) involved in RNA pol I transcription, and CSB, has recently been reported. CSB and G9a have been shown to be in the same complex by using cellular extracts with overexpressed CSB or G9a protein and reciprocal co-IP of CSB and G9a, respectively. Accordingly, mono- and dimethyl transferase activity was shown to associate with immunoprecipitated CSB (Yuan et al., 2007). A direct interaction between G9a and CSB was identified by the binding of purified CSB to beads with immobilized Flag-G9a purified from HEK293T cells. The CSB associated G9a was found to methylate H3K9 and was required for RNA pol I transcription (Yuan et al., 2007).

CSB in the nucleosome remodeling and deacetylation complex: The nucleosome remodeling and deacetylation complex (NuRD), which displays ATP dependent nucleosome remodeling activities as well as histone deacetylase activity, was recently shown to interact with CSB. NuRD is involved in regulation of rDNA transcription by modulating chromatin structure (Xie et al., 2012). Tandem affinity purification of CSB followed by mass spectrometry identified all components of NuRD to be present in complex with CSB. The interaction was supported by co-IP of CSB using an antibody against CHD4, a component of NuRD, in a nuclear extract. Additionally, it was shown that knock down of CSB decreased the binding of CHD4 to the rDNA promoter region in a ChIP assay. This suggests that the interaction between CSB and NuRD is important for the complex binding to the rDNA promoter (Xie et al., 2012). However, a direct protein-protein interaction has not yet been demonstrated.

CSB dependent recruitment of p300, HMGN1 and XAB1 to stalled RNA pol II: The histone acetyl transferase p300 is recruited to stalled RNA pol II upon UV irradiation. This recruitment depends on CSB but not CSA. High mobility group nucleosome binding domain 1 protein (HMGN1) is recruited to RNA pol II after UV irradiation and the recruitment depends on both the CSB and CSA protein. Furthermore, in ChIP assays using antibodies for CSB or RNA pol II for IP, XAB2 (tetratricopeptide (TRP) protein), which is involved in TCR, was found to be pulled down with CSB and RNAPol II. The association of XAB2 to RNA pol II was dependent on both CSA and CSB (Fousteri et al., 2006) similar to the situation for HMGN1. However, it cannot be excluded that other proteins or DNA are mediating the interactions. Nakatsu and co-workers also found CSB to be in complex with XAB2 and RNA pol II when they performed IP's on cell extracts (Nakatsu et al., 2000). However, for p300, HMGN1 and XAB2, respectively, no direct interaction to CSB protein has been reported. In conclusion CSB forms complexes with multiple proteins involved in chromatin remodeling, and in many cases the interactions are direct. The functional role of CSB in chromatin remodeling is still debated, but the interactome suggests roles for CSB in RNA pol I and RNA pol II transcription

2.2.1.6 CSB interacts with endonuclease XPG: The protein XPG is encoded by the *XPG (ERCC5)* gene, and is involved in NER, where it functions as an endonuclease that cleaves 3' of the lesion (O'Donovan et al., 1994). In addition to its role in NER, XPG has been shown to play a role in BER by stimulating the glycosylase activity of NTH1 and lack of XPG results in decreased thymine-glycol incision capacity (Dianov et al., 2000; Klungland et al., 1999). A direct interaction between XPG and CSB has been detected by co-IP of *in vitro* translated XPG and 9E10 epitope tagged CSB (Iyer et al., 1996). A direct interaction

between CSB and XPG was also observed by Far Western and mapped to the C-terminal end of XPG. Functionally, XPG was found to slightly stimulate the ATPase activity of CSB when using DNA containing a 10-nucleotide bubble structure, but not when double stranded DNA was used (Sarker et al., 2005). This stimulatory effect was not observed in another study (Berquist et al., 2012). When IP of CSB from HeLa cell extract was performed under physiological salt conditions, a complex was pulled down, which was able to reconstitute NER in the absence of XPG. The same IP was not able to reconstitute NER in the absence of XPA, XPC, RPA or XPF, indicating the presence of XPG but not XPA, XPC, RPA or XPF in the complex (Bradsher et al., 2002). In ChIP assays XPG was found to co-IP with CSB and RNA pol II after UV irradiation and the precipitation of XPG with RNA pol II was dependent on CSB, suggesting that CSB mediates the contact (Fousteri et al., 2006). However, due to the limitations of the ChIP assay, it cannot be excluded that other proteins or DNA mediates the contact. Purified XPG is able to bind to a stalled RNA pol II *in vitro* in the absence of CSB, which argues against a mediator role for CSB (Sarker et al., 2005); and IP of CSB from whole cell extract did not co-IP with XPG (van Gool et al., 1997). The functional consequence of the interaction between XPG and CSB is not clear, but lack of CSB results in reduced incision of lesions by TC-NER (Anindya et al., 2010). This suggests that interaction of XPG with CSB is playing a role during TC-NER.

2.2.1.7 CSA and CSB interacts: Clinical differences between *CSA*-deficient and *CSB*-deficient patients have not been observed, but the *CSA* and *CSB* gene products have quite different biochemical characteristics and different functions. Therefore, it is interesting that a direct physical interaction between *in vitro* transcribed and translated *CSA* protein and 9E10 tagged *in vitro* translated *CSB* protein was reported more than 10 years ago, and this finding was supported by a yeast-2-hybrid assay (Henning et al., 1995). The direct interaction was supported by the finding that a moderate two fold stimulation of the dsDNA dependent ATPase activity of *CSB* was observed upon addition of *CSA* (Tantin et al., 1997). By using tandem affinity purification followed by mass spectrometry analysis of immunoprecipitated *CSA*, *CSB* was found to be present in a *CSA* containing complex (Fei and Chen, 2012). A functional relevance for an interaction is supported by results from wild type human cells as well as from *CSB* deficient cells, where it has been shown that *CSA* translocates to the nuclear matrix in response to UV irradiation in a *CSB* dependent manner (Kamiuchi et al., 2002). Additionally, the *CSA* protein associates with the chromatin insoluble fraction after UV irradiation in a *CSB* dependent manner (Fousteri et al., 2006), thus supporting a role for *CSB* in translocating *CSA* to the chromatin in response to UV lesions. In cellular extracts with overexpression of *CSA*, *CSA* has been found in complex with *CSB* with increased co-IP after UV irradiation. Interestingly, treatment of the cells with a proteasome inhibitor prolonged this interaction (Groisman et al., 2006).

In an earlier study using HeLa whole cell extracts, *CSA* and *CSB* were present in different fractions upon ion-exchange chromatography and reciprocal co-IP from whole cell extracts did not detect an interaction between *CSA* and *CSB* (van Gool et al., 1997). However, lack of UV irradiation prior to harvest could explain the lack of interaction. Interestingly, a *CSB* and *CSA* interaction was detected in another study, in the absence of UV irradiation (Latini et al., 2011) but the extent of interaction after UV irradiation was not studied.

The interaction between *CSB* and *CSA* has not been mapped per se, but a recent study of the interaction between *CSB* and p53 found that upon IP of p53 from cell extracts, *CSA* was co-purified with the p53-*CSB* complex. However, in a *CSB* deficient cell line, still expressing *CSB*-PGBD3, *CSB*-PGBD3 was pulled down with p53, but *CSA* was no longer present in the complex. This indicates that the N-terminal 465 amino acids of *CSB* are not sufficient for interaction with *CSA*, but suggests that the carboxyl terminal region of *CSB* is required for *CSA* interaction (Latini et al., 2011).

In conclusion several results support a model where CSA and CSB interact, with increased level after UV irradiation, and that the interaction between CSA and CSB is important for the recruitment of CSA to relevant complexes. Thus, the association of CSA and CSB under various conditions is an open and interesting question going forward.

2.2.2 CSB interaction with proteins involved in mitochondrial transcription—

Recently, a direct physical interaction between CSB and mitochondrial transcription factor A (TFAM) has been identified (Berquist et al., 2012). Purified CSB and TFAM interact *in vitro* as determined by co-IP. Interestingly, the interaction was found to stimulate the ATPase activity of CSB 4.5 fold at a four molar ratio of CSB:TFAM - the most significant stimulation of the ATPase activity of CSB reported so far. The stimulation was found not to be a consequence of TFAM distortion of the DNA substrate, thereby supporting a direct functional interaction between CSB and TFAM. Additionally, CSB was able to remove TFAM bound to DNA in an ATP independent manner. Mitochondrial transcription factor 2B and mitochondrial RNA polymerase was found to moderately stimulate the ATPase activity of CSB (1.7 fold and 2.0 fold, respectively) using purified proteins, suggesting possible interactions with those proteins as well. In a reconstituted mitochondrial transcription assay a 5 to 35 fold increase in the formation of long transcripts was observed in the presence of CSB suggesting that CSB stimulates the elongation of mitochondrial RNA polymerase. Support for the observation that CSB stimulates mitochondrial transcription also comes from quantitative long range PCR analysis of mitochondrial transcripts where CSB deficient cell lines were found to have fewer long transcripts than CSB wild type complemented cells. The involvement of CSB was found to have both an ATPase dependent and ATPase independent component, as a CSB ATPase dead mutant partially complemented the transcriptional defect in the CSB deficient cells (Berquist et al., 2012).

The novel role for CSB in mitochondrial transcription opens for interesting new perspectives regarding the understanding of the relation between CSB function and CS pathology. It also provides evidence for the importance of CSB in maintaining mitochondrial function.

2.3 CSB interacts with p53 tumor suppressor protein

The p53 transcription factor and tumor suppressor has been implicated in biological processes, such as DNA repair and aging. It plays a critical role in maintaining genome integrity and is activated by various mechanisms, including DNA damage. It has been known for a long time that p53 physically interacts with CSB via the C-terminal domain of p53 (Wang et al., 1995). When immunoprecipitating p53 from cell extracts, CSB as well CSB-PGBD3 were pulled down. This suggests that either the N-terminal 465 amino acids of CSB are sufficient for interaction with p53 or that PGBD3 interacts with p53 (Latini et al., 2011). However, in a recent study Lake et al. mapped the interaction between CSB and p53 to the C-terminal region and to a fragment containing the helicase motifs (Lake et al., 2011). Additionally, using *in vitro* binding and ChIP approaches, Lake and co-workers demonstrated that CSB facilitates the sequence-independent association of p53 with chromatin when p53 concentrations are low. Interestingly, elevated concentrations of p53 prevents CSB from binding to nucleosomes by occluding a nucleosome interaction surface on CSB (Lake et al., 2011). Additionally, it was found that CSB competes with p300 in binding to p53 so that in the presence of excessive CSB, p53 does not bind p300 (Filippi et al., 2008).

CS cells display elevated and persistent levels of p53 and this was recently shown to be due to insufficient ubiquitination of p53. CSB proteins associate in a complex with p53 and Mdm2 (the major E3 ubiquitin ligase for p53); this interaction stimulates the ubiquitination of p53 in an Mdm2-dependent manner. Tandem affinity purification and

immunoprecipitations combined with mass spectrometry studies also indicated that CSB associates with a Cullin Ring Ubiquitin Ligase complex (Latini et al., 2011).

Although the function of the CSB-p53 interaction in the context of DNA repair remains elusive, it has been suggested that CSB plays an important role in keeping a balance between cellular aging and cancer susceptibility by regulating p53 activity (Frontini and Proietti-De-Santis, 2012)

2.4 Interaction between CSB and BRCA1

The product of the *BRCA1* gene is involved in susceptibility to breast and ovarian cancer and its product regulates the cellular response to DNA double-strand breaks. However, BRCA1 has also been implicated in excision repair of DNA damage. Recently, it was demonstrated that BRCA1 accumulates immediately at UV-irradiated sites in wild type and CSA deficient cells but not in CSB deficient cells. BRCA1 was also shown to physically interact with and polyubiquitinate CSB and this polyubiquitination and subsequent degradation of CSB occurred after UV irradiation, even in the absence of CSA. In addition it was shown that suppression of BRCA1 expression inhibits TC-NER of UV lesions (Wei et al., 2011).

BRCA1 seems to be involved in TC-NER of UV-damage in a CSB dependent but CSA independent manner, and a BRCA1 ubiquitination pathway for CSB may exist alongside the CSA-dependent pathway

2.5 CSB interacts with kinases *in vitro* and *in vivo*

The protein tyrosine kinase c-Abl may also play a role in DNA repair as a regulator/coordinator of the DNA damage response. The kinase is activated in response to genotoxic or oxidative stress and DNA damage. Using pull-down and co-IP experiments we have shown that the N-terminal region of CSB (aa 2-341) interacts with the SH3 domain of c-Abl *in vitro* and *in vivo*. In addition, by combining SDS-PAGE with MALDI-MS analysis of CSB that was *in vitro* phosphorylated by c-Abl, we demonstrated that CSB is phosphorylated at Tyr932. The subcellular localization of CSB to the nucleus and nucleolus was shown to be altered after phosphorylation by c-Abl, and c-Abl-dependent phosphorylation of CSB increased in cells treated with hydrogen peroxide (Imam et al., 2007). In another study, we previously showed that CKII can phosphorylate recombinant CSB protein *in vitro* and reported that the ATPase activity of CSB is affected by this phosphorylation (Christiansen et al., 2003).

2.6 Interaction between Ubiquitin and CSB

Many proteins can be post-translationally modified by ubiquitin. Several ubiquitinated proteins contain a C-terminal ubiquitin binding domain (UBD), and in CSB this region spans from amino acid 1400 to 1428 (Fig. 1). Thus, in pull down assays using a GST tagged UBD fragment of CSB, the fragment was able to bind ubiquitinated proteins from a human cell extract. Reciprocally, wild type, but not UBD mutated CSB, was able to bind immobilized GST tagged ubiquitin (Anindya et al., 2010). It is currently not known in which protein-protein interaction(s) the use of UBD is relevant. But since an UBD deleted CSB protein is not able to perform damage incision or complement a CSB patient cell line with regard to transcription recovery and cell survival after UV irradiation it is likely that UBD of CSB may have a role in TC-NER (Anindya et al., 2010).

2.7 Interaction of CSB with TRF2 *in vivo*

TRF2 is a duplex telomeric DNA binding protein essential for telomere protection. Based on co-IP and immunoblotting experiments Batenburg et al. recently showed that a small

percentage of endogenous TRF2 (estimated to be 1–5%) physically interacts with CSB (Batenburg et al., 2012). Several domains of CSB were shown to be engaged in its interaction with TRF2, whereas a central TRF homology domain of TRF2 was shown to be required and sufficient for binding CSB. Using immunofluorescence staining the authors also found that CSB localizes at a small subset of human telomeres and that CSB is required for preventing the formation of telomere dysfunction-induced foci (TIF). Interestingly, introduction of wild-type CSB into CS cells promoted telomerase-dependent telomere lengthening (Batenburg et al., 2012). Taken together, these findings suggest that CSB is involved in maintaining telomere length and stability. For an overview of the described CSB interaction partners see Fig. 3 and Table 1.

3 CSA protein

The *CSA* gene (*ERCC8*) is located on chromosome 5q12–q13, was cloned in 1995, and encodes a protein of 396 amino acids and 44 kDa. The *CSA* gene product (CSA) belongs to the ‘WD repeat’ (W= tryptophane, D= aspartic acid) family of proteins. According to its primary sequence, CSA contains five WD repeat motifs (Henning et al., 1995), and two additional WD repeats were predicted in CSA by sequence alignment using the COB LATH method (Zhou and Wang, 2001). The WD repeats are believed to form circularized beta propeller structures forming a scaffold for protein-protein interactions. Interestingly, all missense mutations detected in CS-A patients are within the WD repeats (Laugel et al., 2010) (Fig. 4). The family of proteins with WD repeats has been found to exhibit structural and regulatory roles but no enzymatic activity. CSA is part of a multisubunit ubiquitin ligase complex, containing Cullin4A, Roc 1 (Rbx1) through interaction with DNA damage binding protein 1 (DDB1) (Groisman et al., 2003). CSA-associated ligase activity is responsible for the degradation of TC-NER proteins at the end of the repair process so transcription can resume. Accordingly, it is demonstrated that CSA physically interacts with CSB, and CSB is ubiquitinated by the CSA ligase complex. Ubiquitinated CSB is degraded by the proteasome in a UV-dependent manner at a late stage of TC-NER (Groisman et al., 2006). The crystal structure of CSA has not yet been determined. CSA has been shown to physically and functionally interact with a number proteins; however, the precise interactions sites of CSA have not been elucidated and the list of interaction partners is very limited compared to CSB (Table 2).

3.1 Interactions of CSA with proteins involved in BER

Studies with CSA knockout (*csa*^{-/-}) mouse embryonic fibroblasts and keratinocytes demonstrated that *csa*^{-/-} mouse cell lines are not sensitive to oxidative agents, IR or paraquat. In the same study, *csa*^{-/-} mouse embryonic stem cells showed slight IR sensitivity (de Waard et al., 2004). The *csa*^{-/-} mouse mimics the human phenotype in terms of the TC-NER repair defect, retinal degeneration and manifestation of UV sensitivity of skin and eyes, but in contrast to CS in humans, they exhibit normal development (van der Horst et al., 2002). CSA knockout mice fed with food containing di(2-ethylhexyl)phthalate that causes elevated levels of oxidative DNA damage in liver did not show any weight reduction (de Waard et al., 2004). In addition, human primary CSA fibroblasts and SV-40 transformed CSA fibroblasts are not sensitive to X-rays and potassium bromide (D’Errico et al., 2007). In contrast, both human primary CSA keratinocytes and fibroblasts are hypersensitive to potassium bromide that specifically induces oxidative DNA damage. Human primary CSA keratinocytes are slightly hypersensitive to X-rays. Furthermore, 8-oxoG accumulates in primary CSA keratinocytes and fibroblasts, and in SV-40 transformed CSA deficient (CS3BE) cells after potassium bromide induced oxidative stress (D’Errico et al., 2007). These findings indicate that the role of CSA in protecting cells from oxidative damage depends on the cell system and oxidative agents used.

CSA cell extracts are proficient in 8-oxoG and 5-hydroxy-2-deoxycytidine (5-OHdC) cleavage activity in an *in vitro* assay (D'Errico et al., 2007; Foresta et al., 2010). On the other hand, the processing of plasmids containing a single 8-oxoG is defective in CSA and CSB cell lines (Spivak and Hanawalt, 2006). A recent paper demonstrated that the CSA protein localized in mitochondria in response to oxidative stress, and is in complex with mitochondrial OGG1 (Kamenisch et al., 2010). That supports the role of CSA in response to oxidative DNA damage. The molecular role of the CSA protein in the repair of oxidative DNA lesions is not yet understood, but it may play a direct role in BER by regulating expression, activity or localization of BER proteins.

3.2 Interactions of CSA with proteins involved in transcription, TC-NER and ubiquitination

Like cells from CSB patients, CSA patient cells are deficient in TC-NER (Venema et al., 1990). However, no XP proteins or RNA polymerases have been identified as interaction partners of CSA. In contrast, the link between CSA and proteins involved in ubiquitination in response to UV irradiation is better established than the link between CSB and ubiquitination.

3.2.1 Interaction between CSA and UVSSA—UVSSA was recently shown to be involved in TC-NER and to interact with CSA (Fei and Chen, 2012). Using tandem affinity purification followed by mass spectrometry, Fei and Chen detected CSA in complex with UVSSA. Additionally, co-IP experiments followed by western blotting of cell extracts from cells overexpressing UVSSA and/or CSA, confirmed that the two proteins are in the same complex. Furthermore, endogenous XPB, CSA and UVSSA from HEK293T cells were found to be present in the same complex by co-IP. The domain of UVSSA responsible for the interaction with CSA was mapped to the N-terminal 200 amino acids. The CSA mutant W361C situated in WD blade 7 of CSA and causing mild UV sensitive syndrome, was found to greatly diminish the interaction between CSA and UVSSA, suggesting that the interaction depends on this amino acid or the surrounding area (Fei and Chen, 2012). Functionally, the interaction between CSA and UVSSA was found to be important for the stabilization of CSB after UV irradiation as mutant CSA or knock down of UVSSA resulted in UV induced degradation of the CSB protein (Fei and Chen, 2012). Accordingly, Zhang and co-workers have shown that CSA is required for recruitment of the UVSSA-USP7 complex to CSB and RNA Pol II in UV-irradiated chromatin and that UVSSA enhances UV-induced translocation of CSA to the nuclear matrix (Zhang et al., 2012).

3.2.2 CSA is in a complex with DDB1, Cullin4A and Roc1—IP of CSA from CSA overexpressing cells followed by mass spectrometry as well as immunoblotting, detected that the CSA protein is in complex with DDB1. Likewise, Cullin4A, an E3 ubiquitin ligase, and Roc1, were shown to be part of the same ubiquitin ligase complex (Groisman et al., 2003). The interaction between Cullin4A/Roc1 and CSA has, however, later been found to depend on DDB1, strongly suggesting that Cullin4A/Roc1 and CSA does not directly interact (Groisman et al., 2006).

3.2.3 CSA is in complex with COP9 signalosome complex—The COP9 signalosome (CSN) is a conserved protein complex found in all eukaryotic cells and it is involved in the regulation of the ubiquitin (Ub)/26S proteasome system. After UV irradiation, CSA was part of the CSN complex, which contains deubiquitinating activity, negatively regulating ubiquitination activity of the DDB1-Cullin4A/Roc1-CSA complex. CSA dissociated from CSN approximately 4 hours after UV irradiation. In experiments using cells with siRNA knock down of the CSN complex, CSN was shown to be important for UV-induced unscheduled DNA synthesis and RNA synthesis recovery after UV irradiation, thus establishing a role for the CSN complex in GGR and TCR (Groisman et al.,

2003). In time-course studies the ubiquitin ligase activity associated with CSA was required for degradation of CSB approximately 3 hours after UV irradiation. The CSA dependent degradation of CSB may be important for RNA synthesis recovery after UV irradiation (Groisman et al., 2006).

3.2.4 CSA interacts with XAB2 and affects HMGN1 association to stalled

RNApol II—CSA has been found to be in complex with XAB2 by co-IP of cellular extracts. Importantly, the interaction in cell extracts is supported by interaction between *in vitro* translated XAB2 and CSA, thus providing evidence for a direct interaction between the two proteins (Nakatsu et al., 2000). The results are supported by the finding in ChIP assays where the recruitment XAB2 to chromatin with stalled RNA pol II depends on CSA. In the same study it was shown that HMGN1 association with stalled RNApol II also depends on CSA (Fousteri et al., 2006).

3.3 CSA is in complex with p53 tumor suppressor protein

CS cells display elevated and persistent levels of p53 and this was recently shown to be due to insufficient ubiquitination of p53. CSA associates, in a CSB dependent manner, in a complex with p53 and Mdm2 (the major p53 E3 ubiquitin ligase); this interaction stimulates the ubiquitination of p53 in an Mdm2-dependent manner. Tandem affinity purification and IP combined with mass spectrometry studies furthermore indicate that CSA and CSB associate with a Cullin Ring Ubiquitin Ligase complex responsible, under certain circumstances, for p53 ubiquitination (Latini et al., 2011). For an overview of the described CSA interaction partners see Fig. 5 and Table 2.

4 Concluding remarks

The list of proteins interacting with CSB and CSA is still growing and we have tried to cover many of these interactions in this review. However, we apologize for not being able to include all reports involving potential interactions. The standing question in the field of research in CS has been and still is whether the syndrome is a consequence of the role of CS proteins in transcription, TC-NER or BER (Fousteri et al., 2006; Proietti-De-Santis et al., 2006; Stevnsner et al., 2008). Additionally, recent evidence supports a role for CS proteins in mitochondria where it seems to be involved in transcription, genome maintenance and super-complex organization in the mitochondrial membrane as well as autophagy (Berquist et al., 2012; Kamenisch et al., 2010; Osenbroch et al., 2009b; Scheibye-Knudsen and Quistorff, 2009; Stevnsner et al., 2002; Aamann et al., 2010). CSB is thus involved in maintaining cellular homeostasis by involvement in nuclear DNA repair and transcription as well as in mitochondrial functions (Fig. 6). The involvement of CSB in mitochondrial DNA metabolism is a new aspect of CSB functions and may be an important one. It is also supported by the clinical phenotype of CSB, which involves many of the signs and symptoms also seen in mitochondrial diseases. The involvement of CS proteins in TC-NER cannot be ignored since TC-NER is the only process CS proteins are known to be essential for. However, the severity of the CS phenotype compared to XP-A patients argues that lack of TC-NER is not solely the basis for the severe CS phenotype. The identification of a patient with UV sensitive syndrome (UVsS) due to a mutation in *CSA* that causes UV sensitivity but not the sensitivity toward oxidative stress seen in cells from CS patients (Nardo et al., 2009) indicates that the neurological problems seen in CS may be due to other defects than TC-NER deficiency. Additionally, the lack of sensitivity toward oxidative stress in cells from a UVsS patient contrasted to the sensitivity toward oxidative stress observed in CS patients suggest that oxidative stress plays a role in development of CS phenotype.

The answer as to which role of the CS proteins is most important for the development of the CS phenotype is complex. All tissues in CS patients are not equally affected by the lack of

CS proteins and the function of the CS proteins could very well differ between different cell types as e.g. the sensitivity of CS cells towards oxidative stress is cell type dependent (de Waard et al., 2004; de Waard et al., 2003). The importance of the interaction partners of CS could therefore also differ from tissue to tissue.

The function of the CSB protein is most likely mediated by two different mechanisms as there is a difference in the involvement of the ATPase activity of CSB in TC-NER, transcription and BER, as discussed above. It has been proposed that the ATPase activity of CSB is involved in a conformational change of CSB, thereby regulating the ability of CSB to stably interact with chromatin (Lake et al., 2010). It is possible that such a conformational change of CSB determines whether CSB is involved in BER or TC-NER.

The multiple interactions of CS proteins with proteins involved in chromatin remodeling raises another possibility of involvement of CS proteins in BER. It would be interesting to address whether the accumulation of oxidative lesions and sensitivity toward ROS forming agents observed *in vivo* in CS cells is merely an effect of the direct stimulation of BER enzymes with CSB. Studies point to a role for chromatin remodeling in BER (Beard et al., 2005; Beard et al., 2003; Menoni et al., 2007) and it is therefore possible that CS proteins in the context of oxidative lesions, in addition to directly stimulating BER enzymes also are involved in recruiting chromatin remodeling factors. The recent data establishing a role for CSB in mitochondrial transcription, stimulation of CSB's ATPase activity by TFAM and the ability of CSB to remove TFAM from DNA could point towards such a bifunctional role of CSB in BER; by stimulating BER enzymes and by increasing the accessibility of BER enzymes to the substrate. Future studies are needed to fully elucidate these possibilities.

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Highlights

- CSA and CSB proteins participate in multiple protein interactions and complexes
- The CSB protein has functions in BER, TC-NER and transcription
- The CSB protein is likely to have bifunctional roles in mitochondria
- The CSA protein is primarily interacting with proteins involved in ubiquitination

A

B

Figure 1.
Motifs in the CSB protein. CSB harbors an acidic region and a glycine rich stretch with unknown function and two putative nuclear localization sequences (NLS) and a nucleotide binding domain (NTB). An ATPase domain including seven helicase-like motifs is present in the center of the protein, with the ATPase activity of CSB being necessary for the involvement in TC-NER. An ubiquitin binding domain (UBD) is found in the C-terminal with likely relevance for involvement in TC-NER. B) The N-terminal of CSB has been found to interact with proteins involved in BER.

Figure 2.

CSB interactions with BER core participants. The interactions between CSB and the DNA glycosylase NEIL1, and co-existence of CSB in complex with OGG1 affects the repair of oxidative lesions at the incision/excision steps in BER. The interaction with APE1 stimulates the formation of single strand breaks ready for elongation by DNA polymerases. PARP-1 is known to bind single strand breaks.

Figure 3.

Proteins interacting or in complex with CSB. CSB directly interacts (showed in black) or is found in complex with (shown in gray) proteins involved in transcription, TC-NER and BER, based on the literature described in the text. The great overlap between functionality of the proteins interacting with CSB supports a complex multifunctional background for Cockayne Syndrome.

Figure 4. Motifs in the CSA protein. Seven Trp-Asp motifs (WD) have been identified in CSA, forming circularized beta propeller structures serving as scaffold for protein-protein interactions.

Figure 5.

Proteins directly interacting or in complex with CSA. CSA directly interacts (showed in black) or is found in complex with (shown in gray) proteins involved in transcription, TC-NER and ubiquitination, based on the literature described in the text. The majority of proteins found in complex with CSA and with functional effect of the interaction are proteins involved in ubiquitination of proteins in response to cellular damage. Additionally, proteins involved in transcription and TC-NER by chromatin remodeling is found to depend on CSA for their recruitment.

Figure 6.

Roles of CSB in maintenance of cellular homeostasis. CSB are involved in multiple functions in the cell all aimed to maintain cellular homeostasis. In the nucleus CSB is involved in transcription thereby involved in maintaining the transcriptome whereas involvement in TC-NER and BER both are related to genomic stability. In the mitochondria, CSB is involved in maintaining the transcriptome through transcription and DNA stability by mtBER. Additionally, CSB are involved in mitochondrial autophagy and bioenergetics.

Table 1

Proteins directly interacting or in complex with CSB

Protein binding partner	Nature of CSB interaction or interaction domain	The nature of functional interaction	References
NEIL1	CSB2-341	Stimulates incision & AP lyase activity of NEIL1	(Muftuoglu et al., 2009)
APE1	Direct interaction	Stimulates APE1 endonuclease activity	(Wong et al., 2007)
PARP1	CSB2-341	Reduces ATPase activity of CSB	(Thorslund et al., 2005)
mtOGG1	Nd	Stimulates gene expression of mtOGG1	(Kamenisch et al., 2010; Stevnsner et al., 2002)
Nuclear OGG1	In the same protein complex	Stimulates gene expression of nuclear OGG1	(Dianov et al., 1999)
c-Abl	CSB2-341	Phosphorylates CSB	(Imam et al., 2007)
RNA pol I	In complex with	Increases transcription by RNA Poll	(Bradsher et al., 2002; Yuan et al., 2007)
RNA pol II	Direct interaction	Putative recruitment of chromatin remodeling enzymes	(Fousteri et al., 2006; Sarker et al., 2005; Tantin et al., 1997; van den Boom et al., 2004; van Gool et al., 1997)
TFIIH	Direct interaction	Helicase function of TFIIH involved in transcription and TC-NER	(Bradsher et al., 2002; Fousteri et al., 2006; Tantin, 1998)
UVSSA	In complex with	Stabilizes CSB in TC-NER	(Nakazawa et al., 2012; Schwertman et al., 2012; Zhang et al., 2012)
Hi stones	Direct interaction	Chromatin structure	(Citterio et al., 2000)
TTF-1	Direct interaction	Modulation of chromatin structure	(Yuan et al., 2007)
G9a	Direct interaction	Modulation of chromatin structure	(Yuan et al., 2007)
NuRD	In complex with	Modulation of chromatin structure	(Xie et al., 2012)
p300	In complex with	Modulation of chromatin structure	(Fousteri et al., 2006)
HMGN1	In complex with	Modulation of chromatin structure	(Fousteri et al., 2006)
XAB2	In complex with	Modulation of chromatin structure	(Fousteri et al., 2006)
XPG	Direct interaction	Stimulation of TC-NER and BER	(Bradsher et al., 2002; Fousteri et al., 2006; Iyer et al., 1996; Sarker et al., 2005)
CSA	Direct interaction	Translocation of CSA, control of ubiquitination of CSB and p53	(Fei and Chen, 2012; Groisman et al., 2006; Henning et al., 1995; Kamiuchi et al., 2002; Latini et al., 2011; Tantin et al., 1997)
TFAM	Direct interaction	Stimulation of ATPase activity of CSB	(Berquist et al., 2012)

Protein binding partner	Nature of CSB interaction or interaction domain	The nature of functional interaction	References
p53	ATPase domain and C-terminal and/or N-terminal	Regulation of p53 level. Reciprocal regulation of chromatin association.	(Filippi et al., 2008; Lake et al., 2011; Latini et al., 2011; Wang et al., 1995)
BRCA1	Direct interaction	Ubiquitination of CSB	(Wei et al., 2011)
TRF2	Multiple domains	Maintenance of telomeres	(Batenburg et al., 2012)

Table 2

Proteins directly interacting or in complex with CSA

Protein binding partner	Nature of CSA interaction or interaction domain	The nature of functional interaction	References
CSB	Direct interaction	Translocation of CSA, control of ubiquitination of CSB and p53	(Fei and Chen, 2012; Groisman et al., 2006; Henning et al., 1995; Kamiuchi et al., 2002; Latini et al., 2011; Tantin et al., 1997)
UVSSA	WD 7	Stabilization of CSB after UV irradiation	(Fei and Chen, 2012)
CSN	In complex with	Stabilization of CSB after UV irradiation	(Groisman et al., 2006)
p53	In complex with	Regulation of p53 level and binding to chromatin	(Latini et al., 2011)
XAB2	Direct interaction	Transcription and TC-NER	(Fousteri et al., 2006; Nakatsu et al., 2000)
HMG1	In complex with	Modulation of chromatin structure	(Fousteri et al., 2006; Nakatsu et al., 2000)
DDB1, Cullin 4A, Roc1	In complex with	Ubiquitination	(Groisman et al., 2006; Groisman et al., 2003)