2018

Mechanisms Underlying Oxidative Stress-Induced Chromatin Association Of Cockayne Syndrome Protein B (csb)

Erica Lee Boetefuer
University of Pennsylvania, ericaboe@sas.upenn.edu

Mechanisms Underlying Oxidative Stress-Induced Chromatin Association Of Cockayne Syndrome Protein B (csb)

Abstract
Cockayne syndrome is a devastating premature aging disorder characterized by extreme sun sensitivity and severe neurological and developmental defects. The majority of Cockayne syndrome cases are due to mutations within the ATP-dependent chromatin remodeler Cockayne syndrome protein B (CSB). CSB functions in transcription regulation and is required for transcription-coupled nucleotide excision repair (TC-NER). Importantly, CSB is also critical for the relief of oxidative stress. Studies suggest CSB may participate in the major repair pathway for oxidative DNA damage repair, base excision repair (BER), yet direct evidence supporting this hypothesis remains elusive and exactly how CSB functions in this process is unknown. CSB is also suggested to function in the relief of oxidative stress by regulating transcription. We demonstrated CSB's interaction with chromatin is stabilized by oxidative stress and the goal of this work is to understand the role of CSB in the relief of oxidative stress by characterizing the mechanisms underlying CSB's oxidative stress-induced chromatin interaction. Using chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) we characterized for the first time CSB's genome-wide occupancy following oxidative stress. This revealed CSB is enriched at the binding motif for the CCCTC-binding factor (CTCF), and further analysis demonstrated CSB and CTCF directly interact and regulate each other's targeting to specific genomic loci in response to oxidative stress. This, in addition to an increase in CSB's occupancy at promoters, suggests CSB and CTCF may cooperate in regulating transcription in response to oxidative stress, perhaps by mediating long-range chromatin interactions. Analysis of CSB mutant constructs revealed CSB's oxidative stress-induced chromatin association occurs by a mechanism distinct from that of its interaction upon UV irradiation, where it is required for TC-NER, in that it does not require CSB's ATP hydrolysis activity and is mediated by it's ATPase and C-terminal domains. While the BER proteins OGG1 and APE1 did not impact CSB-chromatin association upon oxidative stress, another DNA repair protein, poly(ADP-ribose) polymerase 1 (PARP1) significantly enhances CSB's interaction with chromatin in both a global and locus-specific manner. Together this work suggests CSB, CTCF, and PARP1 cooperate in the relief of oxidative stress by regulating transcription and facilitating DNA repair.

Degree Type
Dissertation

Degree Name
Doctor of Philosophy (PhD)

Graduate Group
Biology

First Advisor
Hua-Ying Fan

Keywords
ATP-dependent chromatin remodeling, Cockayne syndrome protein B (CSB), DNA damage repair

MECHANISMS UNDERLYING OXIDATIVE STRESS-INDUCED CHROMATIN ASSOCIATION OF COCKAYNE SYNDROME PROTEIN B (CSB)

Erica L. Boetefuer

A DISSERTATION

in

Biology

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2018

Supervisor of Dissertation

_____

Hua-Ying Fan, Ph.D.

Associate Professor of Internal Medicine, University of New Mexico

Graduate Group Chairperson

_____

Michael Lampson, Ph.D.

Associate Professor of Biology

Dissertation Committee
Doris Wagner, Ph.D., Robert I. Williams Term Professor of Biology
Shelley L. Berger, Ph.D., Daniel S. Och University Professor of Cell & Developmental Biology
Brian D. Gregory, Ph.D., Associate Professor of Biology
Maya Capelson, Ph.D., Assistant Professor of Cell & Developmental Biology

ACKNOWLEDGMENT

First, I would like to thank my advisor, Dr. Hua-Ying Fan. Thank you for your enthusiasm and for taking me on as your first graduate student. Your suggestions always made my work better and allowing me to learn your thought processes has helped me immensely. I truly appreciate the time you dedicated to helping me succeed. Thank you, especially, for supporting me from afar and helping me through this last year. I wish you all the best for your time in New Mexico!

I would like to thank my committee members, Drs. Doris Wagner, Shelley Berger, Brian Gregory, and Maya Capelson. You always had great suggestions and were enthusiastic about my project from start to finish. Thank you for your help in making some very difficult decisions and for supporting me through it all.

To Dr. Robert Lake— thank you, Bob, for all of your help. You were always patient with me and helped me with whatever I needed. I learned so much about doing science from you, and I really enjoyed working together. Thank you also to Dr. Pei-Fang Tsai for sharing a bay with me, helping me learn the ropes in the Fan lab, and always taking the time to discuss my project.

To my cohort: ***Drs.*** Ruby O'Lexy, Riley Graham, Farid Abdul Halim, and Mitchell Johnson. First, congratulations to all of you! I'm so proud of what you have accomplished and I know your futures are bright. It has been so much fun getting to know all of you and working our way through grad school together. Thank you for your friendship and all of our cohort get-togethers and for making my time at Penn special.

Thank you to Dr. Ken Zaret and all of the Zaret lab members, Meilin Fernandez Garcia, Dr. Katherine Palozola, Jessica Grindheim, Kelsey Kaeding, Megan Federick, Dr. Dario Nicetto, Dr. Jonathan Lerner, Dr. Ryan McCarthy, Naomi Takenaka, Ann O'Brien Jenkins, and Greg Donahue. Thank you for accepting me into your lab for the past year— it was comforting knowing I had a home with your lab, and I enjoyed being a part of your lab meetings and events. Over the years I got to know many of you, and I always enjoyed discussing science with you and

developing friendships outside of the lab as well. Thank you also to Dr. Marisa Bartolomei and the Bartolomei lab for being a great neighboring lab and endless source of entertainment. Thank you, especially, to Dr. Jennifer Meyers SanMiguel, Dr. Frances Xin, Aimee Juan, Dr. Joanne Thorvaldsen, Dr. Lisa Vrooman, Dr. Laren Riesche, and Christopher Krapp for your friendships and for always being there for me when I needed advice.

In addition to those mentioned above, I have received support, mentorship, and friendship from so many people in the Biology Department and the Epigenetics Institute. Thank you to the Biology Department Graduate Chair, Dr. Michael Lampson, and Department Chair, Dr. Brenda Casper. Thank you Sophia Castro-Anderson for all of your administrative support and for chatting with me at your desk. Thank you also to Robin Sherwood for your help over the past year and always making sure everything is organized. Thank you to the many professors for whom I have been a teaching assistant. Drs. Doris Wagner, Shelley Berger, Payel Sen, Nicholas Betley, Dustin Brisson, Michael Lampson, Kimberly Gallagher, Mark Goulian, Greg Guild, Wei Guo, Karen Hogan, Linda Robinson, and Tatyana Svitkina— being your TA was such a valuable experience and has been one of my favorite parts of graduate school. I learned so much and enjoyed working with all of you. Thank you to all of my students over the years— I enjoyed teaching and mentoring you and appreciate all of your hard work! Thank you also to the students and professors for selecting me for teaching awards and for giving me the confidence in myself as a teacher and mentor. Thank you to Dr. Mecky Polschröder and Dr. Mia Levine for your mentorship and always checking in and talking with me when I needed advice.

Thank you so much to my friends in the Biology department and on the 9th floor of Smilow. I have been so lucky to be surrounded by so many truly amazing people. I am continuously impressed by each and every one of you and feel so lucky to have had your support over the years. I enjoyed all of our happy hours, barbeques, and retreats and loved getting to know you. I am particularly grateful for the environment on the 9th floor for everyone being so welcoming, always willing to help, talk about science, or just chat.

A very special thank you to Colleen Gasiorowski. Colleen was an integral part of the Biology Department and her kindness and enthusiasm was contagious. Colleen helped me on so many occasions and was always a pleasure to talk to. Colleen's presence, often sitting in the audience knitting, entertaining introduction of the new graduate students at the department retreat, many e-mails, and unwavering support of her students will be greatly missed.

I would like to thank my friends for being there for me through it all. Danielle Fitzgeorge, Brigid Donlevie Dux, Adrienne Crivaro, Claire Crivaro, Rachael Green-Howard, Anna Huber, Dr. Marina Boushra Freiberg, Dr. Megan Mathews, and Emily Gorrin— your friendship has meant the world to me. We always have so much fun when spending time together and I am so happy all of you are in my life.

Finally, I would like to thank my family. Thank you, Mom and Dad, for everything. Your support has meant everything to me and I would not be here without you. I loved living so close to you in Philadelphia and being able to come home to spend time with you for dinner, hanging out, game nights, and family gatherings. I am so grateful for all that you have given me. Thank you for encouraging me to chase my dreams and for supporting me through it all. Thank you to my sister for always being a phone call away. You work so hard and it's been nice having a sister going through grad school at the same time to commiserate when it's hard but also to celebrate our achievements. You are going to make an amazing physical therapist and I know we'll make the most of getting together for trips and holidays! Thank you to my aunts, uncles, and cousins for the fun times from our crazy family Thanksgivings to days down the shore, may there be many more to come! Thank you Grandmom for your love and kindness always. Thank you Nana for inspiring my love of travel and crafts and for your great stories. Lastly, to my Grandad, I know if you were here you would be so proud and would give me the biggest hug of all. I miss you and I love you always.

ABSTRACT

MECHANISMS UNDERLYING OXIDATIVE STRESS-INDUCED CHROMATIN ASSOCIATION OF COCKAYNE SYNDROME PROTEIN B (CSB)

Erica L. Boetefuer

Hua-Ying Fan, Ph.D.

Cockayne syndrome is a devastating premature aging disorder characterized by extreme sun sensitivity and severe neurological and developmental defects. The majority of Cockayne syndrome cases are due to mutations within the ATP-dependent chromatin remodeler Cockayne syndrome protein B (CSB). CSB functions in transcription regulation and is required for transcription-coupled nucleotide excision repair (TC-NER). Importantly, CSB is also critical for the relief of oxidative stress. Studies suggest CSB may participate in the major repair pathway for oxidative DNA damage repair, base excision repair (BER), yet direct evidence supporting this hypothesis remains elusive and exactly how CSB functions in this process is unknown. CSB is also suggested to function in the relief of oxidative stress by regulating transcription. We demonstrated CSB's interaction with chromatin is stabilized by oxidative stress and the goal of this work is to understand the role of CSB in the relief of oxidative stress by characterizing the mechanisms underlying CSB's oxidative stress-induced chromatin interaction. Using chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) we characterized for the first time CSB's genome-wide occupancy following oxidative stress. This revealed CSB is enriched at the binding motif for the CCCTC-binding factor (CTCF), and further analysis demonstrated CSB and CTCF directly interact and regulate each other's targeting to specific genomic loci in response to oxidative stress. This, in addition to an increase in CSB's occupancy at promoters, suggests CSB and CTCF may cooperate in regulating transcription in response to oxidative stress, perhaps by mediating long-range chromatin interactions. Analysis of CSB mutant constructs revealed CSB's oxidative stress-induced chromatin association occurs by a mechanism distinct from that of its interaction upon UV irradiation, where it is required for TC-NER, in that it does not require CSB's ATP hydrolysis activity and is mediated by it's ATPase and C-terminal domains. While the BER

proteins OGG1 and APE1 did not impact CSB-chromatin association upon oxidative stress, another DNA repair protein, poly(ADP-ribose) polymerase 1 (PARP1) significantly enhances CSB's interaction with chromatin in both a global and locus-specific manner. Together this work suggests CSB, CTCF, and PARP1 cooperate in the relief of oxidative stress by regulating transcription and facilitating DNA repair.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 3: PARP1 facilitates chromatin association of Cockayne syndrome group B protein during oxidative stress

LIST OF ILLUSTRATIONS

Chapter 1: Introduction

Chapter 2: The CSB Chromatin Remodeler and CTCF Architectural Protein Cooperate in Response to Oxidative Stress

Chapter 3: PARP1 facilitates chromatin association of Cockayne syndrome group B protein during oxidative stress

CHAPTER 1: INTRODUCTION

1.1. Cockayne Syndrome and Cockayne Syndrome Protein B (CSB)

1.1.1. Cockayne syndrome

Cockayne syndrome (CS) is a rare, autosomal recessive progeroid disorder characterized by severe neurological and developmental defects as well as an extreme sensitivity to sunlight (Nance & Berry, 1992). Children with Cockayne syndrome have an average lifespan of only 12 years of age and display a prematurely aged appearance with characteristic facial features, including sunken eyes (Figure 1) (Nance & Berry, 1992). A Cockayne syndrome diagnosis is made based upon a failure to thrive, growth retardation, and short stature as well as neurological abnormalities (Nance & Berry, 1992). Neurological deficiencies include delayed psychomotor development, such as walking and speech impairment, intellectual disabilities, and tremors as well as underlying widespread neuronal demyelination and brain abnormalities, including calcifications, cerebral atrophy, and white matter abnormalities (Nance & Berry, 1992). Other common features include cataracts, cavities, hearing loss, and gait abnormalities (Nance & Berry, 1992). There are three sub-classes of Cockayne syndrome, types I, II, and III (Nance & Berry, 1992). Cockayne syndrome type I is “classical” Cockayne syndrome where features of Cockayne syndrome become apparent around one to two years of age. Cockayne syndrome type II is a more severe form of the disease with abnormalities present at or soon after birth. Lastly, Cockayne syndrome type III is a milder and late-onset form of the disease. While these distinctions are made clinically, no underlying genetic or biochemical explanation for the differences in disease severity has been defined (Nance & Berry, 1992).

1.1.2. Identification of genes responsible for Cockayne syndrome

The physician E. A. Cockayne, after whom Cockayne syndrome is named, first described this disorder in 1936 (Cockayne, 1936) and the genes responsible for Cockayne syndrome have

since been identified. As patients demonstrated photosensitivity, fibroblasts from Cockayne syndrome patients were tested and demonstrated an increased sensitivity to the killing effects of UV irradiation (Lehmann, 1982) as well as a failure to recover RNA synthesis following UV irradiation (Mayne & Lehmann, 1982). UV irradiation induces bulky DNA lesions, such as UV-induced cyclobutane pyrimidine dimers (CPD), (6,4)-photoproducts, and DNA crosslinks, which are repaired by nucleotide excision repair (NER) and are known to block transcription machinery (Giorno & Sauerbier, 1976). As cells from Cockayne syndrome patients are able to remove pyrimidine dimers from bulk DNA, Mayne and Lehmann hypothesized Cockayne syndrome cells had a defect in preferential repair of transcriptionally active DNA (Mayne & Lehmann, 1982). This was found to be the case by Venema et al. (1990), who observed a defect in the repair of transcriptionally active genes, demonstrating the genetic defect associated with Cockayne syndrome was within a transcription-coupled nucleotide excision repair process (TC-NER) (Venema, Mullenders et al., 1990).

Cell fusion assays identified at least two complementation groups which complemented the Cockayne syndrome defect: complementation group A (CS-A) and complementation group B (CS-B) (Lehmann, 1982; Tanaka et al., 1981). Troelstra et al. cloned the major gene responsible for Cockayne syndrome in 1992 (Troelstra et al., 1992). This gene identified was termed excision repair cross-complementing group 6 (*ERCC6*) for its ability to complement the UV sensitivity of the UV-sensitive Chinese hamster ovary (CHO) mutant UV61 (Troelstra et al., 1992). *ERCC6* is located on chromosome 10q11.23 and encodes a 1,493 amino acid protein with a molecular weight of 168 kDa (Troelstra et al., 1992). This protein was named Cockayne syndrome protein B (CSB), and mutations within *CSB/ERCC6* account for approximately 80% of all Cockayne syndrome cases (Licht et al., 2003; Mallery et al., 1998). The remaining ~20% of Cockayne syndrome cases are due to mutations within *CSA/ERCC8*, the gene encoding Cockayne syndrome protein A (CSA) (see section 1.1.5. for more details).

An additional transcript was also described, which is created by alternative splicing of CSB's exon five to a transcript within intron five of CSB. The result of this alternative splicing

event is a fusion protein of CSB's first five exons to the PiggyBac-like transposase, *PGBD3* (CSB-PGBD3) (Newman et al., 2008). This fusion protein is expressed to levels similar to that of CSB and is also evolutionarily conserved as long as CSB, suggesting an advantageous function in cells (Newman et al., 2008). Subsequent research conducted on this fusion protein suggests it may function in the immune and antiviral response (Bailey et al., 2012).

1.1.3. Cockayne syndrome protein B is an ATP-dependent chromatin remodeler

CSB is divided into three domains: a highly conserved central ATPase domain, which contains seven conserved helicase motifs, and the less well conserved N- and C-terminal domains (Figure 2) (Troelstra et al., 1992). CSB was found to contain two putative nuclear localization signals (NLS) as well as a stretch of acidic amino acids in its N-terminus and a ubiquitin binding domain (UBD) in its C-terminus, both of unknown function (Figure 2) (Anindya et al., 2010; Troelstra et al., 1992). Cockayne syndrome protein B (CSB) was subsequently classified as a member of the SWI2/SNF2 family of ATP-dependent chromatin remodelers, which is conserved from yeast to human (Flaus et al., 2006; Hopfner et al., 2012). While SWI2/SNF2 proteins are defined by their helicase motif-containing central ATPase domain, no helicase activity has been observed for CSB or any SWI2/SNF2 protein (Pyle, 2008; Selby & Sancar, 1997b; Yusufzai & Kadonaga, 2008). ATP-dependent chromatin remodelers use the energy of ATP hydrolysis to alter contacts between DNA and the histone proteins around which DNA is wrapped (Clapier & Cairns, 2009; Eisen et al., 1995; Flaus et al., 2006). Nucleosomes are the basic unit of chromatin structure, consisting of ~150 base pairs of DNA wrapped around a core octamer of histone proteins. ATP-dependent nucleosome remodeling activity of SWI2/SNF2 proteins results in different changes to nucleosome structure pictured in Figure 3 (Clapier & Cairns, 2009; Fan et al., 2004). These proteins play critical roles in a number of cellular processes, including transcription, replication, recombination, and DNA repair. While no helicase activity has been observed for CSB, CSB does have DNA- and nucleosome-stimulated ATPase

activity and functions in altering nucleosome position (Cho et al., 2013; Citterio et al., 1998; Citterio et al., 2000; Muftuoglu et al., 2006; Selby & Sancar, 1997b).

The N- and C-terminal domains of SWI2/SNF2 remodelers often contain domains important for targeting these proteins to specific chromatin regions; however, these domains are notably absent in CSB (Figure 2). Therefore, a major effort in the field, including the research presented in this dissertation, has been to characterize the mechanisms underlying CSB's interactions with chromatin.

1.1.4. CSB Mutations

The types of mutations within CSB that have been reported in Cockayne syndrome patients are missense mutations and deletion mutations, most of which occur in the ATPase domain with few reported in the C-terminus, as well as stop codon or frame shift mutations, which occur throughout the protein (Lake & Fan, 2013). Those that occur in the N-terminus frequently result in a truncated, non-functional CSB protein. Mutations within the ATPase domain differentially impair CSB's ATPase activity (Lake et al., 2010). Importantly, the severity and disease classification of Cockayne syndrome does not correlate with the mutation type (Licht et al., 2003).

The photosensitivity seen in Cockayne syndrome patients is similar to that of a related DNA repair disorder, Xeroderma pigmentosum (XP). In addition to sun sensitivity, patients with Xeroderma pigmentosum suffer pigmentation abnormalities, a significant predisposition to skin cancer, and, often, progressive neurological degeneration (Cleaver & Kraemer, 1989). Unlike Xeroderma pigmentosum and other DNA repair disorders, cancer has never been reported in a Cockayne syndrome patient (Nance & Berry, 1992). The genes responsible for Xeroderma pigmentosum are required for general genome nucleotide excision repair, which removes bulky DNA lesions from the genome as a whole (Bootsma & Hoeijmakers, 1994).

1.1.5. CSB's role in transcription-coupled nucleotide excision repair

Transcription-coupled nucleotide excision repair (TC-NER) is a sub-class of nucleotide excision repair specific to the actively transcribed strand of DNA (Svejstrup, 2002). CSB is the first protein recruited to RNA polymerase II (RNA pol II) stalled at bulky DNA lesions (Fousteri et al., 2006). CSB is required to recruit downstream repair factors, including core NER factors and Cockayne syndrome protein A (CSA), which is part of a ubiquitin ligase complex (Fousteri et al., 2006; Groisman et al., 2003). CSB's ATPase activity is required for chromatin association following UV irradiation (Lake et al., 2010) and CSB's remodeling activity is required for transcription to resume following repair (Brosh et al., 1999).

It is important to note that CSB's role in UV DNA damage repair is likely not the major factor underlying the pathology of Cockayne syndrome (Brooks, 2013). While this can account for the photosensitivity of patients with Cockayne syndrome, it does not explain the severe neurological and developmental defects and additional disease features of the disorder nor the early aging phenotype. This is particularly evident in comparison to the related DNA repair disorder described in section 1.1.4, Xeroderma pigmentosum. The UV sensitivity of Cockayne syndrome patients is mild compared to patients with Xeroderma pigmentosum and, importantly, Cockayne syndrome patients do not have an increased risk of cancer (Nance & Berry, 1992). It is likely that CSB's role in transcription regulation (discussed in section 1.1.6.) as well as alternate DNA damage repair pathways, such as oxidative DNA damage repair (discussed in section 1.2), contributes to the severity of the Cockayne syndrome phenotype.

1.1.6. CSB and transcription regulation

CSB has been hypothesized to play a role in transcription regulation and involvement of CSB in this process came from several experimental findings. In 1997, purification of CSB from whole cell extracts demonstrated that CSB is found in a large (>700 kDa) complex containing RNA polymerase II (RNA pol II) (van Gool et al., 1997). Analysis of intact and permeabilized cells demonstrated cells deficient in CSB display reduced transcription elongation (Balajee et al., 1997). CSB was found to interact with RNA pol II *in vitro* and also as part of an elongation

complex *in vivo* (Tantin et al., 1997). Importantly, CSB was demonstrated to directly stimulate transcription elongation (Selby & Sancar, 1997a).

It wasn't until recently that our group demonstrated for the first time that CSB is directly involved in regulating transcription (Lake et al., 2014). We conducted CSB chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) and identified genomic loci bound by CSB under replicative cell growth conditions (Lake et al., 2014). ChIP-seq analysis also demonstrated CSB is enriched at promoters and enhancers, suggesting a function for CSB in transcription initiation in addition to its previously described role in transcription elongation (Lake et al., 2014; Selby & Sancar, 1997a; Tantin et al., 1997). We utilized reverse transcriptase qPCR (RT-qPCR) and a CSB null cell line to analyze genes occupied by CSB and demonstrated that CSB directly regulates the expression of nearby genes (Lake et al., 2014). We also examined the function of CSB's remodeling activity in this process. First, we demonstrated by MNase qPCR that CSB can directly remodel nucleosomes at its binding sites, including those at which CSB was shown to regulate transcription. We also utilized a remodeling deficient CSB mutant, CSBΔN1, which is capable of ATP hydrolysis but unable to couple this with its nucleosome remodeling activity (Cho et al., 2013). This demonstrated that CSB is capable of regulating transcription in both nucleosome remodeling-dependent and –independent manners, suggesting CSB may alter transcription by directly remodeling nucleosomes or, similar to its function in TC-NER, serve as a recruitment factor to regulate transcription via factor binging (Lake et al., 2014).

Based on these findings it is likely that CSB's role in transcription contributes to the severe disease pathology of Cockayne syndrome. As we learn more about CSB's role in transcription regulation, findings support long-standing hypotheses that Cockayne syndrome is not only a DNA repair disorder but also a transcription disorder (Bootsma & Hoeijmakers, 1994; Brooks, 2013; Chalut et al., 1994; Friedberg et al., 1994).

Recent studies have also made progress in characterizing how CSB's function in transcription regulation may underlie some of the neurological phenotypes of Cockayne syndrome (Ciaffardini et al., 2014; Y. Wang et al., 2014; Y. Wang et al., 2016). A connection

between CSB and neuronal gene expression was described in 2014 by microarray analysis, which demonstrated CSB-dependent regulation of more than 1,200 genes, including a significant number of gene classes characterized as neuron-related (Y. Wang et al., 2014). Microarray analysis was also performed in postmortem cerebella from Cockayne syndrome patients and demonstrated 4,130 genes were differentially regulated by >1.5 fold, including genes related to the nervous system (Y. Wang et al., 2014). CSB has also been implicated in neuronal differentiation and neurite maintenance, which is hypothesized to occur through an active role of CSB in transcription regulation of key neuronal genes (Ciaffardini et al., 2014; Y. Wang et al., 2014). Understanding CSB's contribution to neuronal gene regulation may provide a platform for a therapeutic intervention targeting the neurological defects of Cockayne syndrome patients. Recent studies have examined candidate genes for bypass of transcriptional regulation by ectopic expression as clinically relevant therapeutic targets for the neural defects of Cockayne syndrome (Y. Wang et al., 2016).

More information on CSB's role in transcription regulation can be found in section 1.3.5. Importantly, CSB also plays a role in regulating transcription in response to oxidative stress as well as following UV irradiation. More details on CSB's role in regulating transcription under these conditions will be discussed in subsequent sections of this dissertation.

1.2. Cockayne Syndrome Protein B (CSB) and the Relief of Oxidative Stress

1.2.1. CSB is required for the relief of oxidative stress

In addition to its role in TC-NER, CSB is also required to relieve oxidative stress. Cells deficient in CSB display increased sensitivity to oxidizing reagents, such as H_2O_2 and λ-irradiation (de Waard et al., 2003; de Waard et al., 2004; Pascucci et al., 2012; Tuo et al., 2001). Recently, our group also showed CSB is required to protect cells from oxidative stress by demonstrating patient-derived CS1AN fibroblasts, which are devoid of any functional CSB, are more sensitive to menadione treatment than cells reconstituted with wild type CSB (see section 2.5.1.) (Lake et al.,

2016). CSB-deficient cells have also been shown to accumulate oxidative DNA damage, including 8-oxoG bases, after λ-irradiation (Muftuoglu et al., 2009; Tuo et al., 2001). Andrade et al. (2012) created induced pluoripotent stem cells (iPCSs) derived from Cockayne syndrome patient fibroblasts and found these iPSCs had increased rates of cell death and, importantly, higher levels of ROS (Andrade et al., 2012). Increased levels of intracellular ROS have also been observed in CSB-deficient cells (Pascucci et al., 2012).

1.2.2. Base excision repair (BER)

Living organisms are constantly exposed to oxidative stress from exogenous (environmental) insults as well as endogenous, intracellular metabolic processes. It is estimated that every cell in the human body has to repair approximately ten to twenty thousand DNA lesions every day or approximately one modification per 10^6-10^7 nucleotides (Lindahl, 1993). Reactive oxygen species (ROS) can result in base lesions, abasic (AP) sites, and DNA strand breaks, and DNA-protein crosslinks as well as other protein and lipid damage (Dizdaroglu, 1992). ROS are constantly generated and cells must combat these damaging agents through direct interception of oxidative species or indirectly through repair of oxidative damage (D'Errico et al., 2013). Oxidative stress is the result of ROS overwhelming cellular defense systems.

Base excision repair (BER) is the major repair pathway for oxidatively damaged DNA, which is mainly comprised of single DNA base changes (Lindahl, 1993). The most common oxidative DNA lesion is 8-hydroxy-7,8-dihydroguanine (8-oxoG), which is thought to cause G→T transversion mutations if left unrepaired (Krokan et al., 1997; Lindahl, 1993). Other common types of oxidative base lesions include 8-hydroxy-7,8-dihydroadenine (8-oxoA), the formamidopyrimidines 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde), and 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol) (Cadet & Davies, 2017; G. L. Dianov et al., 2000; Muftuoglu et al., 2009). BER is also utilized to remove uracil in DNA (G. Dianov et al., 1992).

The majority of BER occurs thorugh short patch BER, which involves removing and replacing a single nucleotide (Figure 4) (G. Dianov et al., 1992; G. Dianov et al., 1998; G. L. Dianov et al., 2000). BER is initiated by a lesion-specific DNA glycosylase, which removes the damaged DNA base by cleaving the bond linking the DNA base to the sugar phosphate backbone (Krokan et al., 1997; Lindahl, 1979; Lindahl, 1993). 8-oxoG glycosylase/apurininc lyase (OGG1) is the glycosylase which catalyzes the removal of 8-oxoG (Klungland & Bjelland, 2007). Other glycosylases include the uracil DNA glycosylase (UDG) (Lindahl, 1974; Lindahl, 1980) and the endonuclease VIII-like (NEIL1) DNA glycosylase, which recognizes FapyGua and FapyAde (Jaruga et al., 2004). Glycosylase removal of the damaged base results in an apurinic/apyrimidinic (AP) or abasic site, which is recognized by apurinic/apyrimidinic endonuclease 1 (APE1) (Wilson & Barsky, 2001). APE1 cleaves the 5' phosphodiester bond, generating a single strand break (SSB) or nick flanked by a 3'-hydroxyl and a 5'-deoxyribose phosphate, which is removed by DNA polymerase β (polβ) by its AP lyase activity (Matsumoto & Kim, 1995; Wilson & Barsky, 2001). It is at this point that BER converges with SSB repair. The remaining nicked DNA serves as template for polβ to incorporate a new nucleotide and DNA ligase III (lig3) to seal the nick (Kubota et al., 1996; Singhal & Wilson, 1993; Sobol et al., 1996; Y. F. Wei et al., 1995). These two proteins are recruited to nicked DNA by interaction with X-ray repair cross-complementing protein 1 (XRCC1) (Caldecott et al., 1994; Cappelli et al., 1997; Kubota et al., 1996; Nash et al., 1997). XRCC1 is one of the first proteins recruited to the resulting nicked DNA and has no enzymatic activity on its own, but instead acts as a scaffold protein to coordinate BER (Kubota et al., 1996). SSBs are also the major substrate for PARP1, which may facilitate recruitment of XRCC1/polβ/lig3 and has also been hypothesized to stabilize the SSB to avoid degeneration to a DSB (for more information, see section 1.2.5.) (G. L. Dianov & Hubscher, 2013).

An alternate pathway, long patch BER, is thought to occur when blocking ends are present at the 5' end of the AP site and cannot be processed by one of the five known end-processing enzymes (G. L. Dianov & Hubscher, 2013; Frosina et al., 1996). This process is

initiated following polβ incorporation of a nucleotide into the nick, but as it cannot be ligated due to the blocked 5' site, the pathway continues via a process similar to that of lagging strand DNA replication (G. L. Dianov et al., 1999; Podlutsky et al., 2001). Polymerase δ is recruited and synthesizes a strand of two to twelve nucleotides in the presence of proliferating cell nuclear antigen (PCNA) and replication factor C (RPC) (Matsumoto et al., 1994). The resulting nucleotide flap is cut off by flap endonuclease I (FEN1) and the nick is sealed by DNA ligase I (Klungland & Lindahl, 1997).

Deficiencies and reduced capacity for BER results in genome instability and has been implicated in a variety of human diseases, including cancer, neurodegeneration, and aging (Bartkova et al., 2005; Kipling & Faragher, 1997; Lombard et al., 2005). It is thought that defects in oxidative DNA damage repair may contribute to the Cockayne syndrome phenotype (G. L. Dianov et al., 1997; Hanawalt, 1994). Though many groups hypothesize CSB plays a role in BER of oxidative DNA damage, direct evidence supporting these hypotheses are limited. Furthermore, the exact function of CSB in this pathway is unclear. The goal of the work presented in this dissertation is to characterize CSB's role in the relief of oxidative stress by understanding how CSB is targeted to chromatin in response to oxidative stress.

1.2.3. CSB null cell extracts display reduced *in vitro* incision of oxidized DNA

Evidence for CSB in BER has been provided by several groups, which report deficient repair of oxidative DNA lesions *in vitro* (G. Dianov et al., 1999; Tuo et al., 2001; Tuo, Jaruga et al., 2002; Tuo et al., 2003). These experiments utilize end-labeled synthetic DNA oligonucleotides consisting of a single damaged base modification in a defined location. Whole cell extracts are incubated with the oligonucleotides and products are run out on gel. The ability of the extracts to excise the modified base lesion coordinates with the amount of cleaved product present in the reaction, which is visible by smaller DNA fragments present further down the gel. Dianov et al. (1999) analyzed whole cell extracts from the patient-derived CSB null cell line, CS1AN, and found incision of 8-oxoG was ~40-50% lower in CSB null cells than in normal lymphoblast cells (G.

Dianov et al., 1999). Importantly, incision activity is increased significantly, by ~60%, when comparing CSB null CS1AN cells to CS1AN cells stably reconstituted with wild type CSB (G. Dianov et al., 1999). Reduced incision activity was also observed for two additional primary fibroblast lines derived from Cockayne syndrome patients compared to normal human fibroblasts (G. Dianov et al., 1999). Interestingly, analysis of whole cell extracts derived from fibroblasts of a family demonstrated ~50% incision activity of a Cockayne syndrome patient compared with a normal sibling and an intermediate phenotype in the parents, predicted to be heterozygous for the CSB allele (G. Dianov et al., 1999). This group did not observe a difference in the incision of uracil or thymine glycol in comparing CSB null CS1AN cells to those reconstituted with wild type CSB (G. Dianov et al., 1999). Interestingly, this group also reported transfection of CS1AN cells with wild type CSB resulted in a 50% increase in OGG1 mRNA as demonstrated by northern blot analysis, suggesting CSB may control OGG1 expression (G. Dianov et al., 1999). This was shown again in 2002 by RT-qPCR and also demonstrated reduced OGG1 mRNA in the presence of CSB^{Q942E}, a helicase motif VI mutant (Tuo, Chen et al., 2002).

Tuo et al. (2001) also observed reduced incision of 8-oxoG in CSB-deficient CS1AN cells compared to CSB^{WT} (Tuo et al., 2001). Analysis of several site-directed mutations within CSB's ATPase domain resulted in reduced incision activity, suggesting a role for CSB's ATP hydrolysis activity in this process (Tuo et al., 2001). However, not all mutations within the ATPase domain affect incision activity, suggesting not all helicase motifs are required and that the effect depends on the type of mutation (Selzer et al., 2002; Tuo et al., 2001). Subsequent studies found whole cell extracts from CSB null cells or from cells containing ATPase domain mutations also demonstrate reduced incision of 8-oxoA, demonstrating CSB contributes to the repair of 8-oxoA as well (Tuo et al., 2002). This has also been demonstrated for fibroblasts from several Cockayne syndrome patient-derived fibroblasts cell lines (Tuo et al., 2003).

As these experiments were all conducted *in vitro* they may not accurately reflect the process as it occurs in cells. Furthermore, CSB is not hypothesized to perform the excision steps reflected in these experiments, and, therefore, exactly how CSB contributes to the results

demonstrated above is unclear. It is also important to note that the above findings are primarily produced from one group and despite convincing evidence that CSB is involved in repair of 8-oxoG and 8-oxoA, not all groups have found this to be true. For example, Osterod et al. (2002) did not see any effect of CSB on the incision of 8-oxoG (Osterod et al., 2002).

1.2.4. CSB interacts with several base excision repair proteins

Another line of evidence supporting the hypothesis that CSB plays a role in base excision repair (BER) is that CSB has been shown to interact with or be in complex with several BER proteins. CSB is found in complex with the DNA glycosylase OGG1 and these two proteins colocalize in cells following λ-irridiation (Tuo et al., 2002). CSB directly interacts with the DNA glycosylase NEIL1 *in vivo* (Muftuoglu et al., 2009). CSB and NEIL1 also colocalize in cells, and CSB has been shown to stimulate NEIL1 incision of FapyGua and FapyAde (Muftuoglu et al., 2009). CSB directly interacts with the AP endonuclease, APE1, *in vitro*, and immunoprecipitation experiments demonstrated CSB and APE1 are found in complex in cells (Wong et al., 2007). Furthermore, CSB stimulates the enzymatic activity of APE1, supporting a biological function of this interaction (Wong et al., 2007). Lastly, CSB interacts with PARP1, which is discussed in section 1.2.5. (Thorslund et al., 2005).

1.2.5. CSB and poly(ADP-ribose) polymerase 1

Poly(ADP-ribose) polymerase 1 (PARP1) is known as a “molecular nick sensor” (de Murcia & Menissier de Murcia, 1994). PARP1 is activated by single strand and double strand DNA breaks and uses the substrate nicotinamide adenine dinucleotide (NAD+) to catalyze the addition of ADP-ribose polymers to protein targets, including itself (Althaus et al., 1999; de Murcia & Menissier de Murcia, 1994). PARP1 consists of a zinc-finger domain containing an N-terminal DNA binding domain (DBD), a central automodification domain, and a C-terminal catalytic domain (Bork et al., 1997; D'Amours et al., 1999; Kraus & Lis, 2003). PARP1’s major role is in single strand break (SSB) repair. PARP1 rapidly detects and binds SSBs, resulting in addition of

poly(ADP)-ribose (PAR) polymers to PARP1 itself as well as other proteins (Satoh & Lindahl, 1992). PARP1 and PARylation increase the rate of SSB repair and, indeed, PARP1 is required for detection and repair of SSBs (Caldecott, 2008; Fisher et al., 2007). PARP1 then functions to recruit the scaffold protein XRCC1 and its interacting partners, and PARP1 may also promote the stability of repair factors at the break. (Caldecott, 2008; El-Khamisy et al., 2003; Hanzlikova et al., 2017). PARP1 also functions in double strand break (DSB) repair and plays a role in nucleotide excision repair via recruitment of repair and chromatin remodeling proteins, opening chromatin and allowing efficient repair (Ray Chaudhuri & Nussenzweig, 2017). In addition to DNA damage repair, PARP1 plays a role in transcription regulation (Kraus & Lis, 2003).

As mentioned above, PARP1 is hypothesized to participate in BER. An intermediate in BER is a single strand nick or single strand break, the major substrate for PARP1 and the point at which BER converges with single strand break repair (Althaus et al., 1999). Similar to its role in in single strand break repair, PARP1 may function to recruit downstream repair factors (I. Ahel et al., 2008; Pleschke et al., 2000). It has been shown that PARP1 interacts with XRCC1 and also that PARP1 is required for XRCC1 foci formation following treatment of cells with H_2O_2 (El-Khamisy et al., 2003; Hanzlikova et al., 2017). PARP1 may also serve to protect the BER nick intermediate from further degeneration into a double strand break (Parsons et al., 2005; Satoh & Lindahl, 1992; Woodhouse et al., 2008). Determining the role of PARP1 in BER has not been straightforward. While some groups report PARP1 enhances the efficiency of BER, other groups have reported PARP1 does not affect BER efficiency or even that the presence of PARP1 may decrease BER kinetics (Allinson et al., 2003; Dantzer et al., 1999; Dantzer et al., 2000; Orta et al., 2014; Reynolds et al., 2015; Strom et al., 2011). Furthermore, groups have reported cells deficient in PARP1 display variable sensitivity to base lesion-inducing agents (Dantzer et al., 1999; de Murcia & Menissier de Murcia, 1994; Pachkowski et al., 2009; Vodenicharov et al., 2000; Z. Q. Wang et al., 1997).

In 2005, CSB was demonstrated to directly interact with PARP1 both *in vitro* and *in vivo* (Thorslund et al., 2005). This interaction is reportedly mediated through CSB's N-terminus *in vitro*,

however whether additional factors mediate the *in vivo* interaction is unclear (Thorslund et al., 2005). CSB can also interact with poly(ADP-ribosyl)ated PARP1 *in vitro* and *in vivo* and this occurs in the presence or absence of oxidative stress as shown by treatment with H_2O_2 (Thorslund et al., 2005). Interestingly, CSB can be poly(ADP-ribosyl)ated by PARP1, however the extent of PARylation is limited as it did not cause a shift in CSB's molecular weight by western blotting (Thorslund et al., 2005). In cells, CSB has been shown to colocalize with PARP1 and PAR following treatment with H_2O_2 (Thorslund et al., 2005). This group also reported poly(ADP-ribosyl)ation of CSB resulted in a reduction of CSB's ATPase activity *in vitro* (Thorslund et al., 2005). Finally, supporting the idea that CSB and PARP1 cooperate in cells, cells deficient in CSB are sensitive to PARP inhibition (Thorslund et al., 2005).

In 2014, Scheibye-Knudsen et al. (2014) sought to examine how CSB and PARP1 regulate each other's activity in response to oxidative DNA damage. CSB is able to displace proteins from DNA (Berquist & Wilson, 2009); therefore, electrophoretic mobility shift (EMSA) was performed to determine whether CSB could impact PARP1-DNA binding. Indeed, incubation of PARP1-DNA with increasing concentrations of CSB altered PARP1's binding pattern, indicating CSB can displace PARP1 from DNA (Scheibye-Knudsen et al., 2014). Inhibition or absence of PARP1 resulted in reduced retention of CSB at locally induced oxidative DNA damage (Scheibye-Knudsen et al., 2014). CSB was also found to bind poly(ADP-ribose) (PAR) and mutation of two PAR binding motifs in CSB's N-terminus resulted in loss of CSB retention at the damage (Scheibye-Knudsen et al., 2014). This work lead researchers to hypothesize CSB is retained at DNA damage via PAR binding and serves to remove PARylated PARP1 from DNA to facilitate downstream repair, providing further evidence and a potential mechanism for CSB's role in oxidative DNA damage repair (Scheibye-Knudsen et al., 2014).

1.2.6. CSB accumulates at sites of locally induced oxidative DNA damage *in vivo*

A key experimental technique that has been heavily utilized by researchers in recent years is inducing local oxidative DNA damage using a photosensitizer. Photosensitizers are

added directly to cells and DNA damage is inflicted by activating the photosensitizer using lasers. Different photosensitizers and laser settings can be used to induce different types of DNA damage. Menoni et al. (2012) demonstrated the effectiveness of this method using the photosensitizer Ro 19-8022. They confirmed the specificity and effectiveness of this technique by showing 8-oxoG accumulates at the site of laser-induced oxidative DNA damage, while the bulky NER substrates, 6,4-photoproducts and CPDs are not present, (Menoni et al., 2012). Using this technique, Menoni et al. (2012) demonstrated accumulation of CSB at sites of locally induced oxidative DNA damage *in vivo* and colocalization with 8-oxoG, supporting a role of CSB in repair of this lesion (Menoni et al., 2012). While OGG1 recruitment to sites of laser-induced 8-oxoG occurred faster than CSB, OGG1 siRNA knockdown did not affect CSB recruitment to the local oxidative DNA damage (Menoni et al., 2012).

1.2.7. Is CSB's role in the relief of oxidative stress dependent upon active transcription?

Whether CSB's role in oxidative DNA damage repair is transcription dependent is a question that had been debated for many years. Using the laser-induced local oxidative DNA damage paradigm described above, Menoni et al. (2012) treated cells with Actinomycin D, a RNA pol I/II inhibitor and demonstrated CSB is still recruited to local 8-oxoG, however transcription inhibition significantly reduced the immobilized fraction of CSB (Menoni et al., 2012). Iyama and Wilson (2016) also compared CSB's response to oxidative DNA damage following treatment with the RNA pol II inhibitor, α-amanitin. This demonstrated CSB's recruitment to oxidative DNA damage was not affected by RNA pol II inhibition; however, retention of CSB at the damage and the total accumulation was slightly lessened (Iyama & Wilson, 2016). Together this work suggests a fraction of CSB may be engaged in a transcription-dependent process in response to oxidative DNA damage.

1.3. Mechanistic Insights into the Regulation of Transcription and Transcription-Coupled DNA Repair by Cockayne Syndrome Protein B

1.3.1. Preface

The manuscript presented in this chapter was accepted for publication *Nucleic Acids Research* on 06 July 2018. It has been reformatted here in accordance with the University of Pennsylvania dissertation formatting guidelines.

1.3.2. Abstract

Cockayne syndrome protein B (CSB) is a member of the SNF2/SWI2 ATPase family and is essential for transcription-coupled nucleotide excision DNA repair (TC-NER). CSB also plays critical roles in transcription regulation. CSB can hydrolyze ATP in a DNA-dependent manner, alter protein-DNA contacts and anneal DNA strands. How the different biochemical activities of CSB are utilized in these cellular processes have only begun to become clear in recent years. Mutations in the gene encoding CSB account for majority of the Cockayne syndrome cases, which result in extreme sun sensitivity, premature aging features and/or abnormalities in neurology and development. Here, we summarize and integrate recent biochemical, structural, single-molecule and somatic cell genetic studies that have advanced our understanding of CSB. First, we review studies on the mechanisms that regulate the different biochemical activities of CSB. Next, we summarize how CSB is targeted to regulate transcription under different growth conditions. We then discuss recent advances in our understanding of how CSB regulates transcription mechanistically. Lastly, we summarize the various roles that CSB plays in the different steps of TC-NER, integrating the results of different studies and proposing a model as to how CSB facilitates TC-NER.

1.3.3. Introduction

Cockayne syndrome protein B (CSB) was identified as an essential component of the transcription-coupled branch of nucleotide excision repair (TC-NER), a process that preferentially removes transcription-blocking DNA lesions (Bohr et al., 1985; Hanawalt & Spivak, 2008; Mayne & Lehmann, 1982; Mellon et al., 1987; Troelstra et al., 1990; Troelstra et al., 1992; Venema, van

Hoffen et al., 1990). Without CSB, there is no preferential repair of lesions on the transcribed DNA strand. Mutations in the gene encoding the CSB protein account for the majority of Cockayne syndrome cases, a devastating premature aging disorder characterized by developmental and neurological defects as well as severe sun sensitivity (Cockayne, 1936; Karikkineth et al., 2017; Nance & Berry, 1992). Previous work demonstrated that CSB is the first protein recruited to RNA polymerase II (RNA pol II) stalled at bulky DNA lesions, where it is required to initiate TC-NER and recruit downstream repair factors (Fousteri et al., 2006; van den Boom et al., 2004). How CSB mediates downstream repair factor recruitment and how CSB's chromatin remodeling activity facilitates efficient repair and allows transcription to resume post-repair was unknown. Recent studies have provided mechanistic insights into how CSB and its biochemical activities may facilitate TC-NER (Cho et al., 2013; Xu et al., 2017).

CSB is found in a complex containing RNA pol II, and *in vitro* reconstitution assays as well as transcription profiling analyses suggest that CSB also plays a role in general transcription regulation (Balajee et al., 1997; Newman et al., 2006; Selby & Sancar, 1997a; Tantin et al., 1997; van Gool et al., 1997). It was not until recently that direct evidence revealed CSB regulates transcription as an ATP-dependent chromatin remodeler (Lake et al., 2014). Additional studies suggest that CSB's function in transcription regulation may underlie some of the neurological phenotypes of Cockayne syndrome (Ciaffardini et al., 2014; Y. Wang et al., 2014; Y. Wang et al., 2016).

CSB is also required for the relief of oxidative stress, and cells deficient in CSB are sensitive to oxidizing agents, accumulate more oxidative DNA damage than CSB expressing cells, and display increased levels of intracellular reactive oxygen species (ROS) (Lake et al., 2016; Muftuoglu et al., 2009; Pascucci et al., 2012; Tuo et al., 2001). Evidence for CSB in base excision repair (BER), the major repair pathway for oxidative DNA damage, has been provided by a number of groups, which report deficient repair of oxidative DNA lesions *in vitro* (G. Dianov et al., 1999; Tuo et al., 2001; Tuo et al., 2002; Tuo et al., 2003). Moreover, CSB has been shown to interact with several proteins involved in BER (Muftuoglu et al., 2009; Tuo et al., 2002; Wong et

al., 2007) and accumulates at sites of oxidative DNA damage (Menoni et al., 2012). However, exactly how CSB participates in BER is unknown. It is important to note that oxidative DNA damage caused by exogenous or endogenous mechanism can also generate substrates that could potentially be repaired by TC-NER (Enoiu et al., 2012; Furuta et al., 2002; Iyama et al., 2015). Indeed, when considering the etiology of Cockayne syndrome, the repair of damaged DNA resulting from mechanisms other than UV irradiation may be more relevant, as CS patients exhibit numerous complications that cannot be attributed to sun exposure (Nance & Berry, 1992).

Here we summarize recent findings on how CSB's biochemical activities are regulated and discuss how CSB may use these different activities to accomplish its biological functions in transcription regulation and TC-NER. The importance of understanding how CSB functions within cells is highlighted by the severity of Cockayne syndrome phenotypes. Ultimately, the results of these studies may lead to therapeutic interventions for Cockayne syndrome patients.

1.3.4. Regulation of CSB's biochemical activity

CSB belongs to the SNF2/SWI2 family of ATP-dependent chromatin remodelers, and these proteins use ATP as energy to alter DNA-histone and/or DNA-protein contacts (Eisen et al., 1995; Flaus et al., 2006; Lake & Fan, 2013). CSB has demonstrated DNA- and nucleosome-stimulated ATP hydrolysis activities as well as DNA strand annealing and exchange activities (Citterio et al., 1998; Muftuoglu et al., 2006; Selby & Sancar, 1997b). Importantly, CSB has been shown to alter nucleosome structure in an ATP-dependent manner (Cho et al., 2013; Citterio et al., 2000). Here, we will review the recent advances in our understanding of the regulation of ATP-dependent chromatin remodeling by CSB.

1.3.4.1. The N-terminal region of CSB couples ATP hydrolysis to chromatin remodeling

Using quantitative restriction enzyme accessibility assays, Cho et al. (2013) found that CSB exposes nucleosomal DNA in an ATP-dependent manner but does so with a maximal rate ten-times slower than that of the human remodeling complex ACF (Cho et al., 2013). Deletion of

the first 454 amino acids abolishes CSB's remodeling activity despite the fact that $CSB^{\Delta 1\text{-}454}$ (CSBΔN) maintains its DNA- and nucleosome-stimulated ATP hydrolysis activity (Figure 5) (Cho et al., 2013). Further deletion analysis of the N-terminal region demonstrated that amino acids 245-365 are critical to couple ATP hydrolysis to chromatin remodeling, as $CSB^{\Delta 245\text{-}365}$ (CSBΔN1) is devoid of any ATP-dependent chromatin remodeling activity, although it still is a robust DNA- and nucleosome-stimulated ATPase (Figure 5) (Cho et al., 2013). This region, termed the N1 region, is rich in basic amino acids without any recognizable motifs.

1.3.4.2. NAP1-like histone chaperones interact with CSB and potentiate the ATP-dependent chromatin remodeling activity of CSB

To understand how CSB's remodeling activity is regulated, Cho et al. (2013) identified the NAP1-like histone chaperones, NAP1L1 and NAP1L4, as new CSB binding proteins (Cho et al., 2013). These two proteins bind to CSB both *in vitro* and in cells, and the N1 region of CSB is critical for this interaction (Cho et al., 2013). Of great interest, NAP1L1 and NAP1L4 substantially increase CSB's remodeling activity to a maximal site-exposure rate constant similar to that of ACF (Cho et al., 2013). Somatic cell genetics further demonstrated that chromatin remodeling by CSB and NAP1L4 is critical for the completion of TC-NER. It was shown that CSBΔN1 fails to completely rescue the UV sensitivity of CSB functional null cells; however, overexpressing NAP1L4 in the presence of CSBΔN1 fully complements the UV sensitivity (Cho et al., 2013). Interestingly, down-regulation of NAP1L2, the brain specific isoform of the NAP1-like proteins, is associated with neurodegenerative diseases, suggesting a biological significance to the CSB-NAP1L interactions in relation to Cockayne syndrome (M. D. Li et al., 2014).

1.3.4.3. DNA interaction and nucleosome remodeling by CSB and NAP1-like proteins – insights from single molecule studies

How do NAP1-like histone chaperones facilitate nucleosome remodeling by CSB? Lee et al. (2017) studied how CSB/NAP1L1 interact with DNA and remodel nucleosomes using single-

molecule approaches, including protein induced fluorescence enhancement (PIFE) and fluorescence resonance energy transfer (FRET) (J. Y. Lee et al., 2017). PIFE assays utilize a fluorophore attached to the DNA as a reporter of a protein binding on the DNA, and the intensity of a fluorophore is enhanced upon binding of a protein in the vicinity of the fluorophore (Hwang & Myong, 2014). FRET is based on the excitation of a donor fluorophore and its concomitant energy transfer to a neighboring acceptor fluorophore, and the efficiency of this transfer is converted to an approximate distance between the two dyes.

Lee et al. (2017) found that CSB interacts with DNA in two principle ways: a rapid simple binding as revealed by PIFE and occasional gross DNA distortion detected by FRET (J. Y. Lee et al., 2017). PIFE results indicated that CSB binds DNA at internal sites and ends without preference, in the presence or absence of ATP. In contrast to the rapid, simple DNA binding, ATP hydrolysis by CSB reduced the propensity of CSB to distort DNA. The latter result is similar to Beerens et al. (2005), in which scanning force microscopy (SFM) demonstrated that a shortening of contour length of a singly-nicked, circular DNA occurred upon CSB binding (Beerens et al., 2005). The shortening of contour length presumably resulted from the wrapping of DNA on CSB. Similar to the gross changes observed by FRET, ATP hydrolysis by CSB reduced the frequency of this DNA wrapping event. However, the shortening of DNA contour length upon CSB binding observed by SFM was dependent on ATP binding, while the gross changes in DNA conformation detected by FRET is independent of ATP binding (Beerens et al., 2005; J. Y. Lee et al., 2017). Whether the difference between these two studies is due to the nature of the DNA used or if these are the same events remains to be determined.

Incubating CSB with NAP1L1 first, before mixing with DNA, induced rare and brief PIFE and no FRET events (J. Y. Lee et al., 2017). Furthermore, PIFE events generated by premixing CSB with NAP1L1 contained well-defined borders in the fluorescence traces (with better defined DNA bound vs. unbound states), in strong contrast to CSB alone, indicating NAP1L1 decreases CSB's interaction with naked DNA. NAP1L1 on its own did not generate PIFE or FRET. Direct visualization of CSB-DNA interactions, by incubating Cy3-labeled CSB with immobilized DNA,

revealed increasing Cy3 signals on DNA over time, indicating that multiple CSB molecules bind to a single DNA fragment. Similar to the PIFE results, inclusion of NAP1L1 reduced the number of CSB-DNA interactions, resulting in well-defined DNA bound vs. unbound states (J. Y. Lee et al., 2017). Together, these results suggest that CSB may multimerize on DNA and that NAP1L1 decreases this tendency (J. Y. Lee et al., 2017).

FRET was also used to monitor remodeling events in real time using mononucleosomes labeled on both DNA and histone H2A (J. Y. Lee et al., 2017). CSB alone or CSB and NAP1L1 were incubated with immobilized mononucleosomes, and remodeling was initiated by adding ATP. Like the human ACF remodeling complex, nucleosome remodeling by CSB or by CSB plus NAP1L1 contains three distinct phases: activation, translocation, and pausing, with the activation step being rate limiting (Blosser et al., 2009; J. Y. Lee et al., 2017). Interestingly, the translocation steps induced by CSB and ACF both have a rate of about two base pairs per second. The major difference is that CSB has higher tendency to pause between two translocation events. Pre-incubating CSB with NAP1L1 increases the activation rate of CSB and decreases the number of pausing events during remodeling. Moreover, the distribution of FRET values created by CSB when NAP1L1 is present is narrower than with CSB alone, consistent with results from bulk experiments revealing that CSB creates more homogenous remodeled products when in complex with NAP1L1 (Cho et al., 2013; J. Y. Lee et al., 2017).

1.3.4.4. Similarities and differences between CSB and the S. pombe yeast homolog Rhp26

While the ATPase domains of CSB and its *S. pombe* homolog, Rhp26, are highly conserved, the flanking N- and C-terminal regions, which are suggested to regulate CSB's enzymatic activity, are shorter and less conserved in Rhp26 (Figure 5) (S. Li, 2015). In contrast to CSB, Rhp26 on its own has little remodeling activity (L. Wang et al., 2014). The N-terminal regions of each protein also function as auto-repressive modules for ATPase activity(L. Wang et al., 2014). However, the N-terminal region of CSB (amino acids 1-454) is also essential for the recognition of UV-induced DNA lesion-stalled transcription as well as ATP-dependent chromatin

remodeling activity (Cho et al., 2013). Wang et al. (2014) identified a conserved “leucine latch” motif in the N-terminus of Rhp26, and this short helix serves to lock Rhp26 in an inactive state (Figure 5) (L. Wang et al., 2014). It would be of interest to determine if ATP hydrolysis by Rhp26 unlocks this inactive state, similar to its human homolog CSB (Lake et al., 2010). Notably, while the N-terminal region of Rhp26 negatively regulates the remodeling activity of Rhp26, this region is dispensable for Rhp26 function in protecting cells from UV-irradiation, as Rhp26ΔN fully complements the UV sensitivity of ΔRhp26, in contrast to its human homolog CSB (Cho et al., 2013; L. Wang et al., 2014). These observations suggest that the highly regulated CSB-chromatin interaction mechanism that is used in mammals is dispensable in *S. pombe.*

The C-terminal regions of both CSB and Rhp26 function as positive regulatory regions, regardless of the detailed mechanism (Figure 5) (Lake et al., 2010; L. Wang et al., 2014). Like CSB, the C-terminal region positively regulates Rhp26’s ATPase activity, as Rhp26ΔC resulted in decreased ATPase activity (L. Wang et al., 2014). However, CSB contains a ubiquitin-binding domain (UBD) in its C-terminal region, which is absent in its yeast homologs (Figure 5). This domain is suggested to be critical for CSB function in TC-NER as well as transcription (Anindya et al., 2010; Epanchintsev et al., 2017) and likely specific to CSB’s role in multicellular organism development.

1.3.5. CSB in transcription regulation

Initial involvement of CSB in transcription regulation came from several lines of evidence. Assays using intact and permeabilized cells revealed that transcription elongation is reduced in CSB cells and that this defect can be complemented by extracts from normal cells (Balajee et al., 1997). This study supported the hypothesis that Cockayne syndrome may in fact be a transcription syndrome in addition to a repair syndrome, and differences in the extent of gene expression defects might account for differences in the severity of Cockayne syndrome (Bootsma & Hoeijmakers, 1994; Chalut et al., 1994; Friedberg et al., 1994).

A role for CSB in transcription was further supported by *in vitro* reconstitution assays with purified components, which revealed that CSB can interact with RNA pol II in isolation and as part of an elongation complex, and that CSB can directly stimulate transcription elongation rates(Selby & Sancar, 1997a; Tantin et al., 1997). Biochemical purification of CSB from whole cell extracts ultimately demonstrated that CSB is indeed part of a large complex (>700 kDa) that contains RNA pol II (van Gool et al., 1997).

1.3.5.1. CSB in transcription regulation during replicative cell growth

Transcription profiling studies supported the notion that CSB participates in transcription regulation during normal cell growth and further revealed that this function of CSB is unlikely general but rather gene-specific (Kyng et al., 2003; Newman et al., 2006). These expression studies implicated CSB in the regulation of genes involved in chromatin structure maintenance and remodeling as well as a variety of metabolic processes. Together, these findings supported the hypothesis that CSB's role in regulating transcription may play a more significant role in the pathology of Cockayne syndrome than previously appreciated.

Deep sequencing of anti-CSB chromatin immunoprecipitated (ChIP-seq) DNA produced the first genome-wide map of CSB occupancy sites in cells during replicative growth (Lake et al., 2014). This study revealed that CSB is significantly enriched at promoters and enhancers, suggesting that CSB may function in transcription initiation, in addition to its previously demonstrated role in transcription elongation (Tantin et al., 1997; van Gool et al., 1997). Motif analysis of CSB-occupied sites revealed that CSB is enriched at sites containing the 12-O-tetradecanoylphorbol-13-acetate (TPA) response element (TRE), which contains the binding motif for activator protein 1 (AP-1). AP-1 is a family of bZIP transcription factors, which include Jun and Fos family members, and these proteins play critical roles in responding to environmental stimuli. CSB was shown to interact with c-Jun and this interaction was found to be critical for CSB recruitment to TRE-containing sites. Side-by side comparisons of wild-type CSB and a chromatin remodeling-deficient CSB derivative (CSBΔN1, Figure 5) revealed that CSB regulates

nucleosome positioning around its binding sites and alters the expression of nearby genes(Lake et al., 2014). Therefore, this study provided the first direct evidence for a function of CSB in regulating transcription through its ATP-dependent chromatin remodeling activity (Figure 6). Nonetheless, not all genes near CSB-occupancy sites were dependent upon CSB's remodeling activity, although gene expression was clearly impacted by CSB loss (Figure 6). Taken together, these observations demonstrate that CSB has both chromatin remodeling-dependent and – independent functions in transcription regulation (Lake et al., 2014). The remodeling independent function suggests that CSB may act as a scaffold to recruit transcriptional regulators (blue, Figure 6), similar to that of its function of recruiting repair factors in TC-NER (Cho et al., 2013). Combining tandem affinity purification (TAP) with mass spectrometry, Nicolai et al. (2015) identified 33 novel CSB interacting partners. These proteins include the SWI/SNF-related SMARCA family of proteins, the transcriptional activator MTA2, and the transcriptional repressors HDAC1 and GATA2A/B (Nicolai et al., 2015). Therefore, CSB may coordinate its own activity with other remodelers, histone modifying enzymes and transcription factors to regulate chromatin structure for transcriptional regulation.

1.3.5.2. CSB in transcription regulation during oxidative stress

NOTE: *The work described in this section is described in full in* ***Chapter 2*** *(Lake et al., 2016).*

CSB has also been suggested to play a role in transcription regulation in response to oxidative stress (Kyng et al., 2003). Kyng et al. (2003) conducted microarray analysis following H_2O_2 treatment in CSB-null or CSB ATPase mutant cell lines and, compared to wild type, found expression changes in 122 out of 6912 genes examined, including genes important for the stress response, transcription, translation, signal transduction, and the cell cycle (Kyng et al., 2003). Lake et al. (2016) examined CSB occupancy at a genome-wide level after treatment with menadione, which induces oxidative stress (Lake et al., 2016). It was found that CSB occupancy was altered, with a significant increase at promoters from 2% in unstressed cells to 11% in cells

experiencing oxidative stress, suggesting that CSB may regulate transcription initiation to mount a response to oxidative stress.

Motif analysis revealed that sites bound by CSB during oxidative stress are enriched for TREs, as in non-stressed cells, and that the percentage of CSB-bound TREs did not change (Figure 6) (Lake et al., 2016). However, binding motifs for the transcriptional regulator CCCTC-binding factor (CTCF) were substantially enriched upon oxidative stress, with an increase from 1% in non-stressed cells to 11% in oxidatively stressed cells. CTCF is involved in transcription regulation and is a key player in regulating long-range chromatin interactions (B. K. Lee & Iyer, 2012; Ong & Corces, 2014). *In vitro* protein-interaction studies using purified proteins revealed that CSB and CTCF directly interact. Additionally, it was found that this interaction is enhanced in cells by oxidative stress (Lake et al., 2016). Using CTCF knockdown, it was found that the CTCF protein is needed to recruit CSB to sites containing the CTCF binding motif upon oxidative stress. Intriguingly, this was also found to be true for other stress-induced CSB occupied loci that do not contain the CTCF binding motif, suggesting that CTCF may also recruit CSB to sites that are near a CTCF binding motif, in addition to sites containing the CTCF motif. Reciprocally, CSB was found to increase CTCF-DNA interaction, both *in vitro* and in cells. Together, these results support the hypothesis that CSB may work with CTCF to organize 3-dimensional chromatin structure to efficiently regulate a transcriptional response to oxidative stress (Figure 6) (Lake et al., 2016). How CSB's enzymatic and protein-recruitment activities contribute to this process remains to be determined.

Given that CSB is proposed to function in oxidative DNA damage repair, it is also possible that the interaction of CSB and CTCF may facilitate the formation of DNA repair hubs in the 3-dimensional chromatin space to efficiently remove oxidative DNA damage. It will be of great interest to determine the extent to which CSB is enriched at sites of oxidative DNA lesions by analyzing DNA mutation signatures associated with CSB-ChIPed DNA from cells exposed to oxidative stress.

1.3.5.3. CSB in transcription regulation in response to UV irradiation

Over thirty-five years ago, it was reported that RNA synthesis fails to recover after UV irradiation in cells from Cockayne syndrome patients (Mayne & Lehmann, 1982). This was initially thought to be the consequence of a block in transcription elongation resulting from defective TC-NER. However, that model was challenged by experiments using *in vitro* transcription systems with nuclear extracts prepared from UV-irradiated or mock-irradiated normal human and Cockayne syndrome cells (Rockx et al., 2000). From this study, it was found that there was a global defect in transcription initiation in Cockayne syndrome cells. This defect was associated with a loss of the hypophosphorylated, transcription-initiating form of RNA pol II with a concomitant increase in the hyperphosphorylated, transcription-elongating form of RNA pol II. Subsequent experiments using ChIP-qPCR confirmed the defect in transcription initiation and further revealed a decrease in the recruitment of RNA pol II to the promoters of certain genes (Proietti-De-Santis et al., 2006).

Recent studies have suggested that the inability to reactivate transcription in Cockayne syndrome cells after UV irradiation may be due, in part, to an inability to relieve general transcriptional repression induced by UV irradiation (Epanchintsev et al., 2017; Kristensen et al., 2013). Kristensen et al. (2013) searched for common factor binding motifs near the promoters of a collection of UV-repressed genes and found that these genes contained binding sites for activating transcription factor 3 (ATF3), a transcriptional repressor that is activated in response to cellular stress (Hai et al., 1999; Hai & Hartman, 2001; Kristensen et al., 2013). In CSB wild-type cells, ATF3 mRNA and protein levels increase and peak approximately eight hours after UV irradiation, which corresponds with maximal repression of genes whose promoters are bound by ATF3 (Kristensen et al., 2013). Between approximately 12 and 24 hours after UV irradiation, ATF3 levels decrease and ATF3 is removed from bound promoters, and this correlates with recruitment of RNA pol II as well as transcription resumption (Figure 6) (Kristensen et al., 2013). In CSB-deficient cells, however, the ATF3 protein and ATF3 occupancy at its target promoters remain high (Kristensen et al., 2013). This work suggested that CSB might be required to remove

ATF3 from its target promoters to allow transcription resumption (Figure 6), and this hypothesis was subsequently tested (Epanchintsev et al., 2017). It was found that CSB collaborates with Cockayne syndrome protein A (CSA) to promote the ubiquitination and degradation of ATF3, thereby allowing transcription to resume (Figure 6). CSA is a WD-40 repeat-containing protein that is part of an E3-ubiquitin ligase complex along with DNA damage binding protein 1 (DDB1) and Cullin 4A (CUL4A) (Groisman et al., 2003; Kristensen et al., 2013; Zhou & Wang, 2001). Like CSB, mutations within CSA can also lead to Cockayne syndrome (Lehmann, 1982). Importantly, using an ATP-deficient CSB derivative (Q678E), these studies revealed that ATP hydrolysis by CSB is not necessary for recruitment of CSB to ATF3-occupied sites or ATF3 ubiquitination; however, ATP hydrolysis was necessary for recruitment of the proteasomal machinery and subsequent ATF3 turnover. It will be of great interest to determine if the chromatin remodeling deficient CSB derivative, CSBΔN1, can support ATF3 degradation, to examine the role that CSB may play in reorganizing the epigenetic landscape for the resumption of transcription initiation after UV-induced genotoxic stress.

It is, however, important to note that while CSB's role in ATF3 release may partially underlie the mechanism of RNA synthesis recovery after UV irradiation, it is unlikely to be the only mechanism. ATF3 is also induced by other genotoxins, such as ionizing radiation and alkylating agents, yet CSB null cells do not show the same level of sensitivity to these genotoxins as they do to UV irradiation (Hai & Hartman, 2001; Ranes et al., 2016; L. Wei et al., 2015; Wong et al., 2007).

1.3.6. Regulation of TC-NER by CSB

CSB is critical for multiple steps of the TC-NER process. Lake et al. (2010) demonstrated that ATP hydrolysis by CSB is essential for CSB to become associated with UV-induced DNA lesion stalled RNA pol II, the critical first step of transcription-coupled DNA repair (Lake et al., 2010). However, chromatin remodeling by CSB is dispensable for this step, since the ATPase-proficient, yet remodeling-deficient CSBΔN1 mutant is capable of stably associating with

chromatin at sites of lesion-stalled transcription after UV irradiation (Cho et al., 2013). Chromatin immunoprecipitation experiments followed by western blot analyses revealed that representative factors of the nucleotide excision repair machinery, the transcription elongation complex, and the CSA-ubiquitin ligase complex are recruited to UV-induced DNA lesion-stalled RNA pol II in the presence of the chromatin remodeling deficient CSBΔN1, arguing against the notion that the function of chromatin remodeling by CSB in TC-NER is to create access for factor binding (Cho et al., 2013). These observations, along with the work of Fousteri et al. (2006), indicate that CSB likely recruits factors for repair and transcription resumption through protein-protein interaction (Fousteri et al., 2006). Given that chromatin remodeling by CSB is required for steps after the recruitment of the TC-NER machinery, chromatin remodeling by CSB likely regulates the chromatin landscape for more efficient DNA repair and/or transcription resumption(Cho et al., 2013). NAP1-like proteins are also expected to contribute to these activities, as their overexpression in the presence of CSBΔN1 fully rescues the UV sensitivity resulting from the loss of CSB (Cho et al., 2013).

CSB without its last 273 amino acids (CSB^{del}) failed to complement the UV sensitivity of the CSB-deficient cell line CS1AN-sv (Anindya et al., 2010). This mutant protein can interact with RNA pol II after UV irradiation and can recruit necessary NER factors; however, the DNA incision step of TC-NER is compromised in the CSB^{del} background (Anindya et al., 2010). A ubiquitin binding domain (UBD) lies within the last 273 amino acids (Figure 5), and a CSB protein with point mutations that disrupt ubiquitin binding (LL to GG) also fails to fully complement the UV sensitivity of CS1AN-sv cells, although the effect on cell survival and RNA recovery is less severe than CSB^{del}. These observations reveal that ubiquitin binding by CSB is important for CSB function in TC-NER (Anindya et al., 2010).

ATP hydrolysis by CSB is important for the initiation of TC-NER, however, the exact role of CSB in this process and how this protein recognizes and interacts with RNA pol II arrested at DNA lesions is unknown. The electron cryomicroscopy (cryo-EM) structure from Xu et al. (2017) revealed that the *S. cerevisiae* CSB ortholog, Rad26, binds DNA upstream of the RNA pol II

elongation complex, making contacts with the upstream DNA duplex region and single-stranded DNA in the upstream fork of the transcription bubble (Figure 7) (Xu et al., 2017). Interestingly, Rad26 caused an 80-degree bend in the upstream duplex DNA, perhaps creating novel interaction surfaces to facilitate repair factor recruitment (Figure 7) (Xu et al., 2017). This DNA distortion observed by cryo-EM might be related to the DNA distortion previously observed by FRET and SFM (Beerens et al., 2005; J. Y. Lee et al., 2017).

By modeling this Rad26 structure with the Snf2 remodeler bound to nucleosomes, Xu et al. (2017) proposed a model whereby Rad26 pulls the DNA template strand away from RNA pol II by translocating on the DNA duplex. This would lead to annealing the strands of the transcription bubble, consistent with the observed strand annealing activity of CSB (Muftuoglu et al., 2006), and promote the forward movement of RNA pol II (Xu et al., 2017). This work also supports a model in which CSB promotes transcription elongation by preventing backtracking and promoting forward movement of RNA pol II when it encounters a non-bulky transcription-stalling signal. However, CSB would fail to promote forward RNA pol II movement in the presence of bulky transcription-blocking lesions (Xu et al., 2017).

We would like to propose a model to account for CSB's function in different steps of TC-NER, based on the collective work from the human and yeast homologs. CSB uses ATP hydrolysis to undergo a conformational change to probe chromatin for lesion-stalled RNA pol II (Figure 7) (Lake et al., 2010; Xu et al., 2017). Once discovered, the N-terminal, substrate-recognition domain would bind to the lesion-stalled RNA pol II complex (Lake et al., 2010). The association of CSB with lesion-stalled transcription would lead to an 80-degree DNA bend (Figure 7). The CSB-chromatin association and resulting DNA conformation would be re-enforced by interaction between chromatin and the C-terminal region of CSB, which is necessary for stable chromatin association (Figure 7) (Lake et al., 2010; Xu et al., 2017). Once stably associated, CSB would function as a scaffold, creating a platform to recruit factors needed to repair DNA and resume transcription (Figure 7) (Fousteri et al., 2006; Lake et al., 2010). This model is supported by the observations that the remodeling deficient CSBΔN1 protein is correctly recruited to DNA

lesion-stalled RNA pol II and can initiate the recruitment of additional protein factors necessary for DNA repair (Cho et al., 2013). Subsequently, CSB facilitates DNA incision by NER factors through its Ub-binding domain (UBD) (Anindya et al., 2010). Removal of RNA pol II is not a prerequisite for the incision events, as strand excision can occur in a cell-free repair system with a stalled polymerase covering the DNA lesion (Selby et al., 1997).

Genome-wide studies have revealed that repair of transcription blocking lesions occurs as waves along gene bodies in the 5'-3' direction (Andrade-Lima et al., 2015; Chiou et al., 2018). Importantly, by controlling these transcriptional waves with the reversible transcription elongation inhibitor 5,6-dicholor-1-β-D-ribofuranosybenzimidazole (DRB), Chiou et al. (2018) have provided strong evidence that a single transcription elongation complex does not progress along a template to engage multiple lesions, but rather dissociates from the template after the dual incisions. RNA pol II dissociation would expose the 3' hydroxyl generated by the incision event to promote new DNA synthesis and ligation (Chiou et al., 2018). RNA pol II removal might be promoted by CSB translocation, by the helicase activity of XPB or XPD, or simply by instability created by fragment removal (Figure 7). Whether the chromatin remodeling activity of CSB is used to create an epigenetic landscape that permits more efficient DNA repair or to facilitate transcription resumption after repair remains to be determined. Future studies examining the structure of the remodeling-deficient CSBΔN1-RNA pol II elongation complex (EC) will provide insights into the functions of ATP hydrolysis by CSB in TC-NER.

1.3.7. Future Perspectives

Over the recent years, we have learned much about how the biochemical activities of CSB are tightly regulated and are used to facilitate TC-NER and transcription regulation; however, there is still much to be learned. For example, given that the chromatin remodeling activity of CSB is crucial for efficient TC-NER, are there different requirements of CSB (or are additional proteins needed) for TC-NER at nucleosome dense as compared to nucleosome-free

regions? Does the enzymatic activity of CSB in any way influence RNA pol II stalled at bulky DNA lesions to permit DNA repair? Does CSB play a role in resetting the epigenetic landscape after TC-NER for transcription resumption? Does CSB-dependent ubiquitinylation account for transcription regulation beyond ATF3? To what extent does CSB organize the three-dimensional chromatin structure to orchestrate DNA repair and transcription regulation during oxidative stress relief? Additional studies using structural, genomic and in vitro reconstituted systems will shed new light on these and other outstanding questions in the coming years.

1.4. Hypotheses and Introduction to Experimental Paradigm

Work presented in this chapter demonstrates that CSB participates in a number of different cellular processes and has both remodeling-dependent and -independent functions. CSB was initially identified as a critical component of TC-NER. Subsequent studies demonstrated CSB also participates in transcription regulation and also suggest a role for CSB in additional DNA repair processes. We recently demonstrated CSB directly participates in transcription regulation via both remodeling-dependent and –independent mechanisms and others have suggested a role for CSB in transcription regulation following UV irradiation. CSB is also required for the relief of oxidative stress; however, exactly how CSB participates in this response is unclear.

The majority of the work in this dissertation is conducted on a Cockayne syndrome patient-derived cell line CS1AN.S3.G2 (CS1AN). The CS1AN cell line is an SV-40 immortalized cell line derived from skin fibroblasts of a three-year-old female with Cockayne syndrome. CS1AN cells contain only one CSB allele coding for a short, non-functional, truncated CSB protein lacking all predicted domains. The mutation was identified as an A to T transversion at nucleotide 1088, which results in a premature stop codon at amino acid 337 (Troelstra et al., 1992). To study oxidative stress, cells are treated with menadione, which induces oxidative stress by creating several ROS, including the superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the highly oxidizing hydroxyl radical ($^{\bullet}OH$) (Cadet & Davies, 2017). In addition to oxidative base lesions, menadione can also generate single strand breaks (Cadet & Davies, 2017).

To understand the role of CSB in the relief of oxidative stress, this dissertation seeks to characterize the mechanisms by which CSB interacts with chromatin in response to oxidative stress. To accomplish this I utilize chromatin association assays, including chromatin immunoprecipitation, to characterize elements underlying CSB's response to oxidative stress. First, genome-wide chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) as well as ChIP-qPCR will reveal targeting mechanisms underlying CSB's oxidative stress-induced targeting to specific genomic loci. I examine CSB mutant constructs to identify the critical determinants of CSB required for its oxidative-stress induced chromatin association and compare

that to its association for TC-NER. I then determine whether base excision repair proteins as well as active transcription by RNA polymerase II affect CSB's oxidative stress-induced chromatin association to work towards understanding whether CSB participates in BER as a means of relieving oxidative stress. I hypothesize characterizing the mechanisms by which CSB is targeted to chromatin during oxidative stress will elucidate mechanisms by which CSB participates in the relief of oxidative stress.

1.5. Figures

Figure 1. Cockayne syndrome phenotype. Photos of Baptiste Bixel (top, 6/24/1993 to 10/29/2003) at age 3, 7, and 10 and Eden Elizabeth Carroll (bottom, 12/2/03 to 7/19/09, age in photos unknown) demonstrate the progression of Cockayne syndrome. Photos downloaded from www.cockayne-syndrome.org.

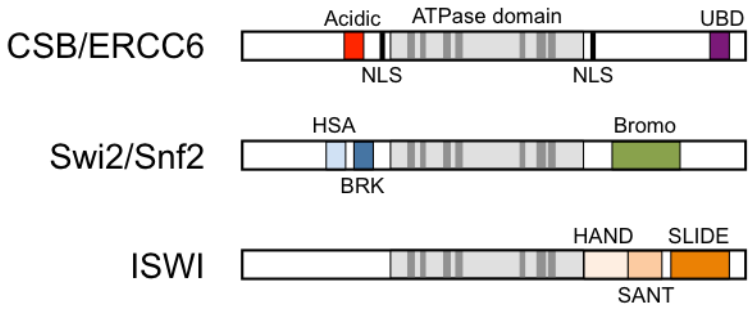

Figure 2. CSB/ERCC6 protein schematic and representative SWI2/SNF2 ATP-dependent chromatin remodelers. Unlike other SWI2/SNF2 remodelers, CSB does not contain targeting domains in its N- or C-terminus. Snf2/Swi2 contains an HSA domain, which functions in interaction with nuclear actin-related proteins and actin, a BRK domain of unknown function, and a bromo domain, which recognizes acetylated proteins. The ISWI HAND/SANT/SLIDE domain is important for nucleosome interaction. Figure adapted from (Lake & Fan, 2013).

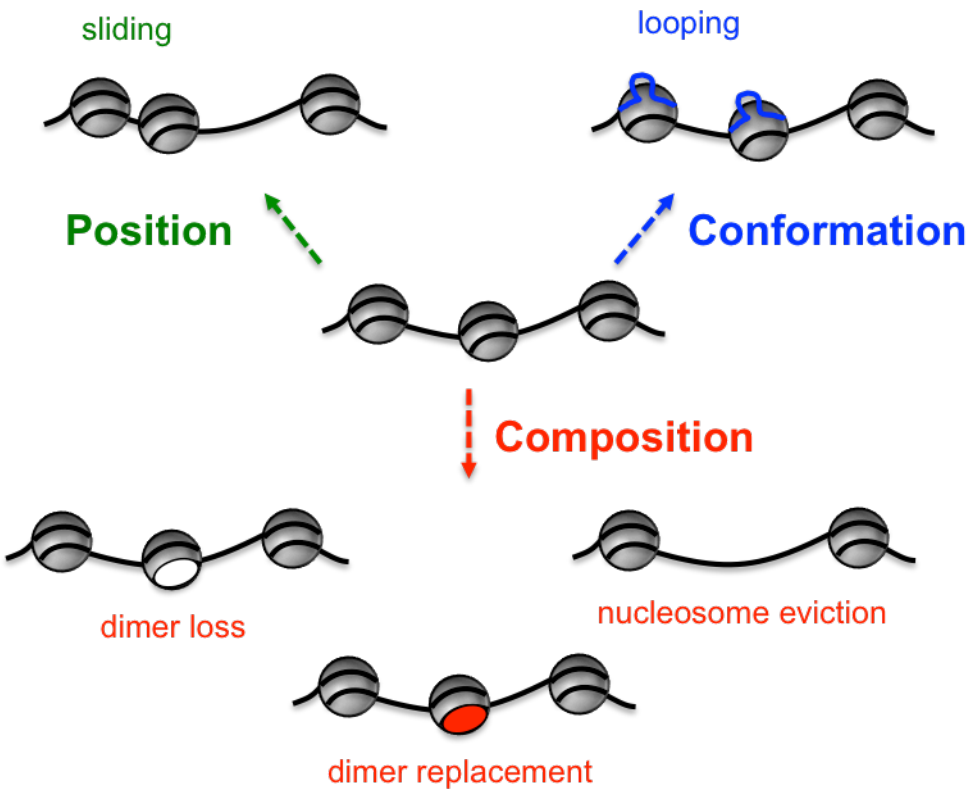

Figure 3. Mechanisms by which ATP-dependent chromatin remodelers alter chromatin structure. This includes translational movement or sliding of a nucleosome, altering nucleosome conformation, or changing nucleosome composition, including removing or replacing dimers and nucleosome eviction. Figure adapted from (Lake & Fan, 2013).

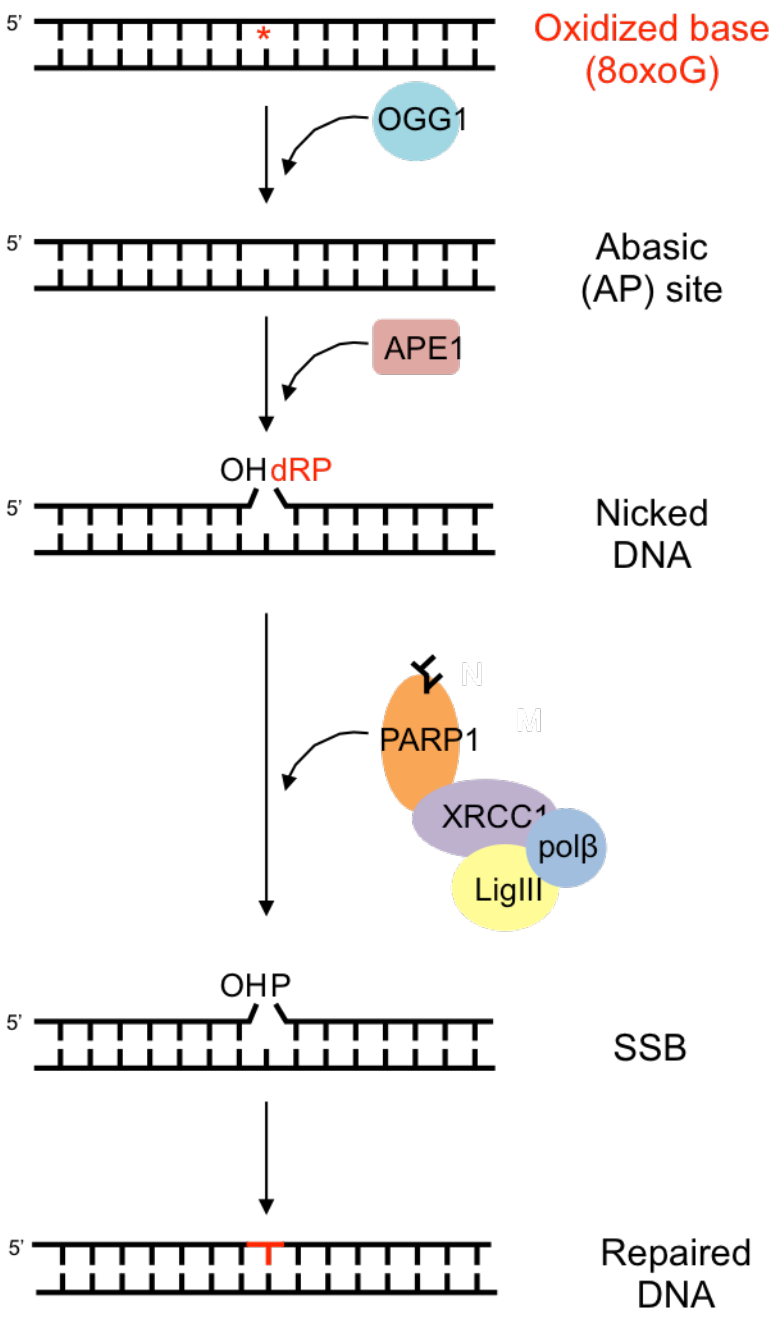

Figure 4. Base excision repair. Schematic depicting the steps of short patch base excision repair. See text for details.

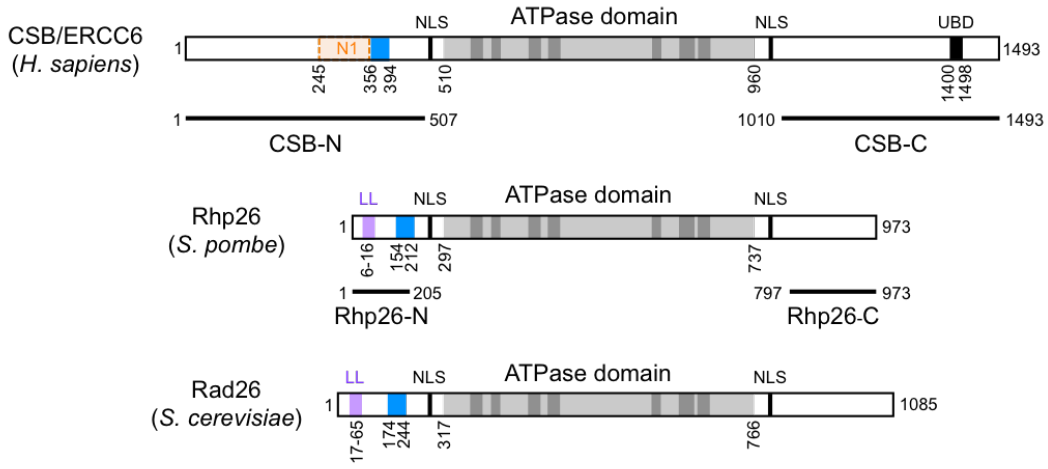

Figure 5. Schematics of the human CSB/ERCC6 and its yeast homologs, Rhp26 and Rad26. ATPase domains are in gray. Leucine latch motifs (LL) are in purple and acidic rich regions are in blue. NLS, putative nuclear localization signal. UBD, ubiquitin binding domain.

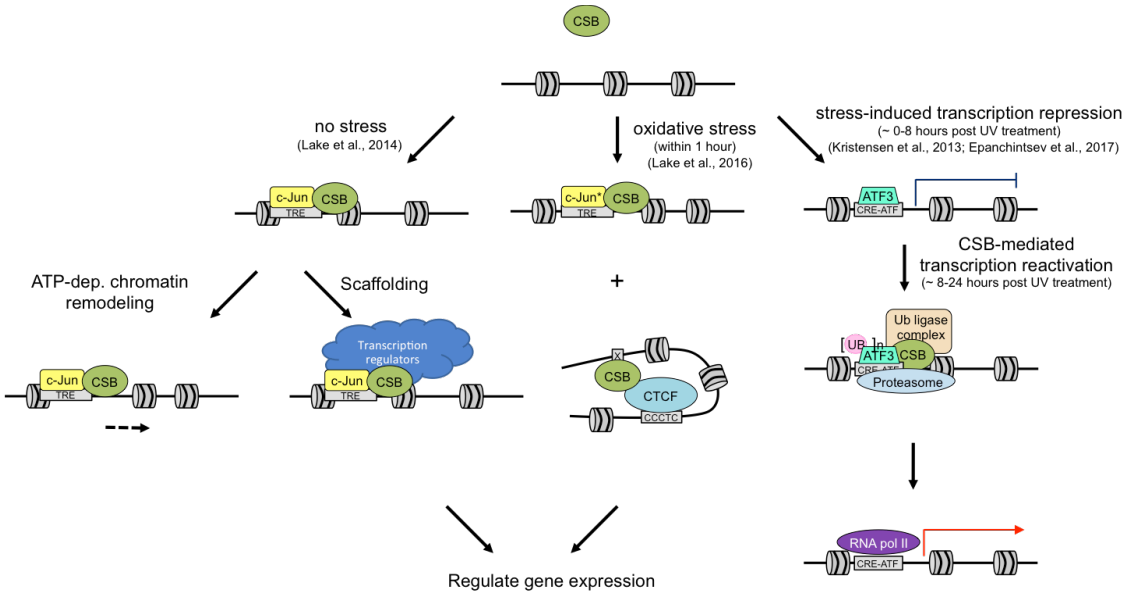

Figure 6. Models for transcription regulation by CSB under different growth conditions. **(Left)** Under non-stressed conditions, CSB is targeted to TREs by the transcription factor c-Jun, where CSB can regulate transcription by both remodeling-dependent and –independent mechanisms. **(Middle)** Targeting of CSB by c-Jun to TREs also occurs in cells following oxidative stress with an up-regulation of c-Jun after stress (c-Jun*). Additionally, following oxidative stress, CSB is also enriched at binding motifs for the architectural protein CTCF. CSB may regulate 3-D chromatin structure by modulating CTCF-chromosome interactions to regulate gene expression. **(Right)** Following UV irradiation and DNA repair, ATF3 is ubiquitinated in a CSB- and CSA-dependent manner and degraded by the proteasome, allowing RNA pol II recruitment and transcription reactivation.

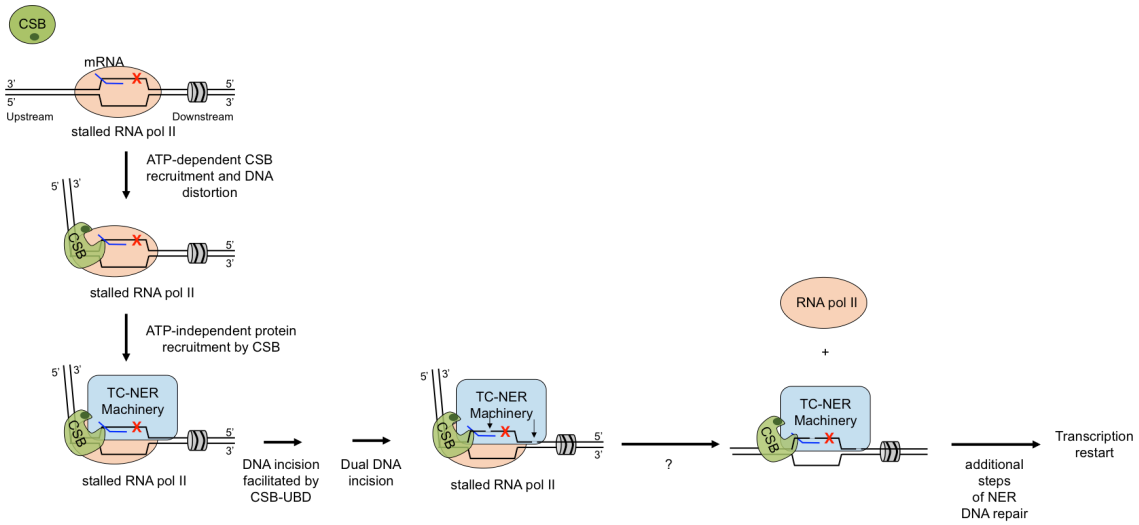

Figure 7. Model for how CSB integrates into different steps of TC-NER. The association of CSB with RNA pol II stalled at a bulky DNA lesion (red X) is stabilized. This step requires the DNA/nucleosome stimulated ATP hydrolysis activity of CSB. CSB interacts with both RNA pol II and duplex DNA upstream of RNA pol II as well as single-stranded DNA within the upstream fork of the transcription bubble. CSB generates an 80-degree bend in the upstream DNA and provides an interaction surface for recruiting additional TC-NER factors. CSB facilitates this step through protein-protein interactions, but not through its ATP-dependent chromatin remodeling activity. The C-terminal Ub-binding domain (dark green oval) of CSB is needed for efficient DNA incision. Dual incisions happen in the presence of RNA polymerase II, which is subsequently removed to permit the remaining steps of the repair processes by a yet to be determined by a mechanism.

CHAPTER 2: THE CSB CHROMATIN REMODELER AND CTCF ARCHITECTURAL PROTEIN COOPERATE IN RESPONSE TO OXIDATIVE STRESS

2.1. Preface

The manuscript presented in this chapter was published by *Nucleic Acids Research* on 18 March 2016 (Lake et al., 2016). It has been reformatted here in accordance with the University of Pennsylvania dissertation formatting guidelines.

2.2. Abstract

Cockayne syndrome is a premature aging disease associated with numerous developmental and neurological abnormalities, and elevated levels of reactive oxygen species have been found in cells derived from Cockayne syndrome patients. The majority of Cockayne syndrome cases contain mutations in the ATP-dependent chromatin remodeler CSB; however, how CSB protects cells from oxidative stress remains largely unclear. Here, we demonstrate that oxidative stress alters the genomic occupancy of the CSB protein and increases CSB occupancy at promoters. Additionally, we found that the long-range chromatin-structure regulator CTCF plays a pivotal role in regulating sites of genomic CSB occupancy upon oxidative stress. We show that CSB directly interacts with CTCF *in vitro* and that oxidative stress enhances the CSB-CTCF interaction in cells. Reciprocally, we demonstrate that CSB facilitates CTCF-DNA interactions *in vitro* and regulates CTCF-chromatin interactions in oxidatively stressed cells. Together, our results indicate that CSB and CTCF can regulate each other's chromatin association, thereby modulating chromatin structure and coordinating gene expression in response to oxidative stress.

2.3. Introduction

Reactive oxygen species (ROS) are constantly generated during aerobic metabolism. When ROS overloads the cellular antioxidant defense systems, the resulting alteration in redox homeostasis leads to oxidative stress (Sena & Chandel, 2012). Oxidative stress has been implicated in the aging process and diseases, such as cancer and neurological disorders. Cockayne syndrome is a premature aging disease associated with neurological and developmental abnormalities as well as sun sensitivity (Nance & Berry, 1992). Although the underlying mechanisms that lead to the diverse features of Cockayne syndrome remain largely unknown, a reduced ability of cells to relieve oxidative stress has been proposed to be a leading cause (Andrade et al., 2012; Cleaver et al., 2013; Pascucci et al., 2012).

Mutations in the Cockayne syndrome group B protein (CSB) account for the majority of Cockayne syndrome cases (Troelstra et al., 1992). CSB belongs to the SWI2/SNF2 ATP-dependent chromatin remodeler family, which is conserved from yeast to human (Lake & Fan, 2013). These proteins alter chromatin structure in an ATP-dependent manner and regulate fundamental nuclear processes, such as transcription and DNA repair. CSB displays ATP-dependent chromatin remodeling activities *in vitro* and in cells (Cho et al., 2013; Citterio et al., 2000; Lake et al., 2014).

CSB functions in transcription regulation, in addition to its better-characterized function in transcription-coupled nucleotide excision repair (Hanawalt & Spivak, 2008; Newman et al., 2006). Transcription profiling assays have indicated that CSB plays a general role in transcription regulation (Newman et al., 2006; Y. Wang et al., 2014), and a direct role of CSB in transcription regulation was demonstrated by identifying genomic occupancy sites of the CSB protein. CSB is enriched at regions with epigenomic features of promoters and enhancers (Lake et al., 2014). Importantly, CSB alters nucleosome structure near its occupancy sites to directly regulate gene expression (Lake et al., 2014).

Upon oxidative stress, CSB-deficient cells display increased cell death as compared to CSB-expressing cells (Kyng et al., 2003; Pascucci et al., 2012; Tuo et al., 2001). Increased ROS levels, altered gene expression and damaged DNA are observed in primary cells, iPS cells and

immortalized cells derived from Cockayne syndrome patients (Andrade et al., 2012; Cleaver et al., 2014; Kirkali et al., 2009; Newman et al., 2006; Osenbroch et al., 2009). To understand further how CSB relieves oxidative stress, we identified sites of genomic CSB occupancy upon oxidative stress using chromatin immunoprecipitation followed by deep sequencing (ChIP-seq). We found that CSB co-localizes with CTCF, a CCCTC-binding transcription factor and a major regulator of long-range chromatin interactions (Ong & Corces, 2014), at a subset of genomic regions upon oxidative stress. We also found that CSB and CTCF directly interact and can regulate each other's chromatin association in response to oxidative stress.

2.4. Materials and Methods

2.4.1. Cell culture and menadione treatment

CS1AN-sv cells and CS1AN-sv cells stably expressing CSB were maintained in DMEM-F12 supplemented with 10% FBS (Cho et al., 2013; Lake et al., 2014; Troelstra et al., 1992). For the ChIP-seq, ChIP-qPCR and co-IP assays, oxidative stress was induced by treating cells with 100 μM menadione in culture medium for 1 hour. For the cell survival and protein-fractionation assays, menadione concentrations are as noted in the text and figures.

2.4.2. Protein fractionation

Equal numbers of cells were seeded onto five 60 mm dishes and allowed to grow overnight until ~80% confluent. Cells were treated with varying concentrations of menadione in growth medium for 1 h or left untreated. Cells were then rinsed with PBS and collected in 200 μl buffer B (150 mM NaCl, 0.5 mM $MgCl_2$, 20 mM HEPES (pH 8.0), 10% glycerol, 0.5% Triton X-100, 1 mM DTT) on ice, as described previously (Lake et al., 2010). Cell lysates were centrifuged at 20 000 × g for 20 min at 4°C, and 150 μl supernatant was added to 50 μl 4× SDS sample buffer; this was the soluble fraction (S). 200 μl 1× SDS sample buffer was added to the pellet, which was then sonicated for 10 s at 25% amplitude using a Branson 101-135-126 Sonifier; this

was the chromatin-enriched fraction (C). The resulting chromatin-enriched fractions were 1.3-fold more concentrated than the soluble fractions. 14 µl of each protein fraction was loaded on the gels. Antibodies used for western blot analysis were as described below. Western blots were developed using SuperSignal West Pico chemiluminescent substrate and imaged with a Fujifilm ImageQuant LAS-4000 imager.

To determine the percentage of CSB co-fractionating with chromatin, western blots were quantified using ImageJ. CSB signals were normalized to respective BRG1 signals. CSB co-fractionating with chromatin was calculated as 'normalized CSB signals in 'C'/(normalized CSB in 'C' + normalized CSB signal in 'S' x 1.3)'.

2.4.3. shRNA knockdown

Mission lenti-viral shRNA expression constructs targeting CTCF (TRCN0000230191) and a non-targeting shRNA (SHC002) were purchased from Sigma-Aldrich. Virus was produced as previously described (Lake et al., 2014). The target cell confluence at the time of infection was ~20%. Infected cells were harvested 5 days post-infection for chromatin immunoprecipitation (ChIP) and western blot analyses.

2.4.4. ChIP-qPCR and ChIP-western analyses

ChIP was carried out following standard protocols. Briefly, 4-million cells were fixed with 1% formaldehyde for 10 min and sonicated on ice at 40% amplitude (30 s on, 90 s off, for a total of 24 min) using a Branson 101-135-126 Sonifier. ChIP was performed using 5 µl of a polyclonal anti-CTCF antibody (Millipore 07–729), 10 µl monoclonal anti-CSB antibody (1B1) (Lake et al., 2014) and 5 µl recombinant protein-G agarose beads (Invitrogen). ChIPed DNA was analyzed by real-time PCR using a 7900HT Fast Real-Time PCR System from Applied Biosystems and SYBR green. Primers were as described in Supplementary Table S7. For all ChIP-qPCR experiments described in this manuscript, menadione treated and untreated cells were examined side-by-side.

For the ChIP-seq experiments, the CSB+M sample was processed alongside one untreated sample, which was previously reported (Lake et al., 2014).

For western blot analyses, ChIP samples were reverse cross-linked in SDS sample buffer at 95°C for 30 min (Cho et al., 2013).

2.4.5. Antibodies

Antibodies used for western blot analysis were rabbit anti-CSB (1:2000) (Yu et al., 2000), rabbit anti-CTCF (1:2000) (Millipore, 07-729), mouse anti-GAPDH (1:10 000) (Millipore, MAB374), rabbit anti-BRG1 (1:1000) (Fan et al., 2005), rabbit anti-acetylated histone H3 (1:1000) (Millipore, 06-599), HRP-conjugated goat anti-rabbit IgG (1:10 000) (Pierce, 31460) and HRP-conjugated goat anti-mouse (IgG+IgM) (1:10 000) (Jackson Laboratory, 115-035-044).

2.4.6. ChIP-Seq and data analysis

ChIP libraries for deep sequencing were constructed and sequenced as described previously (Lake et al., 2014). The resulting sequencing reads were mapped and peaks were identified as described in (Lake et al., 2014). Raw and processed files (GSE50925) have been deposited at the Gene Expression Omnibus (GEO) repository. CSB ChIP-seq data from untreated cells were previously published (GEO:GSE50171) (Lake et al., 2014).

ChIP sequencing reads within a 200 bp region around a peak center from the two cell populations were compared. If the difference between signal intensities was 4-fold or greater and the *P*-value for that difference was ≤0.0001, the peak was classified as ‘significantly induced by menadione’ (blue) ‘or’ significantly repressed by menadione (green)’ (Figure 2A). The remaining occupancy sites were classified as common (red) (Figure 2A).

The genomic distribution of CSB occupancy was classified using the gene annotation tool from UCSC RefGene as follows: (i) promoter (from −1 kb to the transcription start site), (ii) TTS (from the transcription termination site to +1 kb), (iii) 5′ UTR, (iv) 3′ UTR, (v) exon, (vi) intron and (vii) intergenic (the rest). The Genomic Regions Enrichment of Annotations Tool (GREAT, version

2.0.2) was used for pathway analysis of CSB occupancy sites, using the 'MSigDB pathways' category (McLean et al., 2010). The assignment of peaks to genes was made using the default setting (proximal 5 kb upstream and 1 kb downstream of a transcription start site, plus a distal extension to the regulatory elements of neighboring genes, up to 1000 kb) (McLean et al., 2010).

2.4.7. Menadione sensitivity assays

100 000 cells were seeded onto 35 mm dishes. Twenty-four hours later, cells were either left untreated or treated with varying amounts of menadione in DMEM/F12 medium supplemented with 10% FBS for 1 h. After treatment, the menadione-containing medium was removed and fresh medium without menadione was added. The cells were subsequently cultured for an additional 24 h before cell viability was assayed. For cells infected with CTCF shRNA-expressing lentivirus, cells were treated with menadione 96 h post-infection, as described above, and assayed for survival 120 h post-infection. The number of viable cells was determined by trypan blue exclusion, using a hemocytometer. Percent survival was calculated as the ratio of treated cells to untreated cells.

2.4.8. Constructs, protein expression and protein purification

CSB expression constructs were as previously described (Lake et al., 2010). For protein expression in SF9 cells, Flag-tagged proteins were purified using M2-affinity chromatography (Fan et al., 2005). MBP and MBP-CTCF (zinc fingers 1–11) were expressed and purified as described previously (Plasschaert et al., 2014).

2.4.9. *In vitro* protein-protein interaction assays

Purified, N-terminally Flag-tagged CSB, CSB-N, or CSB-C were incubated with MBP-CTCF immobilized on amylose beads at 4°C for 1 hour in PBS containing 0.5% Triton X-100 and 10 μM $ZnSO_4$. The resulting amylose beads were washed with PBS + 0.5% Triton X-100 + 10 μM $ZnSO_4$, and proteins were eluted in SDS sample buffer by heating beads at 95°C for 5 min.

2.4.10. Gel shift assays

A 200 bp DNA fragment containing a CTCF-binding site was generated by PCR in the presence of ^{32}P-dATP (Supplementary Figure S4B). Proteins were mixed with 1 nM ^{32}P-labeled DNA at the indicated concentrations. Binding reactions were carried out in 30 mM HEPES (pH7.9), 60 mM NaCl, 6% glycerol, 6 mM $MgCl_2$, 100 μM $ZnSO_4$ and 0.02% NP40 at 30°C for 10 min. Reactions were loaded directly onto a 5% polyacrylamide gel prepared with 0.5× TBE. Gels were imaged using a Typhoon Trio (GE).

2.5. Results

2.5.1. Oxidative stress induces changes in the genomic localization of CSB

To induce oxidative stress in cultured cells, we used menadione, which generates free radicals through redox cycling (Watanabe et al., 2004). To validate this system, we first determined if CS1AN-sv cells, which do not have functional CSB, were more sensitive to oxidative stress than CS1AN-sv cells reconstituted with CSB^{WT} (Figure 1A). As predicted, CS1AN-sv cells were more sensitive to menadione treatment than CSB-reconstituted CS1AN-sv cells.

We next determined if menadione treatment altered the CSB-chromatin interaction, using a fractionation protocol we have previously described (Figure 1B) (Lake et al., 2010). A 1-h menadione treatment at 50 μM and 100 μM induced the co-fractionation of CSB and chromatin, while this treatment did not have an apparent impact on another ATP-dependent chromatin remodeler, BRG1 (Figure 1B and C). As shown in Figure 1C, cells treated with 100 μM menadione for 1 h displayed a maximal increase in the amount of CSB co-fractionating with chromatin. Accordingly, we used 100 μM menadione to determine the genomic localization of CSB upon oxidative stress. Of note, menadione continuously generates reactive oxygen species (ROS) in cells through redox cycling (Watanabe et al., 2004). Consequently, in the cell-survival assays shown in Figure 1A, even though fresh medium was added to cells after one hour of

menadione treatment, ROS can still be generated during the 24-h incubation in growth medium. Therefore, it is difficult to draw direct comparisons between menadione concentrations used in the survival assays (Figure 1A) to those used to induce CSB-chromatin co-fractionation (Figure 1B and C).

To determine the genomic localization of CSB upon oxidative stress, we performed CSB-ChIP-seq from cells treated with 100 μM menadione for 1 h. The resulting sequencing reads were mapped to the human genome, and peaks were identified using HOMER with a default option on ChIPed samples against matching input samples (Heinz et al., 2010). In total, we recovered 19 063 CSB peaks in cells treated with menadione (Figure 2A, Supplementary Table S1).

We subsequently compared CSB occupancy in cells with or without menadione treatment. To do this, we compared signal intensities over a 200-bp region in cells treated with menadione to that in cells without treatment (Lake et al., 2014). If the difference between signal intensities was 4-fold or greater and the *P*-value for that difference was <0.0001, the signal was classified as menadione induced or repressed (blue or green, respectively, Figure 2A); the remaining signals were classified as common (red, Figure 2A). Among them, we identified 7070 CSB-occupancy sites induced by menadione treatment and 9163 CSB-occupancy sites repressed by menadione treatment, corresponding to ~40% of total CSB-binding sites in each of the growth conditions (Figure 2A and Supplementary Tables S2–S4).

ChIP-qPCR was used to validate the ChIP-seq results at seven regions (Figure 2B and C). ChrX-1, chr17-1, chr19-2 and chrX-2 represent regions of menadione-induced CSB occupancy, and Chr12-7, chr2-2 and chr7-1 represent regions that are occupied by CSB but unaffected by menadione treatment (common). ChIP-qPCR confirmed that the occupancy of CSB at chrX-1, chr17-1, chr19-2 and chrX-2 was induced by menadione treatment: the increase in CSB enrichment at these sites in response to menadione was >4-fold, with *P*-value <0.01 (Figure 2C). CSB occupancy at chr12-7 was unaffected by menadione. Chr2-2 and chr7-1 were also occupied in both growth conditions, albeit with a slight decrease after menadione treatment. This slight reduction was significant, as the *P*-value was <0.01; however, these occupancy sites were

considered common, as the signal intensities from the ChIP-seq results were within a 4-fold difference (Figure 2A and C).

2.5.2. Promoter occupancy by CSB is increased upon oxidative stress

We then classified the CSB occupancy sites into seven functional categories, using the UCSC RefSeq gene annotations (Figure 2D-F). Previously, we found a modest but significant enrichment of CSB at promoter regions in unchallenged cells (Lake et al., 2014). Interestingly, upon menadione treatment, we observed further enrichment of CSB at promoters: from 2% of total CSB binding sites locating at promoter regions in untreated cells to 11% in menadione treated cells (*P*-value <1e−310 using Bernoulli's test) (Supplementary Figure S1A). Among CSB occupancy sites induced by a 1-h, 100 μM menadione treatment, 18% of them were located at promoters while the genomic distribution of promoters is only 1%. The fraction of promoter-occupied sites dropped to 5% among the 'common' peaks and 1% among the 'repressed' peaks (Figure 2D and E and Supplementary Tables S2–S4). These observations support a role of CSB in transcription regulation upon oxidative stress.

To gain insight into the molecular functions of genes that lie close to CSB occupancy sites, we searched for overlaps with the Molecular Signatures Pathways Database (MSigDB) using the Genomic Regions Enrichment of Annotations Tool (GREAT) (McLean et al., 2010). The top terms associated with total CSB occupancy in cells treated with menadione involve the roles of gene expression, cell cycle control, spliceosome, and protein metabolism (Supplementary Figure S1B).

We also determined cellular pathways enriched in the list of genes whose promoters are occupied by CSB upon oxidative stress, using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang da et al., 2009a; Huang da et al., 2009b). The top five KEGG pathways enriched are listed in Supplementary Table S5; they are proteasome, spliceosome, RNA degradation, oxidative phosphorylation and Alzheimer's disease.

2.5.3. CSB is enriched at CTCF-binding sites upon oxidative stress

To better understand the mechanisms that regulate CSB occupancy at specific genomic regions upon oxidative stress, we used HOMER to identify DNA-binding motifs enriched at CSB occupancy sites (Heinz et al., 2010). As previously reported, CSB was found to occupy c-Jun/AP1-binding sites (Lake et al., 2014); however, the percentage of CSB-occupied c-Jun/AP1-binding motifs did not change in response to oxidative stress (Supplementary Table S6). Strikingly, CTCF-binding motifs became substantially enriched upon oxidative stress (Figure 3A). In unstressed cells, only 1% of the CSB-occupancy sites contained a CTCF-binding motif, similar to that of the genomic distribution (i.e. background) (Figure 3A). On the other hand, in stressed cells, 8.3% of the CSB-occupancy sites contained CTCF-binding motifs, which is about eight-fold over the background (Figure 3A). Additionally, CTCF-binding motifs are present in 11% of the menadione-induced CSB-occupancy sites (Figure 3A). This observation suggests that CTCF may function with CSB in response to oxidative stress.

To test the hypothesis that CTCF collaborates with CSB to protect cells from oxidative stress, we first determined if cells with decreased CTCF protein levels were more sensitive to ROS. As shown Supplementary Figure S2, cells expressing CTCF shRNA had an approximately 70% reduction in CTCF protein levels as compared to cells expressing a control shRNA. Furthermore, these cells displayed a slight increase in menadione sensitivity as compared to cells expressing a control shRNA (as a paired *t*-test on CSB^{wt} cells with and without CTCF shRNA expression had a *P*-value of 0.08 at 20 μM menadione) (Figure 3B). These results suggest a potential function of CTCF in protecting cells from oxidative stress. Of note, decreasing CTCF levels to 20% in the CSB-null cell line did not further increase ROS sensitivity.

2.5.4. CTCF regulates a subset of CSB occupancy sites upon oxidative stress

To test the hypothesis that CTCF can alter the genomic occupancy of CSB upon oxidative stress, we selected six sites from our CSB ChIP-seq data that displayed menadione-induced CSB occupancy (Figures 2A and 3D); chr5-5, chr12-34, and chr17-4 contained CTCF-

binding motifs while chrX-2, chr17-1, and chrX-1 did not contain CTCF-binding motifs. The chr3-t locus was chosen as a control for ChIP efficiency, since CSB occupancy at this site did not change upon oxidative stress (Figure 3D). ChIP-qPCR confirmed increased CSB occupancy at these six sites in cells treated with menadione as compared to untreated cells.

To test if CTCF contributed to CSB occupancy at these sites, we performed CSB ChIP-qPCR with cells expressing CTCF shRNA (Figure 3C and D). We first confirmed that cells expressing control shRNA and CTCF shRNA had the same amounts of CSB (Supplemental Figure S3). We found that reducing the CTCF protein level by ~65% selectively reduced CSB occupancy at these sites in menadione treated cells (Figure 3D), indicating that CTCF positively regulates CSB occupancy at these sites upon menadione treatment. Significantly, CTCF knockdown did not decrease basal CSB occupancy. These results together demonstrate that, in response to oxidative stress, CTCF not only regulates CSB occupancy at sites containing CTCF-binding motifs but also at sites devoid of CTCF-binding motifs. Moreover, these results suggest that CTCF may directly interact with CSB.

2.5.5. Oxidative stress enhances CBS and CTCF interaction

To learn more about how CTCF regulates CSB occupancy upon oxidative stress, we determined if CTCF interacts with CSB by co-immunoprecipitation, using lysates prepared from 293T cells expressing a Flag-tagged CTCF protein. As shown in Figure 4A, Flag-CTCF co-immunoprecipitated with CSB, and this interaction increased by about 4-fold after treatment with 100 μM menadione for 1 h. Notably, similar amounts of Flag-CTCF were used in the immunoprecipitation experiments, yet anti-Flag antibodies precipitated less Flag-CTCF from menadione treated cells, suggesting that some of the Flag epitope was occluded in these cells. However, more CSB co-purified with Flag-CTCF in cells treated with menadione, indicating that oxidative stress increases CSB–CTCF interactions in cells.

2.5.6. CSB directly interacts with CTCF *in vitro*

To determine if CSB and CTCF interact directly, we expressed and purified Flag-tagged CSB and a maltose-binding protein-tagged CTCF derivative (MBP-CTCF), containing the central 11 zinc fingers (aa 269–579) (Figure 4B) (Plasschaert et al., 2014). As revealed by Coomassie staining, MBP-CTCF directly bound to CSB, while MBP alone did not (Figure 4C, compare lane 6 to 9), indicating that the 11 Zn-fingers of CTCF is sufficient for CSB interaction *in vitro*. Additionally, the C-terminal 483 amino acids of CSB, which lie outside the central catalytic domain, were sufficient for CTCF binding (compare lane 8 to 11). No interaction was detected between the zinc-fingers of CTCF and the first 507 amino acids of CSB (compare lane 7 to 10). Nonetheless, it was previously demonstrated that an endogenously generated CSB-fusion protein, composed of the N-terminal region of CSB fused to a PiggyBac transposase (CSB-PGBD3), was enriched at sites containing CTCF-binding motifs during replicative cell growth (Gray et al., 2012). Taken together, these observations suggest that CSB and CTCF directly interact and that the interface between these two proteins is multivalent (see discussion) (Gray et al., 2012).

2.5.7. CSB positively regulates CTCF-DNA interactions *in vitro*

Given that CSB binds DNA in a sequence-independent manner and CSB directly interacts with CTCF, it is formally possible that CSB may also regulate the interaction of CTCF with DNA (Cho et al., 2013). To test this hypothesis, we used a 200-bp DNA fragment that contains one perfect, core CTCF-binding site in our *in vitro* protein-DNA binding assays (Figure 4D and E and Supplementary Figure S4B). By incubating MBP-CTCF with radiolabeled DNA, we obtained distinct MBP-CTCF•DNA complexes using electrophoretic mobility shift assays (Figure 4D, lanes 6–9). Distinct complexes were not observed with MBP alone (Figure 4D, lanes 1–4), indicating the protein-DNA complexes in lanes 7–9 were mediated through CTCF.

We next investigated the effect of CSB on the CTCF–DNA interaction. In the presence of 6 nM MBP-CTCF and 1 nM DNA, no clear MBP-CTCF•DNA complex was observed (Figure 4E, lane 1). However, when we included increasing amounts of CSB into the reactions, increasing

amounts of DNA-protein complexes were detected (Figure 4E, lanes 2–5). Two observations indicate these DNA-protein complexes contain MBP-CTCF: (i) an anti-MBP antibody can recognize the two bands marked by '•' and '••' (Supplementary Figure S4A), and (ii) given that CSB binds DNA in a sequence-independent manner, DNA-protein complexes containing only CSB would resolve as smears in a native polyacrylamide gel (Figure 4E, lanes 7–10) (Lake & Fan, 2013). It is not yet clear why we observed two prominent bands, marked by one and two dots, in the mobility shift assays (Figure 4E). Each of these bands contain the CTCF protein, as they can be super-shifted by and anti-CTCF antibody (Supplementary Figure S4A). Given that two bands appeared in the absence of the CSB protein (Figure 4D), it is unlikely that one of these bands in Figure 4E represents a trimeric CSB–CTCF–DNA complex. Possible explanations for their origin could be different CTCF–DNA stoichiometries, resulting from the binding of a second CTCF protein to an imperfect CTCF-binding site imbedded in the DNA fragment, or different CTCF–DNA conformations, resulting from additional CTCF–DNA contacts that might occur outside of the consensus-binding site. Nonetheless, these results indicate that CSB facilitates the interaction of CTCF with DNA.

2.5.8. CSB augments CTCF-chromatin interactions in cells

We next used ChIP-qPCR to determine if CSB can regulate the interaction of CTCF with chromatin in cells. We randomly selected six CSB occupied sites that contained CTCF-binding motifs and displayed increased CSB occupancy upon oxidative stress, based on our CSB ChIP-seq data. For these assays, the myc promoter, a known CTCF target, was used to control for CTCF-ChIP efficiency. ChIP assays were performed in CS1AN-sv cells and CS1AN-sv cells reconstituted with CSB^{WT}. As shown in Figure 5A, we observed a significant increase in CTCF occupancy at chr20-50, chr5-5, chr12-34, chr2-9, chr12-8, and chr17-4 in a menadione-dependent manner in CSB^{WT}cells. Strikingly, in cells without CSB (CS1AN-sv cells), we did not observe significant menadione-dependent changes in CTCF occupancy at these six sites. No changes were observed for CTCF occupancy at the myc promoter.

2.6. Discussion

Previously, we found that CSB is enriched at genomic regions containing epigenetic features of enhancers and promoters during replicative cell growth (Lake et al., 2014). We also found that CSB can alter chromatin structure near its occupancy site to regulate transcription (Lake et al., 2014). In this study, we found a dramatic increase in CSB occupancy at promoters upon oxidative stress: about 20% of the CSB occupancy sites that are induced in oxidatively stressed cells lie in promoter regions as compared to the genomic distribution of promoters, which is only 1%, supporting a function of CSB in transcription regulation upon oxidative stress (Figure 2). The top terms associated with total sites of CSB occupancy in cells treated with menadione involve the roles of gene expression, cell cycle control, spliceosome, and protein metabolism (Supplementary Figure S1B), suggesting that CSB might play a general role in regulating RNA and protein homeostasis as well as cell division in response to oxidative stress. Pathway analysis of the genes with their promoter regions occupied by CSB upon oxidative stress suggests that CSB might also control energy and ROS production by regulating the oxidative phosphorylation machinery at the transcriptional level (Supplementary Table S5). Indeed, defects in mitochondrial function have been associated with cells lacking functional CSB (Scheibye-Knudsen et al., 2013). Of note, we currently cannot exclude the possibility that 100 μM menadione might induce a CSB response that is not only related to the relief of oxidative stress but also to those related to cell death, resulting from excessive oxidative stress. Future ChIP-seq studies examining CSB occupancy in response to different menadione doses will help to distinguish between these different CSB functions.

CSB has been suggested to participate in the repair of oxidized bases (Khobta & Epe, 2013; Menoni et al., 2012; Stevnsner et al., 2008; Wong et al., 2007), and this study does not exclude this possibility. Oxidized DNA lesions would be, to a large degree, randomly distributed throughout the genome, and the association of CSB with oxidized DNA would, therefore, not resolve as defined anti-CSB ChIP-seq peaks.

We have shown that CTCF directly interacts with CSB and impacts CSB occupancy at specific genomic regions upon oxidative stress (Figure 3), revealing a novel mechanism by which the activity of this chromatin remodeler can be regulated. Although we have identified sites which contain the CTCF-binding motif, and to which CSB demonstrated CTCF-dependent occupancy (chr5-5, chr12-34 and chr17-4, Figure 3D), the level of CSB occupancy at these sites is 5–15-fold less than that of CSB at the chrX-2, chr17-1 and chrX-1 loci, which do not contain a CTCF-binding motif. These observations suggest that CSB is recruited to the latter sites through another mechanism. Remarkably, we also observed CTCF-dependent CSB enrichment at sites without CTCF-binding motifs, such as chrX-2, chr17-1 and chrX-1, suggesting that CTCF may stabilize CSB occupancy at these sites. Taken together, the physical and functional interaction between CSB and CTCF that is greatly enhanced upon oxidative stress may have the potential to establish DNA loops to regulate gene expression in response to oxidative stress (Figure 5B).

Another transcript originating from the CSB locus generates a protein composed of the N-terminal 465 residues of CSB fused to a piggyBac transposase (CSB-PGBD3) (Newman et al., 2008). Strikingly, CSB-PGBD3 was found enriched at sites containing CTCF-binding motifs (Gray et al., 2012). However, the association of CSB-PGBD3 with CTCF-binding sites is different from that of CSB, as it occurs in the absence of oxidative stress (Gray et al., 2012). The results of that study suggested that the N-terminal region of CSB could interact with CTCF and that CTCF and CSB-PGBD3 may play roles in chromosomal looping during replicative cell growth. Given that we did not see a direct interaction between the N-terminal 507 residues of CSB and the 11 Zn-fingers of CTCF (Figure 4), these results suggest that CSB-N likely interacts with full-length CTCF or a region that flanks the central CTCF Zn-finger domain. During replicative cell growth, the N-terminal region of CSB occludes a chromatin interaction surface in the C-terminal region (Lake et al., 2010). This occlusion might, in part, explain why CSB-PGBD3 association with CTCF-binding motifs occurs in the absence of stress, while the association of CSB with CTCF-binding motifs preferentially occurs upon oxidative stress.

Collectively, these observations suggest that CSB and CTCF have at least two regions of contact. Therefore, long-range chromosomal interactions that might be mediated by CSB and CTCF (Figure 5B) may be asymmetric, with the central CTCF Zn-finger domain binding to a CTCF motif at one end, CSB binding to a regulatory site at the other end, and a strong protein bridge between the ends mediated by two interactions: one between the CTCF zinc finger domain and the CSB C-terminal region, and the other between part or all of CTCF and the CSB N-terminal region. Moreover, such looping would be further reinforced as CSB and CTCF can, reciprocally, stabilize each other's binding to DNA: CSB can enhance CTCF binding to a CTCF motif *in vitro* (Figure 4D and E), menadione strongly induces the CSB-CTCF interaction in cells (Figure 4A), and even a modest CTCF knockdown (~65%) can reduce menadione-inducible CSB binding to sites that lack a CTCF motif (Figure 3D).

The basis for the oxidative stress-enhanced CSB-CTCF interaction remains to be determined. Change in post-translational modification is one possibility, as stress-associated changes have been observed for both CSB and CTCF (Imam et al., 2007; Thorslund et al., 2005; J. Wang et al., 2012). For example, upon oxidative stress, CSB has been suggested to be poly(ADP-ribosyl)ated and phosphorylated, and CTCF is found to be de-sumoylated.

The only other chromatin remodeler that has been shown to interact with CTCF is the chromodomain–helicase–DNA-binding protein 8 (CHD8), and this interaction is critical for CTCF-dependent insulator function (J. Wang et al., 2012). Intriguingly, CHD8 also associates with the zinc-fingers of CTCF, as do several other proteins (e.g. Sin3A and YB-1), indicating that the zinc-finger region of CTCF can support both protein–DNA and protein–protein interactions (Chernukhin et al., 2000; Lutz et al., 2000; J. Wang et al., 2012). Although all 11 zinc-fingers of CTCF could, in principle, associate with DNA, a CTCF–DNA association requires only a subset of zinc-fingers (Filippova et al., 1996; Guo et al., 2015). Therefore, some of the CTCF–zinc-fingers may be free to interact with proteins, such as CSB. Indeed, the consensus CTCF sequence that was recovered from our ChIP-seq analysis contained only the core CTCF-binding site. Nonetheless, whether or not a specific CTCF-binding site in the genome can allow for both

CTCF–DNA and CTCF–CSB interactions may depend upon the number of DNA–CTCF Zn-finger contacts made at that site (Guo et al., 2015), as well as the relative affinities of specific CTCF Zn-fingers for DNA versus CSB.

CTCF plays a fundamental role in organizing long-range chromatin structure (Ong & Corces, 2014). Chromosome conformation capture-based studies have revealed that chromatin fibers can be organized into different topologically associating domains, termed TADs. Strikingly, ~85% of CTCF occupancy sites lie within TADs and ~15% lie at TAD borders. CTCF has long been known to function in transcriptional activation as well as repression. One current model to explain the multiple CTCF functions is that CTCF promotes interactions between transcription regulatory elements within a TAD and precludes interactions between regulatory elements of different TAD (Ong & Corces, 2014). More recently, Li *et al*. have shown that the heat shock response can cause widespread rearrangement of 3D chromatin organization and lower the CTCF occupancy at the boundaries of TADs, leading to a decrease in intra-TAD interactions and an increase in new inter-TAD interactions (L. Li et al., 2015).

It remains to be determined how CSB associates with only a subset of CTCF binding sites. Regardless of the mechanism that imparts CTCF-binding site specificity, our observation that CSB can regulate CTCF-DNA interactions *in vitro* and in cells supports a hypothesis that CSB and CTCF can reciprocally regulate each other's interactions with chromatin, leading to the establishment of new, long-range chromosome associations upon oxidative stress. It is clear that modulating the association of CTCF with chromatin can have profound impacts on chromatin organization, which in turn can influence fundamental processes, such as transcription. Accordingly, we would like to speculate that the ATP-dependent chromatin remodeler CSB cooperates with CTCF to protect cells from oxidative stress by regulating long-range chromosomal interactions. Future experiments using chromatin conformation capture-based approaches will offer more insights into the functions of the CSB-CTCF collaboration during oxidative stress.

2.7. Figures

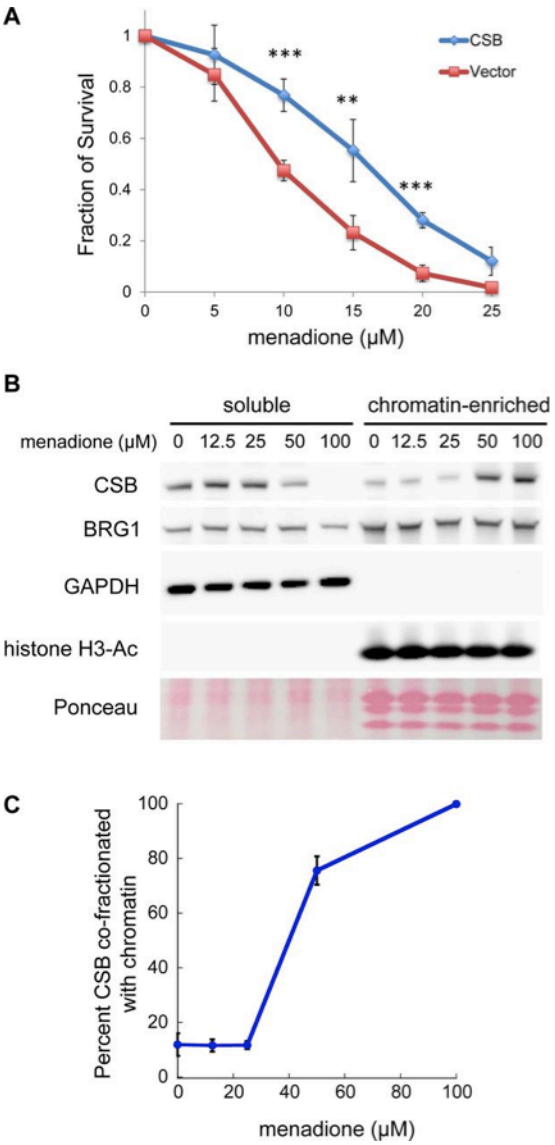

Figure 1. Menadione sensitivity assays. (**A**) CS1AN-sv cells were reconstituted with CSB^{WT} or an empty vector. Stable cell lines expressing transgenes were assayed for viability 24 h after a 1-h menadione treatment with the indicated menadione concentrations. Shown are means ± standard errors of the mean (SEM) (*n* = 5). A paired *t*-test was used to determine if the difference in menadione sensitivity of CS1AN cells before and after CSB add-back was significant. Triple asterisks indicate *P* values < 0.001, and double asterisks indicate *P* values < 0.01. (**B**) Analysis of CSB partitioning in cells after a 1-h menadione treatment, with menadione concentrations as indicated. Western blots were probed with antibodies as noted. BRG1 was used as a loading control. GAPDH and acetylated histone H3 were used as markers for soluble and chromatin-enriched fractions, respectively. Total core histones were visualized by Ponceau S staining. (**C**) Quantification of CSB levels in the soluble versus chromatin-enriched fraction. Shown are means ± SEM (*n* = 4).

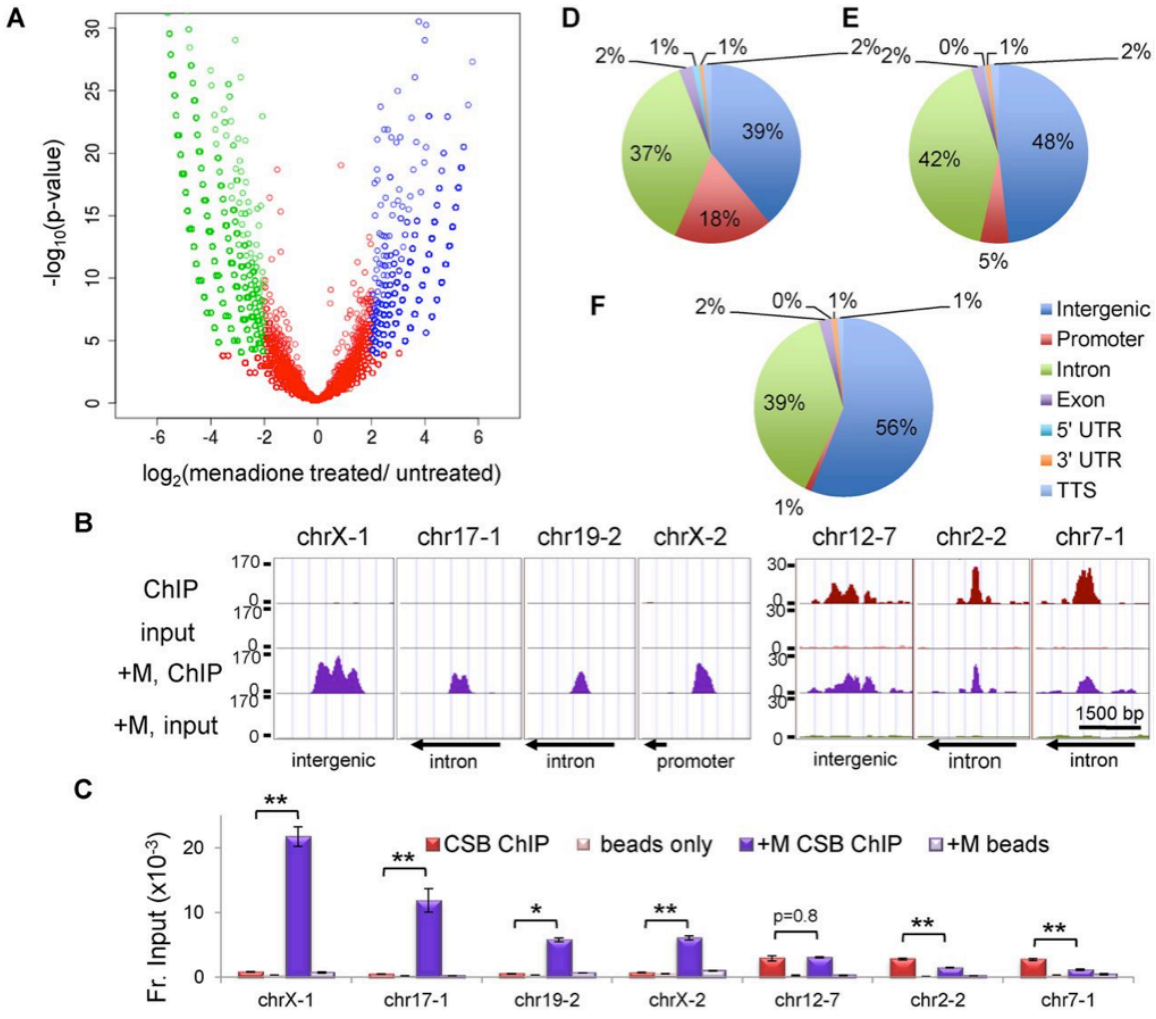

Figure 2. Comparison of CSB occupancy in cells with or without menadione treatment. (**A**) A volcano plot showing the correlation between CSB ChIP-seq results from cells with or without a 1-h menadione treatment (100 μM). (**B**) Screen shots of CSB ChIP-seq results from seven genomic regions, displayed using the UCSC genome browser. The y-axis is number of normalized sequencing reads. The x-axis represents the genomic coordinates; chrX-1, chrX:73766518–73766600; chr17-1, chr17:49770395–49770537; chr19-2, chr19:45793789–45793877; chrX-2, chrX:48568220–48568299, chr12-7, chr12:13679173–13679256; chr2-2, chr2:180325437–180325517; chr7-1, chr7:2001695–2001793. The directions of nearby transcription (arrows) are noted at the bottom. (**C**) Validation of CSB ChIP-seq results by ChIP-qPCR. Bar graphs showing CSB ChIP-qPCR results with matched beads-only controls. Shown are means ± SEM (*n* = 3). A paired t-test was used to determine if the difference in CSB enrichment before and after menadione treatment was significant. Single asterisks indicate *P* values < 0.05, and double asterisks indicate *P* values < 0.01. (**D**–**F**) Genomic distribution of CSB occupancy sites. The genome was divided into seven categories, as defined by the UCSC RefSeq gene annotation. (D) Menadione-induced CSB occupancy. (E) Common CSB occupancy. (F) Menadione-repressed CSB occupancy.

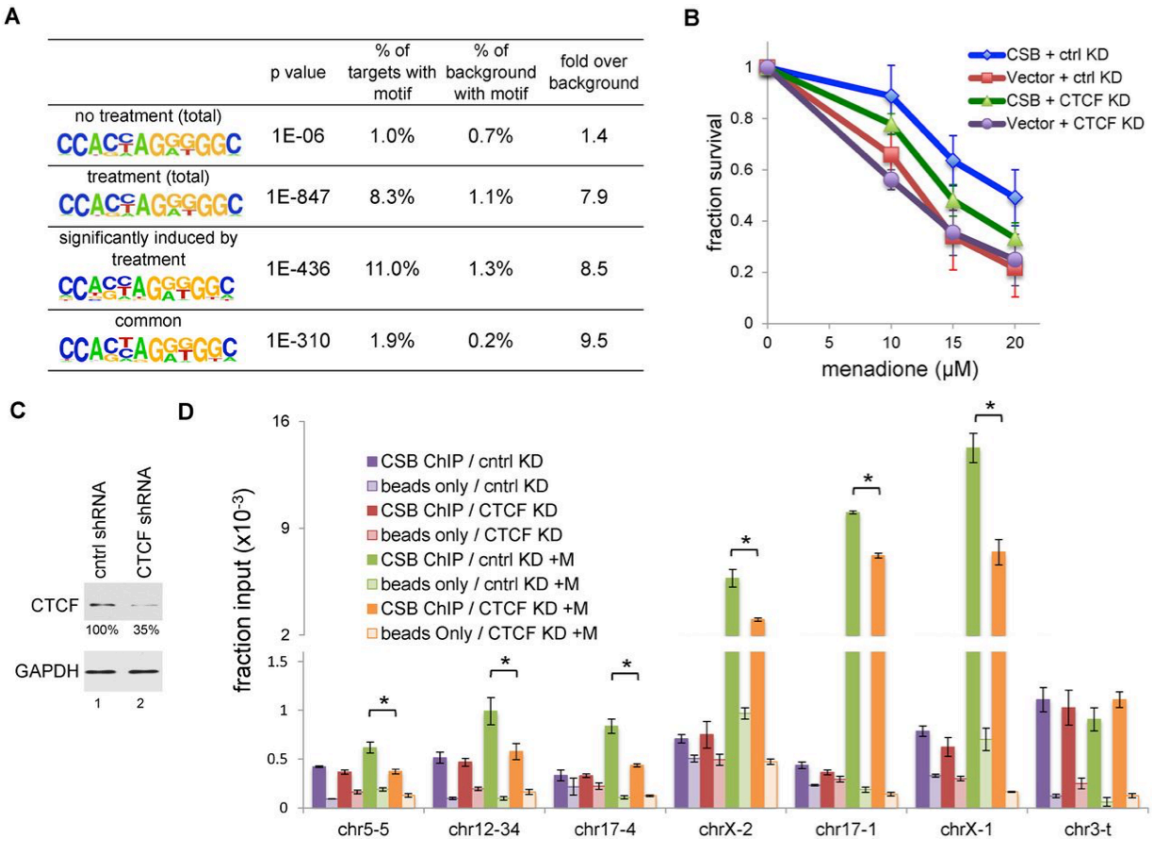

Figure 3. CTCF collaborates with CSB in response to oxidative stress. (**A**) Motif analysis of CSB ChIP-seq data. (**B**) Menadione sensitivity assays on CSB expressing and non-expressing (Vector) cells with decreased CTCF levels. Shown are means ± SEM (*n* = 4). (**C**) Western blot showing a reduction in the CTCF protein level in cells expressing CTCF shRNA. Relative CTCF levels are shown below the CTCF blot. (**D**) CSB ChIP-qPCR assays in cells infected with lentivirus expressing control or CTCF shRNA, with or without with a 1-h menadione treatment (100 μM). Shown are means ± SEM (*n* = 3). A paired *t*-test was used to determine if the difference in CSB enrichment with and without CTCF shRNA treatment was significant. Asterisks indicate *P*-values < 0.05.

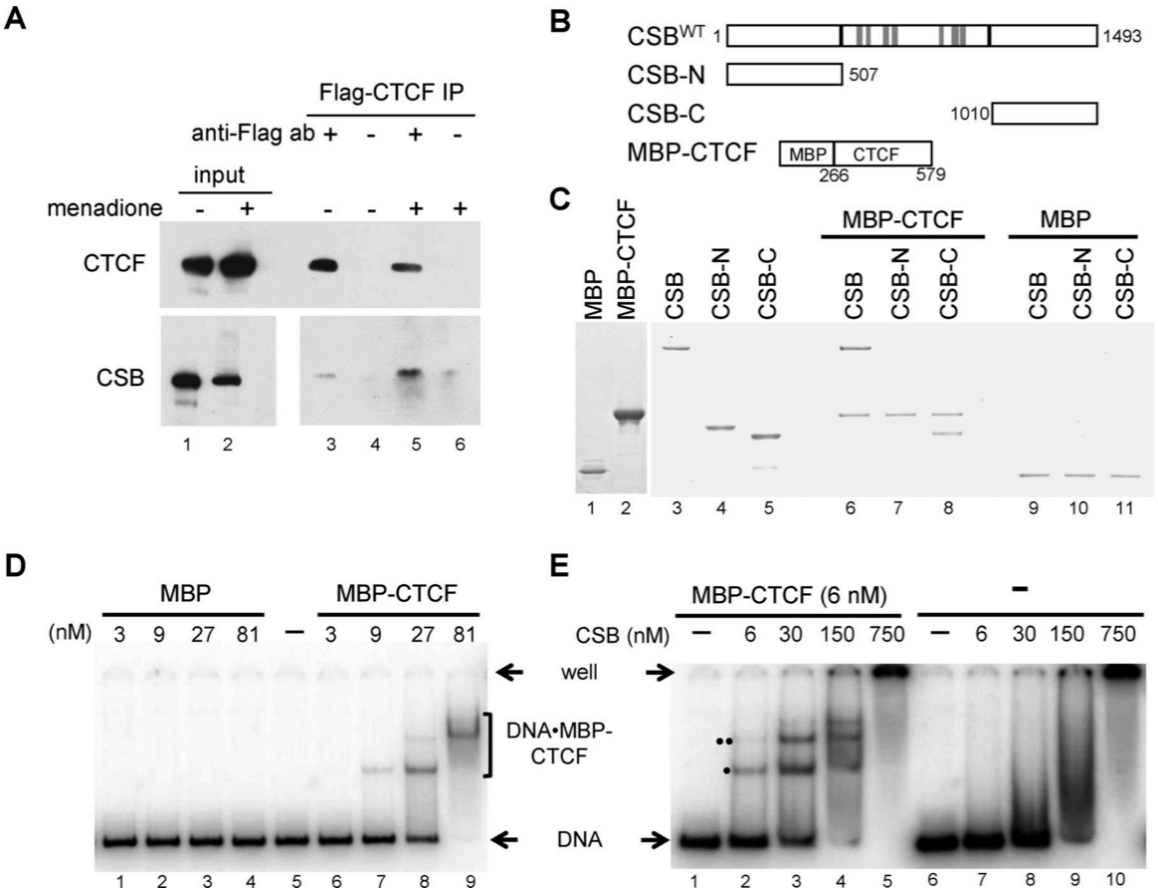

Figure 4. CSB interacts with CTCF in cells and *in vitro*. (A) Co-immunoprecipitation of CSB and CTCF in 293T transiently transfected with Flag-tagged CTCF, with or without a 1-h treatment of 100 μM menadione. 3.3% of the lysates used for IP were loaded as input. (B) Schematics of recombinant proteins used in (C–E). All CSB derivatives were N-terminally tagged with the Flag epitope. (C) Coomassie-stained gel showing that CSB directly interacts with CTCF. CSB-C, but not CSB-N, is sufficient for the CTCF association. MBP was used as a negative control. (D and E) EMSA assays showing that CSB enhances CTCF association with DNA. (D) Varying amounts of purified MBP-CTCF (lane 2 in C) or MBP (lane 1 in C) were incubated with a ^{32}P-labeled, 200 bp DNA fragment containing a CTCF-binding motif (Supplementary Figure S4B). Protein–DNA complexes were resolved in a native 5% polyacrylamide gel. (E) Varying amounts of purified CSB were incubated with the radiolabeled DNA fragment in the presence or absence of MBP-CTCF. Reactions were subsequently resolved in a 5% native polyacrylamide gel. Protein–DNA complexes marked by '•' and '••' contain the MBP-CTCF protein, as they interacted with an anti-MBP antibody (Supplementary Figure S4A).

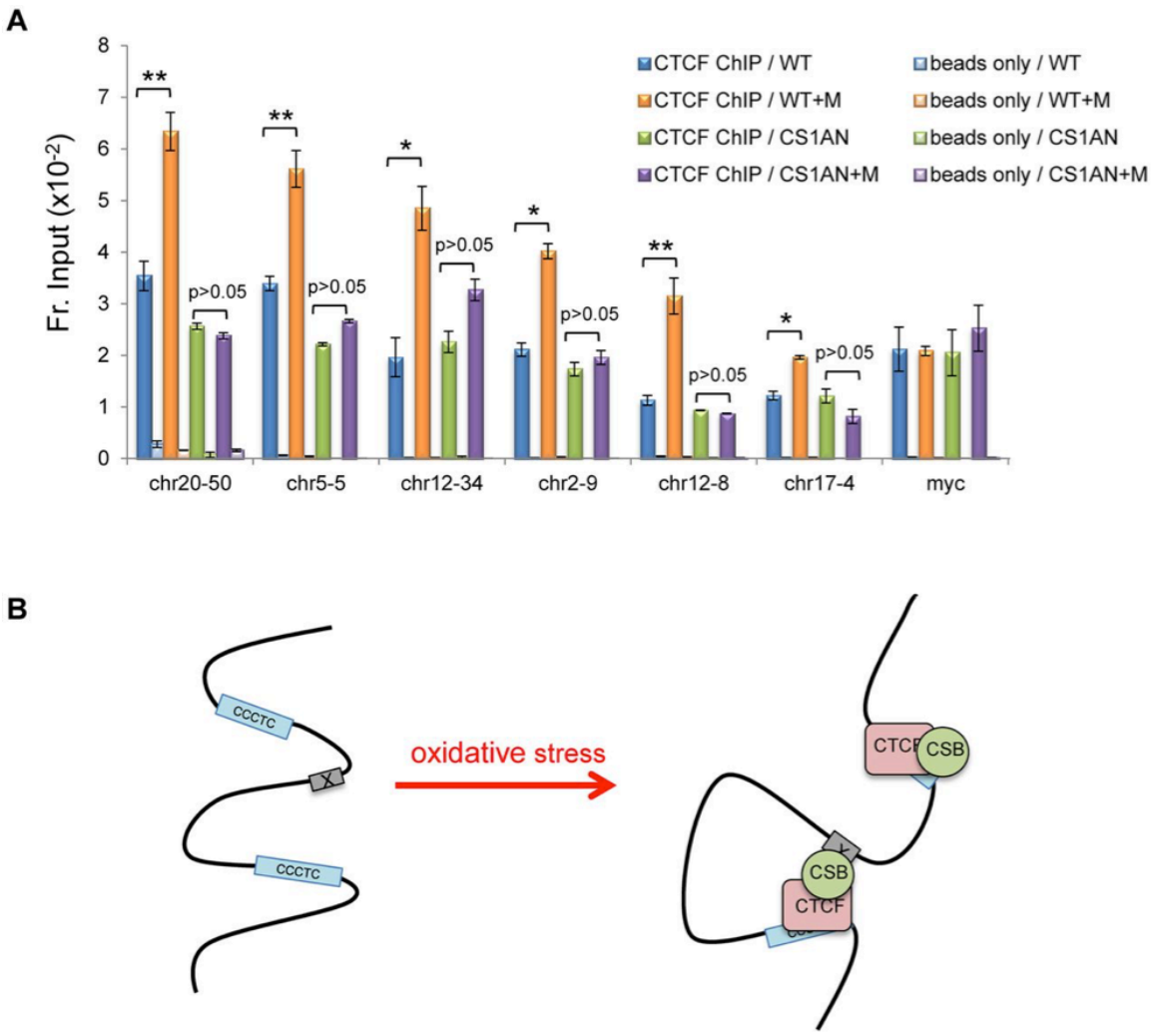

Figure 5. CSB regulates a subset of CTCF occupancy sites upon oxidative stress. (**A**) CTCF ChIP-qPCR assays in CSB expressing (WT) and non-expressing (CS1AN) cells, with or without a 1-h menadione treatment (100 μM). Shown are means ± SEM (*n* = 3). A paired *t*-test was used to determine if the difference in CTCF enrichment before and after menadione treatment was significant. Single asterisks indicate *P* values < 0.05, and double asterisks indicate *P* values < 0.01, as determined by a paired *t*-test. (**B**) Model depicting possible modes of CSB–CTCF chromatin association in response to oxidative stress. CTCF can recruit CSB to CTCF-binding sites or enhance the association of CSB with distal sites. See text for details.

2.8. Supplementary Data

Supplementary data is available at *Nucleic Acids Research* online (Lake et al., 2016).

CHAPTER 3: PARP1 FACILITATES CHROMATIN ASSOCIATION OF COCKAYNE SYNDROME GROUP B PROTEIN DURING OXIDATIVE STRESS

3.1. Preface

The manuscript presented in this chapter has been submitted for publication and is currently under review with *The Journal of Biological Chemistry*. It has been reformatted here in accordance with the University of Pennsylvania dissertation formatting guidelines.

3.2. Abstract

Cockayne syndrome protein B (CSB), an ATP-dependent chromatin remodeler, relieves oxidative stress by regulating DNA repair and transcription. While CSB is proposed to participate in base excision repair (BER), the primary DNA repair pathway for oxidative DNA damage, exactly how CSB participates in this process is unknown. Additionally, it is unclear whether CSB participates in other repair pathways during oxidative stress. Here we address these questions by examining how CSB is targeted to chromatin in response to oxidative stress created by menadione treatment, both globally and locus specifically. We find menadione-induced global CSB-chromatin association is mechanistically distinct from UV-induced CSB-chromatin association, in that its ATPase activity is dispensable. We demonstrate PARP1 enhances the kinetics of global CSB-chromatin association induced by menadione treatment, and we find the major BER enzymes, OGG1 and APE1, have no influence on this association. We do not see an increase in γ-H2AX, a marker for double-strand DNA breaks, in menadione-treated cells and, therefore, our results support a model whereby PARP1 localizes to single-strand breaks and recruits CSB to participate in DNA repair. Furthermore, we find that this global CSB-chromatin association occurs independently of transcription elongation by RNA polymerase II. However, in contrast to global chromatin association, we show that both PARP1 knockdown and transcription elongation inhibition interfere with the recruitment of CSB to specific genomic regions upon

menadione treatment. This latter observation supports the hypothesis that CSB is also targeted to specific genomic loci to participate in transcription regulation in response to oxidative stress.

3.3. Introduction

Cockayne syndrome is a devastating recessive disorder characterized by features of premature aging, extreme sun sensitivity, and neurological and developmental abnormalities (Lehmann, 1982; Nance & Berry, 1992). The majority of Cockayne syndrome cases are the result of mutations within the gene encoding Cockayne syndrome protein B (CSB), an ATP-dependent chromatin remodeler (Citterio et al., 2000; Eisen et al., 1995; Troelstra et al., 1992). CSB plays a role in transcription regulation (Balajee et al., 1997; Lake et al., 2014; Newman et al., 2006; Selby & Sancar, 1997a; Tantin et al., 1997; van Gool et al., 1997) and is essential for transcription-coupled nucleotide excision repair (TC-NER) (Bohr et al., 1985; Hanawalt & Spivak, 2008; Mayne & Lehmann, 1982; Mellon et al., 1987; Troelstra et al., 1990; Troelstra et al., 1992; Venema et al., 1990). CSB also contributes to the relief of oxidative stress by regulating DNA repair as well transcription (Khobta & Epe, 2013; Kyng et al., 2003; Lake et al., 2016); however, mechanisms underlying these activities remain elusive. Cells deficient in CSB show increased sensitivity to oxidizing agents (Lake et al., 2016; Pascucci et al., 2012; Tuo et al., 2001), accumulate oxidative DNA damage (Muftuoglu et al., 2009; Tuo et al., 2001) and display increased levels of intracellular reactive oxygen species (ROS) (Pascucci et al., 2012).

The major repair pathway for oxidative DNA damage is base excision repair (BER) (G. L. Dianov & Hubscher, 2013). BER is initiated by a substrate-specific DNA glycosylase that removes the oxidized base. This is followed by cleavage of the sugar-phosphate backbone and excision of the remaining apurinic-apyrimidinic site (AP site) by apurinic-apyrimidinic endonuclease 1 (APE1), or in some cases glycosylases with inherent endonuclease activity. The resulting nicked DNA is recognized by and activates poly(ADP-ribose) polymerase 1 (PARP1), which uses NAD+ to catalyze the addition of poly(ADP-ribose) (PAR) polymers to itself as well as other proteins. PARP1 is hypothesized to recruit proteins important for DNA repair, such as the scaffold protein

XRCC1. PARP1 may also serve to stabilize nicked DNA, preventing degradation of single strand breaks into double strand breaks (Abbotts & Wilson, 2017; G. L. Dianov & Hubscher, 2013; El-Khamisy et al., 2003; Parsons et al., 2005; Satoh & Lindahl, 1992). The remaining gap is filled by DNA polymerase β and ligation is performed by DNA ligase IIIα (Lig3). An alternative pathway, long-patch BER, is initiated by blocked 5'-ends during nick repair.

Evidence for a role of CSB in BER has been provided by several groups, which report that cellular extracts from CSB null cells demonstrate reduced incision activity of oxidative DNA lesions *in vitro* (G. Dianov et al., 1999; Khobta & Epe, 2013; Tuo et al., 2001; Tuo et al., 2002; Tuo et al., 2003). Recent findings by Menoni et al. (2012) provide support for the notion that CSB functions in the repair of oxidized DNA, by demonstrating that CSB accumulates at sites of locally induced oxidative DNA damage in cells (Menoni et al., 2012). CSB has also been shown to physically and functionally interact with several key BER proteins, such as OGG1 and APE1 (Tuo et al., 2002; Wong et al., 2007). CSB has also been found to associate with PARP1 and PARP1 has been shown to poly(ADP-ribosyl)ate CSB (Thorslund et al., 2005). Recently, Scheibye-Knudsen et al. (2014) demonstrated PARylated PARP1 is required for retaining CSB at sites of oxidative DNA damage and hypothesized that CSB participates in PARP1 displacement from damaged DNA to facilitate repair (Scheibye-Knudsen et al., 2014).

Under replicative cell growth conditions, CSB interacts with chromatin very dynamically, and only 10% of CSB stably associates with chromatin (Lake et al., 2010). In response to UV DNA damage, where CSB is employed for TC-NER, the situation is reversed, and 90% of CSB becomes associated with chromatin while 10% of CSB remains soluble. Recently, we demonstrated that oxidative stress also stabilizes the association of CSB with chromatin on a global level (Lake et al., 2016). In addition, we found that oxidative stress induces the occupancy of CSB at specific genomic loci, including loci containing the binding motif for the chromatin architectural protein CCCTC-binding transcription factor (CTCF) (Lake et al., 2016). Importantly, we found that CSB and CTCF reciprocally regulate each other's site-specific, chromatin association in response to oxidative stress and that these two proteins directly interact (Lake et

al., 2016). These observations suggest a role for CSB in regulating higher-order chromatin structure in during oxidative stress.

In this study, we further characterize the mechanisms by which CSB stably associates with chromatin, both globally and locus-specifically, in response to oxidative stress.

3.4. Results

3.4.1. Oxidative stress induces stable CSB-chromatin association

CSB interacts with chromatin dynamically. During replicative cell growth, ~10% of CSB co-fractionates with chromatin (Fig. 1A-B) (Lake et al., 2016). However, when cells are treated with menadione, which creates oxidative stress by producing reactive oxygen species (Aherne & O'Brien, 2000), a substantial increase in CSB-chromatin association is observed (Fig. 1A-B) (Lake et al., 2016). To dissect the mechanisms by which menadione induces the global association of CSB with chromatin, we used the patient derived, CSB-deficient CS1AN-sv cell line that has been stably reconstituted with wild-type CSB (CS1AN-CSB^{WT}) (Fig. S1A-B). CSB's expression level in CS1AN-CSB^{WT} cells is within two-fold of that of the human fibroblast cell line MRC5 (Fig. S1A) (Cho et al., 2013). We examined the time dependence of CSB-chromatin association in CS1AN-CSB^{WT} cells treated with 100 μM menadione and found that ~90% of CSB co-fractionates with chromatin within 30 minutes (Fig. 1A-B). As previously demonstrated, the partitioning between soluble and chromatin fractions of another ATP-dependent chromatin remodeler, BRG1, was not grossly altered by menadione treatment and, therefore, BRG1 was used as a protein loading control for normalization (Fig. 1A) (Lake et al., 2016). Acetylated histones H3 as well as Ponceau S staining of total histone proteins were used as controls to examine chromatin fractionation efficiency (Fig. 1A). Additionally, as expected, the active form of RNA polymerase II was in the chromatin fraction, while GAPDH was in the soluble fraction. The CTCF protein, which was previously shown to increase its association with CSB in response to menadione treatment (Lake et al., 2016), was chromatin-associated regardless of menadione treatment (Fig. 1A).

We next examined how two other DNA repair proteins behaved in this fractionation assay (Fig. 1A). Menadione treatment induced the chromatin association of XRCC1, a scaffolding protein involved in DNA repair (Fig. 1A-B). We found that PARP1 was present in both the soluble and chromatin fractions and its partitioning between these two fractions was not significantly changed by menadione treatment (Fig. 1A-B). In addition, we did not observe an apparent change in the levels of the classic marker for DNA double-strand breaks, γ-H2AX, after menadione treatment (Fig. 1A).

To further demonstrate that oxidative stress increases CSB-chromatin association, we performed anti-CSB chromatin immunoprecipitation (ChIP) followed by western blot analysis, using an antibody against histone H3. We found a more than five-fold increase of histone H3 co-immunoprecipitating with CSB in cells treated with menadione than in untreated cells, demonstrating that menadione treatment increases CSB's association with chromatin (Fig. 1C).

3.4.2. ATP hydrolysis by CSB is dispensable for menadione-induced chromatin association

Stable CSB-chromatin association can also be induced by UV irradiation, and this association requires ATP hydrolysis by CSB to relieve auto-repression (Lake et al., 2010). We next determined if menadione-induced stable CSB-chromatin association is also ATP-dependent. To accomplish this, we used the CSB-deficient CS1AN-sv cell line reconstituted with a CSB protein harboring a patient derived mutation, CSB^{R670W}, which is devoid of ATPase activity (Figs. 1D and S1A-B) (Lake et al., 2010). In sharp contrast to UV-induced CSB-chromatin association, menadione-induced stable association of CSB^{R670W} with chromatin was kinetically similar to CSB^{WT}. This result reveals that ATP hydrolysis by CSB is dispensable for global CSB-chromatin association in response to menadione treatment.

3.4.3. Oxidative stress-induced CSB-chromatin association is mediated through its ATPase domain and C-terminal region

To dissect further the mechanism by which CSB becomes stably associated with chromatin in response to oxidative stress, we analyzed a set of CSB deletion-derivatives (Fig. 2). All mutant proteins were stably expressed in CS1AN-sv cells and nuclear localized (Fig. S1A-B). CSBΔN, which is devoid of its N-terminal region but has intact ATPase and C-terminal domains, co-fractionates with chromatin, even in the absence of genotoxic stress (Lake et al., 2010). However, unlike UV-induced CSB-chromatin association, menadione treatment resulted in a further increase in the association of CSBΔN with chromatin (Fig. 2B). This result suggests that CSB senses oxidative stress through its ATPase and/or C-terminal domains.

Deleting the last 484 amino acids of CSB (CSBΔC) abolishes the ability of CSB to associate with chromatin in response to UV irradiation (Lake et al., 2010). In contrast, CSBΔC still responds to menadione treatment, however, the fraction of CSBΔC associating with chromatin was lower at the 20- and 30-minute time points (Fig. 2C). This observation suggests that the C-terminal region of CSB contributes to menadione-induced CSB-chromatin association. However, CSB-C alone did not bind chromatin as well as full-length CSB when cells were within the first 10 minutes of menadione treatment, suggesting that the ATPase domain contributes to menadione-induced CSB-chromatin association (Fig. 2D). The N-terminal region of CSB (CSB^{1-507}) showed little chromatin association, even after 30 minutes of menadione treatment (Fig. 2E). Together these findings support a model in which oxidative stress-induced CSB-chromatin association is mediated through the ATPase domain and the C-terminal region of CSB. Moreover, the results reveal that menadione-induced chromatin association of CSB does not rely upon ATP-dependent relief of auto-repression.

3.4.4. Menadione-induced global CSB-chromatin association does not require active transcription by RNA polymerase II

Another key factor underlying UV–induced CSB-chromatin association is active transcription. Inhibition of RNA polymerase II (RNA pol II) transcription elongation by 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) prevents stable CSB-chromatin association induced by UV irradiation (Lake et al., 2010). We, therefore, examined whether CSB-chromatin

association induced by menadione treatment also requires active RNA pol II transcription. CS1AN-CSB^{WT} cells were exposed to DRB or a DMSO control for 1 hour prior to treatment with menadione for 20 minutes. As demonstrated in Fig. 3A-B, DRB did not significantly alter the stable association of CSB with chromatin that is induced by menadione on a global level. However, as previously observed, similar DRB treatment prevented UV-induced CSB-chromatin association (Fig. 3C). This finding indicates that stable CSB-chromatin association resulting from oxidative stress is regulated by a mechanism that is distinct from UV-induced association.

3.4.5. APE1 and OGG1 are dispensable for global menadione-induced CSB-chromatin association

CSB has been suggested to relieve oxidative stress by both facilitating base-excision repair and regulating transcription of specific genes. Therefore, the menadione-induced global CSB-chromatin association would be expected to represent, to a large degree, sites of oxidized DNA. Accordingly, we used the chromatin-fractionation assay to dissect the mechanism by which menadione induces CSB-chromatin association.

As CSB directly interacts with the major apurinic/apyrimidinic endonuclease APE1 (Wong et al., 2007), we hypothesized that APE1 may recruit CSB to sites of APE1-mediated DNA strand breaks to facilitate APE1 activity in cells treated with menadione. If this hypothesis were correct, we expect to find less CSB co-fractionating with chromatin in cells with decreased APE1 levels. To test this hypothesis, we reduced the level of the APE1 protein using shRNA and determined its consequence on the amount of CSB co-fractionating with chromatin (Fig. 4A-D). As shown in Fig. 4A, we were able to reduce APE1 protein levels to less than 30%; however, we did not observe a significant change in menadione-induced CSB-chromatin association. This result suggests that APE1 is not crucial for recruiting CSB to chromatin when cells are treated with menadione (Fig. 4B-D).

OGG1, a glycosylase, initiates the base excision repair of 7,8-dihydro-8-oxoguanine (8-oxoG), the major oxidized DNA lesion. Given that CSB has been reported to be in complex with OGG1 (Tuo et al., 2002), we next tested if the global recruitment of CSB to chromatin is mediated

by OGG1. To accomplish this, we reduced OGG1 protein levels using shRNA-targeting OGG1 (Fig. 4E) and determined its impact on the levels of CSB co-fractionating with chromatin in response to menadione treatment (Fig. 4F-H). Reduction of OGG1 levels to 10% did not significantly reduce the level of CSB co-fractionating with chromatin in cells treated with menadione (Fig. 4E-H), arguing against the possibility that OGG1 is responsible for the global recruitment of CSB to chromatin when cells are treated with menadione (Fig. 4F-H). Of note, we did observe a small but significant increase in CSB-chromatin co-fractionation in OGG1 knockdown cells as compared to control cells following treatment with menadione for 30 minutes (Fig. 4H). This observation suggests that OGG1 may prevent a fraction of CSB recruitment to chromatin, either directly or indirectly, through a mechanism that awaits to be determined.

Together the findings shown in Fig. 4 argue against the possibility that APE1 or OGG1plays a major role in global recruitment of CSB to chromatin upon oxidative stress. Additionally, these results suggest the possibility that CSB may function upstream of these two proteins in base-excision DNA repair.

3.4.6. PARP1 facilitates CSB-chromatin association induced by menadione treatment

Another candidate protein for targeting CSB to chromatin in response to oxidative stress is PARP1, as it not only interacts with CSB but also poly(ADP)ribosylates CSB (Thorslund et al., 2005). Therefore, we examined CSB-chromatin association following control or PARP1 shRNA knockdown (Fig. 5). Fig. 5A is a representative western blot showing the level of PARP1 knockdown, which was routinely about 90%. We found that PARP1 knockdown significantly reduced the kinetics of CSB-chromatin association following menadione treatment (Fig. 5B-D). To confirm this finding, we repeated the experiments in control and PARP1 knockdown cells, either untreated or treated with menadione for 20 minutes (average 96% knockdown, n=11). We found a drop from 40% CSB co-fractionating with chromatin in cells treated with control shRNA to 17% in cells treated with PARP1 shRNA (Fig. S2A). A difference was observed whether or not we

used BRG1 to normalize protein levels (comparing Fig. S2A to S2B). Together, these results indicate that PARP1 enhances the kinetics of menadione-induced CSB-chromatin association.

PARP1 could facilitate menadione-induced CSB-chromatin association through its ability to directly interact with CSB. Alternatively, PARP1 might do so through its enzymatic activity. To determine the contribution of PARP1's enzymatic activity in menadione-induced CSB-chromatin association, we treated cells with the potent PARP inhibitor, KU-0058948 (Fig. 5E-H). Cells treated with KU-0058948 had less poly(ADP-ribosyl)ation activity, as demonstrated by western blot analysis using an anti-PAR antibody (Fig. 5E). We did not observe a significant change in the kinetics of CSB-chromatin association induced by menadione treatment, suggesting that PARP1 may influence CSB-chromatin recruitment through direct protein-protein interaction.

3.4.7. PARP1 facilitates the recruitment of CSB to specific genomic loci induced by menadione treatment

ChIP-seq experiments revealed that menadione treatment also increases the occupancy of CSB at specific genomic loci (Lake et al., 2016). To determine if PARP1 participates in recruiting CSB to these loci in response to oxidative stress, we used ChIP-qPCR to examine CSB occupancy at four of these sites (chrX-1, chrX-2, chr17-1, and chr19-2) in cells treated with shRNA targeting PARP1 (Fig. 6A). When the PARP1 protein level was reduced to 15% of its normal level, the occupancy of CSB at these genomic loci induced by menadione treatment was significantly reduced (Fig. 6A) (Lake et al., 2016). On the other hand, the occupancy of CSB at chr12-7, a site that is bound by CSB but unaffected by menadione treatment, was not altered by a decrease in PARP1 protein levels (Fig. 6A). These results indicate that PARP1 plays a key role in facilitating the recruitment of CSB to specific genomic loci in response to oxidative stress, in addition to playing a role in influencing the kinetics of global CSB-chromatin association following oxidative stress.

We next determined if the locus-specific CSB occupancy relies upon the enzymatic activity of PARP1. As shown in Fig. 6B, after treating cells with KU-0058948, we observed a significant decrease in CSB occupancy at chrX-1 and chrX-2, but not chr17-1 and chr19-2. These

results indicate that the enzymatic activity of PARP1 contributes to the recruitment of CSB to specific loci, but only at a subset of its occupied sites. Interestingly, we found that treating cells with the transcription elongation inhibitor DRB significantly decreased menadione-induced site-specific CSB occupancy at all four sites examined, further supporting the notion that CSB functions in transcription regulation when cells are under oxidative stress (Fig. 6C).

3.5. Discussion

In this study, we demonstrated that the global chromatin association of CSB induced by oxidative stress does not require ATP-dependent relief of auto-repression (Fig. 1) and, therefore, is distinct from the mechanism by which UV irradiation induces CSB-chromatin association for its essential function in TC-NER (Lake et al., 2010). We also showed that the ATPase domain and the C-terminal region of CSB are required for timely association of CSB with chromatin in response to oxidative stress (Fig. 2). The auto-repressive, N-terminal region of CSB (CSB-N), which is also essential for substrate discrimination during UV-induced CSB-chromatin association, is dispensable for menadione-induced CSB-chromatin association (Fig. 2). Importantly, we find that PARP1, which responds to both single-strand and double-strand DNA breaks (Ray Chaudhuri & Nussenzweig, 2017), enhances the kinetics of global CSB-chromatin association induced by oxidative stress (Fig. 5). However, we observed no significant increase in the level of γ-H2AX, a marker for DNA double-strand breaks, in cells treated for 30 minutes with menadione (Fig. 1A). Together, these results support the notion that PARP1 functions in the recruitment of CSB to single-strand DNA breaks upon oxidative stress (Fig. 7A). The majority of single-strand DNA breaks that CSB responds to are unlikely the product of base excision repair (BER), as menadione-induced global CSB-chromatin association is independent of OGG1 and APE1 (Fig. 4). These observations, therefore, suggest that PARP1 may enhance the recruitment of CSB to sites of single-strand DNA breaks directly generated by reactive oxygen species through menadione treatment (Fig. 7A) (Aherne & O'Brien, 2000). Accordingly, we would like to propose that one major function of CSB in cells exposed to oxidative stress is to cooperate with

PARP1 in single-strand DNA break repair. This model is consistent with the observation by Menoni et al. (2012), where OGG1 is not required for the recruitment of CSB to locally induced oxidative DNA damage generated by photo-activation of Ro-19-8022 (Menoni et al., 2012). Furthermore, we found that PARP1's enzymatic activity is not required for menadione-induced CSB-chromatin association, suggesting that the enhanced chromatin association kinetics mediated by PARP1 is likely the result of direct protein-protein interaction (Fig. 5). Indeed, CSB has been shown to directly interact with the PARP1 protein (Thorslund et al., 2005). However, it is also possible that another protein that is recruited by PARP1 may function to enhance the CSB-chromatin association (Fig. 7A). Furthermore, the DNA-binding regions within the ATPase and C-terminal domains of CSB play important roles in mediating this association, as the N-terminal region alone (CSB-N), which is capable of interacting with PARP1 (Thorslund et al., 2005), fails to associate with chromatin upon oxidative stress (Fig. 2E). Lastly, although DNA lesion-stalled transcription is the primary signal that promotes CSB-chromatin association in response to UV irradiation, transcription elongation is not required for menadione-induced global CSB-chromatin association (Fig. 3).

We also found that $CSB^{\Delta 245\text{-}365}$ (CSBΔN1), a CSB derivative that is devoid of any chromatin remodeling activity (39), cannot complement the menadione sensitivity of CSB-deficient cells (Fig. 7B). This indicates that the chromatin remodeling activity of CSB is required for CSB's function in the repair of menadione-induced DNA damage or the transcriptional response to oxidative stress, or both. In the case of DNA repair, CSB may function to replace PARP1 once recruited to a single-strand break to facilitate DNA repair, as proposed by Scheibye-Knudsen et al. (2014) (Scheibye-Knudsen et al., 2014). Alternatively, CSB may facilitate single-strand break repair by opening up chromatin structure. Indeed, prior studies have shown that PARP1 can recruit other chromatin-remodeling complexes, such as ALC1, CHD2, and SNF2h, to facilitate DNA repair (D. Ahel et al., 2009; Gottschalk et al., 2009; Luijsterburg et al., 2016; Smeenk et al., 2013).

Previously, we had shown that menadione treatment promotes the occupancy of CSB at specific loci throughout the genome, and this site-specific occupancy likely reflects a role that CSB plays in mounting a transcriptional response to oxidative stress (Lake et al., 2016). Here, we have shown that decreasing PARP1 protein levels can decrease this enhancement of site-specific occupancy (Fig. 6A). Given that PARP1 has been suggested to participate in transcription elongation (Gibson et al., 2016), it is likely that PARP1 knockdown reduces transcription elongation, which then leads to decreased CSB occupancy specific sites (Fig. 6A). This hypothesis is supported by our observation that inhibiting RNA pol II transcription elongation with DRB also decreases the enhancement of site-specific CSB occupancy induced by menadione (Fig. 6C). Interestingly, the enzymatic activity of PARP1 is only required at a subset of the loci examined (Fig. 6B). This is consistent with the notion that the requirement for PARP1 activity in transcription is context dependent (Kraus & Lis, 2003) and suggests that PARP1 enhances CSB occupancy on chromatin through both activity-dependent and -independent mechanisms.

We previously demonstrated that CSB and CTCF can reciprocally regulate each other's occupancy at specific genomic loci upon oxidative stress, and we hypothesized that CSB may cooperate with CTCF by altering 3-D genome organization to facilitate the relief of oxidative stress (Lake et al., 2016). While the role of this 3-D genome organization may be to regulate gene expression, this study also opens up the possibility that the 3-D chromatin organization mediated by CTCF and CSB may facilitate the formation of DNA repair hubs for the repair of single-strand DNA breaks localized by PARP1.

3.6. Experimental Procedures

3.6.1. Cell culture and treatment protocol

CS1AN-sv cells and CS1AN-sv cells stably expressing CSB or mutant CSB proteins were maintained in DMEM-F12 supplemented with 10% FBS (Cho et al., 2013; Lake et al., 2014). 293T cells were maintained in DMEM supplemented with 10% FBS. All cells were cultured at

37°C in 5% CO_2. CS1AN-sv cells stably expressing CSB, CSB^{R670W}, CSBΔN, CSBΔC were expressed as previously described (Lake et al., 2010). CS1AN cells stably expressing CSB-N and CSB-C were generated by transfecting cells with CSB-N or CSB-C expression plasmids and selecting with 600 µg/mL G418 (Lake et al., 2010). Oxidative stress was induced by treating cells with 100-µM menadione (MP Biomedicals, 102259). The PARP inhibitor, KU-0058948 hydrochloride (Axon Medchem, 2001), was used at a final concentration of 1 µM for 1 hour (Hanzlikova et al., 2017). RNA pol II transcription elongation was inhibited by treating cells with 50 µM 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB, Sigma-Aldrich, D1916) for one hour prior to treatment with menadione (Lake et al., 2010). Menadione was added directly to the DRB- or KU-0058948 containing medium. For the UV control experiment, cells were treated with 50 µM DRB for 1 hour and then irradiated with 100 J/m^2 UV (245 nm) using a Stratalinker (Lake et al., 2010). Cells were allowed to recover for one hour prior to processing.

3.6.2. Protein fractionation and western blotting

Equal numbers of cells were seeded onto 60 mm dishes and allowed to grow overnight to ~80% confluence. Media was changed on all plates and cells were left untreated or treated with 100 µM menadione for indicated times. Cells were lysed and proteins were fractionated as described previously (Lake et al., 2016; Lake et al., 2010). Briefly, cells were rinsed with PBS, collected in 200 µl buffer B (150 mM NaCl, 0.5 mM $MgCl_2$, 20 mM HEPES (pH 8.0), 10% glycerol, 0.5% Triton X-100, 1 mM DTT) on ice and centrifuged at 15,000 rpm for 20 minutes at 4°C. 150 µl supernatant was added to 50 µl 4x SDS sample buffer (soluble fraction, ‘s’) and 200 µl 1xSDS sample buffer was added to the pellet, which was sonicated for 10 seconds at 25% amplitude with a Branson 101-135-126 Sonifer (chromatin-enriched fraction, ‘c’, 1.3 times more concentrated than ‘s’). Proteins were run on a NuPAGE™ 4-12% Bis-Tris protein gel (Invitrogen, NP0323BOX) with the BenchMark™ pre-stained protein ladder (Invitrogen, 10748-010) and gels are labeled with molecular weight markers (kDa). Western blots were developed using SuperSignal West Pico or Dura chemiluminescent substrate (ThermoFisher Scientific, 34580 and

34075), and imaged with a Fujifilm ImageQuant LAS-4000 imager or developed using a Kodak Processor M35A. To determine the percentage of CSB co-fractionated with chromatin, images were scanned and quantified using ImageJ. Determination of percent CSB co-fractionated with chromatin was calculated as previously described by normalizing respective to BRG1 and adjusting for the 1.3-fold more concentrated chromatin-enriched fraction (Lake et al., 2016).

3.6.3. Lentiviral shRNA knockdown

Mission shRNA targeting OGG1 (TRCN0000314740), APE1 (TRCN0000007958), PARP1 (TRCN0000007932) (Ma et al., 2012; Wu et al., 2017), and a non-targeting shRNA (SHC002) were from Sigma-Aldrich. Virus was produced as previously described (Lake et al., 2016). Briefly, virus was produced by co-transfecting 293T cells with shRNA and the third generation lentiviral packaging plasmids pMGLg-RRE, pRSV-REV, and pMD2.G/VSV. Media was changed 24 hours after transfection and virus-containing medium was collected 24 hours later. The target cell confluence at time of infection was ~20%. Media was changed 24 hours after infection, and cells were harvested at 72 hours (PARP1, APE1) or 96 hours (OGG1) post-infection.

3.6.4. ChIP-western and ChIP-qPCR analyses

Chromatin immunoprecipitation (ChIP) was carried out as previously described (Lake et al., 2014; Lake et al., 2016). ChIP was performed using approximately 4 million cells, 5 µl monoclonal anti-CSB antibody (1B1) (Lake et al., 2014) and 5 µl protein-G agarose beads (Invitrogen, 15920010). Real-time PCR was done using a 7900HT Fast Real-Time PCR System (Applied Biosystems) and SensiFAST^{TM} Sybr Hi-Rox Mix (Bioline, BIO-92020) following manufacturer instructions. Primers are listed in Table S1. The real-time PCR data were analyzed using $\Delta\Delta$Ct method (Schmittgen & Livak, 2008).

For ChIP-western analysis, ChIP was conducted as above following treatment with 100 μM menadione for 30 minutes. Samples were reverse cross-linked in 1xSDS sample buffer at 95°C for 30 minutes and immediately ran on a gel (Cho et al., 2013).

3.6.5. Antibodies

Antibodies used for western blot analysis were rabbit polyclonal anti-CSB antibodies to the N-terminus (Jasmine) or C-terminus (Libra) (both used at 1:2000) (provided by Dr. Weiner, University of Washington) (Lake et al., 2010), rabbit polyclonal anti-BRG1 (1:2000) (provided by Dr. Kingston, MGH) (Lake et al., 2010), rabbit polyclonal anti-XRCC1 (1:1000) (Cell Signaling Technology, 2735), rabbit polyclonal anti-PARP1 (1:1000) (Cell Signaling Technology, 9542), rabbit polyclonal anti-γ-H2A.X (1:1000) (Cell Signaling Technology, 2595), rabbit polyclonal anti-CTCF (1:2000) (Millipore, 07-729), mouse monoclonal anti-RNA polymerase II (1:500) (Covance, H5), rabbit polyclonal anti-acetyl-Histone H3 (1:1000) (Millipore, 06-599), rabbit polyclonal anti-histone H3 (1:2000) (Cell Signaling Technology, 9715), mouse monoclonal anti-GAPDH (1:10,000) (Millipore, MAB374), rabbit polyclonal anti-OGG1 (1:10,000) (Abcam, ab124741), rabbit polyclonal anti-APE1 (Cell Signaling Technology, 4128S), HRP-conjugated goat anti-rabbit IgG (1:10,000) (Pierce, 31460), and HRP-conjugated goat anti-mouse (1:10,000) (Jackson Laboratory, 115-035-044). ChIP was performed using the N-terminal anti-CSB antibody 1B1 (10). Poly ADP-Ribose (PAR) was analyzed using mouse monoclonal anti-PAR (1:1000) (Tulip BioLabs, #1020/N) and peroxidase-conjugated AffiniPure goat anti-mouse IgG, Fcγ Subclass 3 Specific (1:2000) (Jackson ImmunoResearch Laboratories, Inc., 115-035-209).

3.6.6. Menadione sensitivity assay

Approximately 100,000 cells were seeded onto 35 mm dishes in DMEM/F12 medium supplemented with 10% FBS and allowed to grow for 24 hours at 37°C. Cells were then given fresh media and left untreated or treated with indicated concentrations of menadione for 1 hour. After 1 hour, menadione-containing medium was removed and fresh medium without menadione

was added. Cells were cultured for an additional 24 hours at which point cell viability was determined by trypan blue exclusion using a hemocytometer. Percent survival was calculated as the ratio of treated cells to untreated cells (Lake et al., 2016).

3.7. Figures

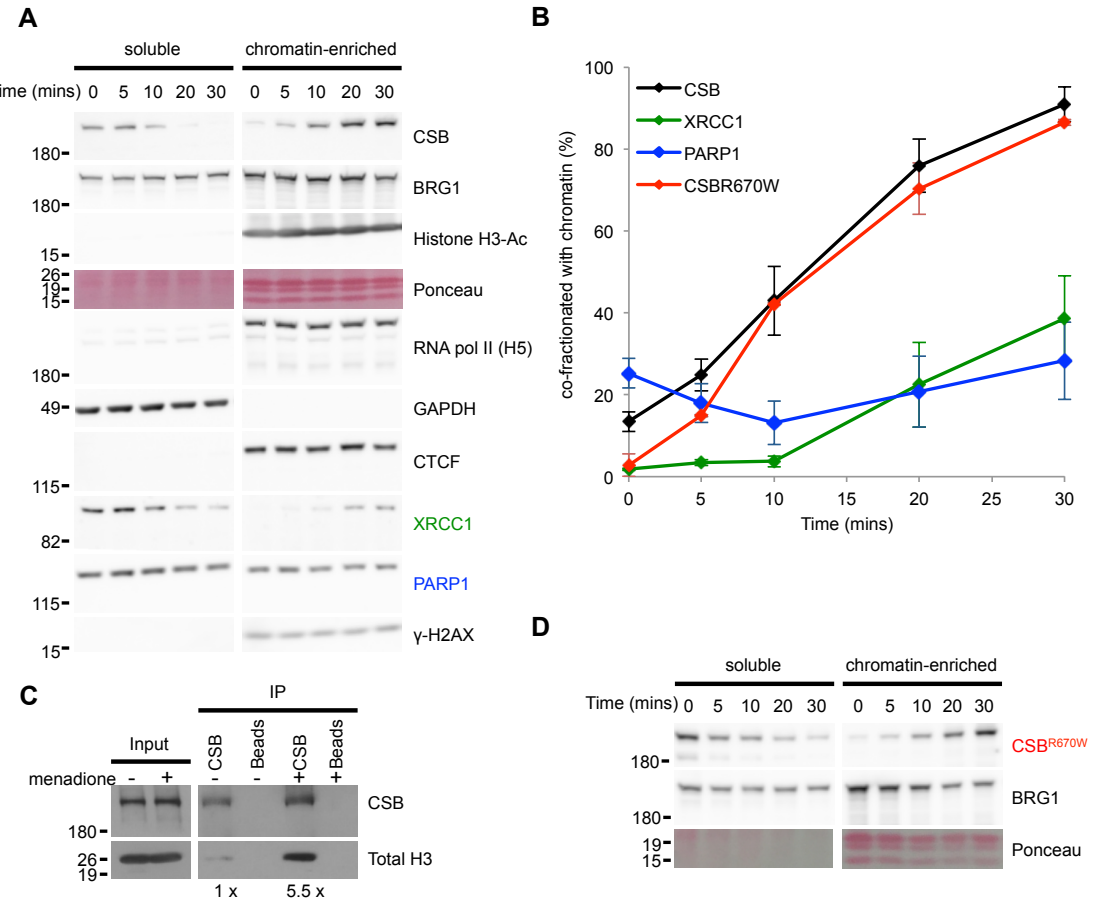

Figure 1. The association of CSB with chromatin in response to menadione treatment occurs independently of ATP hydrolysis. (**A**) Protein fractionation assay in CS1AN-CSB^{WT} cells following treatment with 100 μM menadione for times indicated. Western blots were probed with antibodies listed. BRG1 was used as a loading control. Acetylated histone H3 and total core histones (visualized by Ponceau S staining) were used as markers for the chromatin-enriched fraction. GAPDH was used as a marker for the soluble fraction. (**B**) Quantification of percent CSB^{WT} (*n*=5), PARP1 (*n*=4), XRCC1 (*n*=4), and CSB^{R670W} (*n*=2) in the chromatin-enriched fraction as a function of time, normalized to BRG1. Error bars represent SEM. (**C**) CSB ChIP-western analysis in CS1AN- CSB^{WT} cells untreated (-) or treated with 100 uM menadione for 30 minutes (+). Numbers at bottom show fold change in histone H3 normalized to CSB (*n*=2). (**D**) Protein fractionation assay in CS1AN-CSB^{R670W} cells following treatment with 100 μM menadione for times indicated (*n*=2). Shown is a representative western blot probed with antibodies to CSB and BRG1, and stained with Ponceau S.

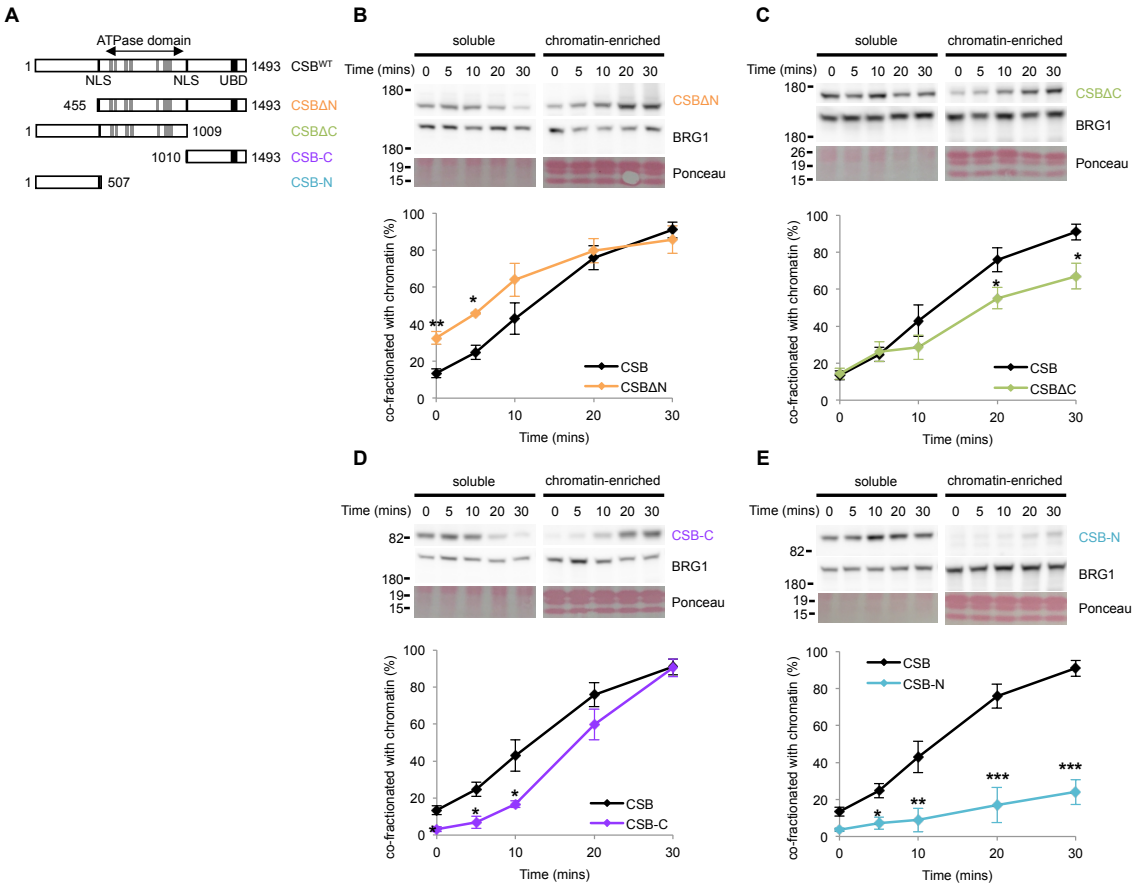

Figure 2. The association of CSB with chromatin in response to menadione treatment is largely mediated through its ATPase domain and C-terminal region. (**A**) Schematic representation of the CSB protein and CSB deletion constructs used in the protein fraction assays. Grey boxes represent the seven conserved helicase motifs, thin black boxes represent the two putative nuclear localization signals (NLS), and the thick black box represents the ubiquitin binding domain (UBD). (**B-E**) Protein fractionation assays demonstrating chromatin association as a function of time in CS1AN-sv cells reconstituted with indicated CSB derivatives, following treatment with 100 μM menadione. CSB^{WT} (*n*=5) (from Fig. 1A) and (**B**) CSBΔN (*n*=2), (**C**) CSBΔC (*n*=3), (**D**) CSB-C (*n*=4), and (**E**) CSB-N (*n*=2). Shown are representative western blots probed with the indicated antibodies and stained with Ponceau S. Plots show quantification of the western blot data with CSB signals normalized to BRG1 signals. Error bars represent SEM. Paired *t*-test compares construct enrichment to CSB^{WT} (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

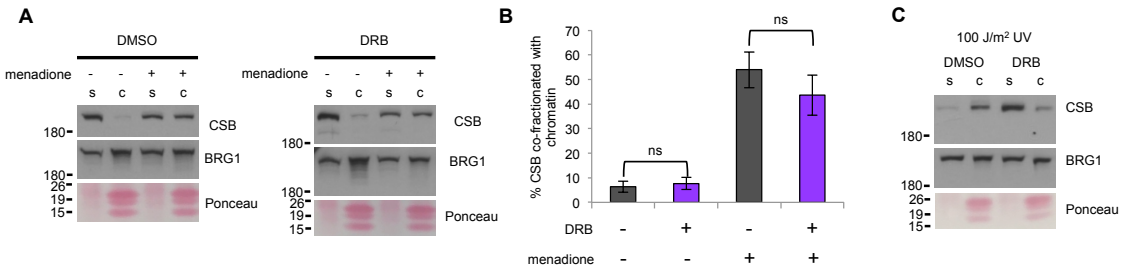

Figure 3. Inhibiting transcription elongation of RNA pol II by DRB does not alter menadione-induced CSB-chromatin association. (**A**) Protein fractionation assay in CS1AN-CSB^{WT} cells. Cells were treated with 50 μM DRB or DMSO for 1 hour followed by 100 μM menadione for 20 minutes. Representative western blot probed with antibodies listed. "S" denotes soluble proteins fraction and "C" denotes chromatin-enriched fraction. (**B**) Quantification of CSB chromatin co-fractionation data in (A) normalized to BRG1 signal intensity. Shown are means ± SEM and paired *t*-test compares enrichment in cells with DMSO to DRB (*n*=3, ns: not significant). (**C**) Protein fractionation assay in CS1AN-CSB^{WT} cells treated with 50 μM DRB or DMSO for 1 hour followed by 100 J/m^2 of UV irradiation. Cells were analyzed 1 hour after UV treatment.

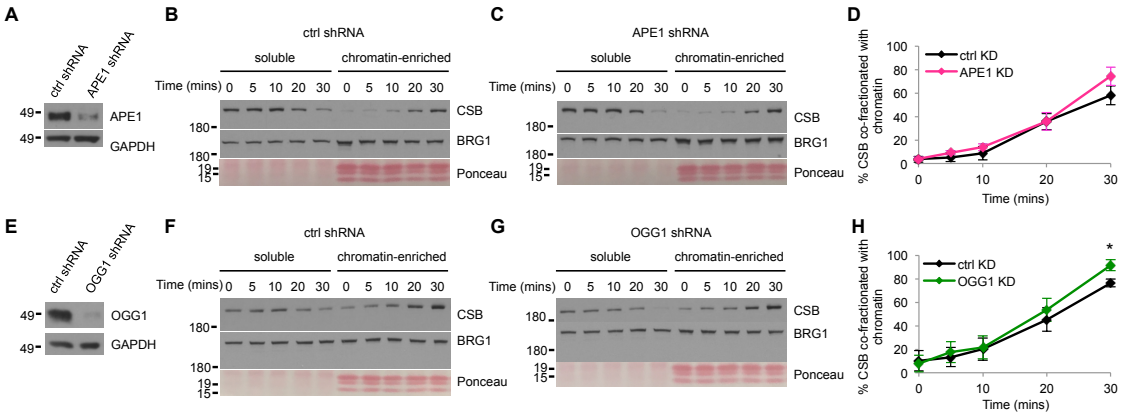

Figure 4. APE1 or OGG1 are dispensable for menadione-induced global CSB-chromatin association. (**A**) Representative western blot revealing the extent of APE1 knockdown (average knockdown 72%, normalized to GAPDH). (**B-C**) Protein fractionation assay revealing CSB-chromatin association as a function of time after menadione treatment in CS1AN-CSB^{WT} cells expressing a control (ctrl) or APE1 shRNA. Representative western blot probed with antibodies listed and stained with Ponceau S. (**D**) Quantification of data in B and C showing percent CSB co-fractionating with chromatin. Error bars represent SEM. Paired *t*-test comparing CSB enrichment in control vs APE1 knockdown (n=4) revealed no significant differences in association kinetics. (**E**) Representative western blot revealing the extent of OGG1 knockdown (average knockdown 90%, normalized to GAPDH). (**F-G**) Protein fractionation assay revealing CSB-chromatin association as a function of time after menadione treatment in CS1AN-CSB^{WT} cells expressing a control (ctrl) or OGG1 shRNA. Representative western blot probed with antibodies listed and stained with Ponceau S. (**H**) Quantification of data in F and G showing percent CSB co-fractionating with chromatin. Error bars represent SEM. Paired *t*-test comparing CSB enrichment in control to OGG1 knockdown (n=4, * $p \leq 0.05$).

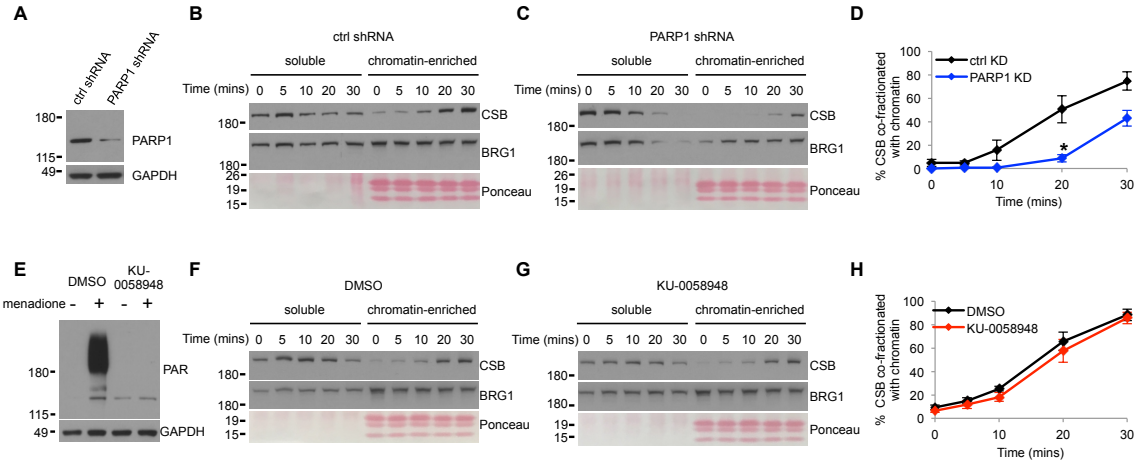

Figure 5. The PARP1 protein, but not its enzymatic activity, is required for efficient, global CSB-chromatin association in response to menadione treatment. (**A**) Representative western blot revealing the extent of PARP1 knockdown (average knockdown 89%, normalized to GAPDH). (**B-C**) Protein fractionation assay revealing CSB-chromatin association as a function of time after menadione treatment in CS1AN-CSB^{WT} cells expressing a control (ctrl) or PARP1 shRNA. Representative western blot probed with antibodies listed and stained with Ponceau S. (**D**) Quantification of data in B and C showing percent CSB co-fractionating with chromatin. Error bars represent SEM. Paired *t*-test comparing CSB enrichment in control vs PARP1 knockdown (*n=3*, * $p \leq 0.05$). (**E**) Western blot probed with an anti-PAR antibody demonstrating PARP1 inhibition by KU-0058948. (**F-G**) Protein fractionation assay of CS1AN-CSB^{WT} cells treated with DMSO (vehicle control) or KU-0058948, followed by the addition 100 μM menadione for the indicated times. Representative western blots probed with antibodies listed and stained with Ponceau S. (**H**) Quantification of data in **F** and **G** showing percent CSB co-fractionating with chromatin. Error bars represent SEM. Paired *t*-test comparing CSB enrichment in DMSO vs KU-0058948 treated cells (*n*=5) revealed no significant difference.

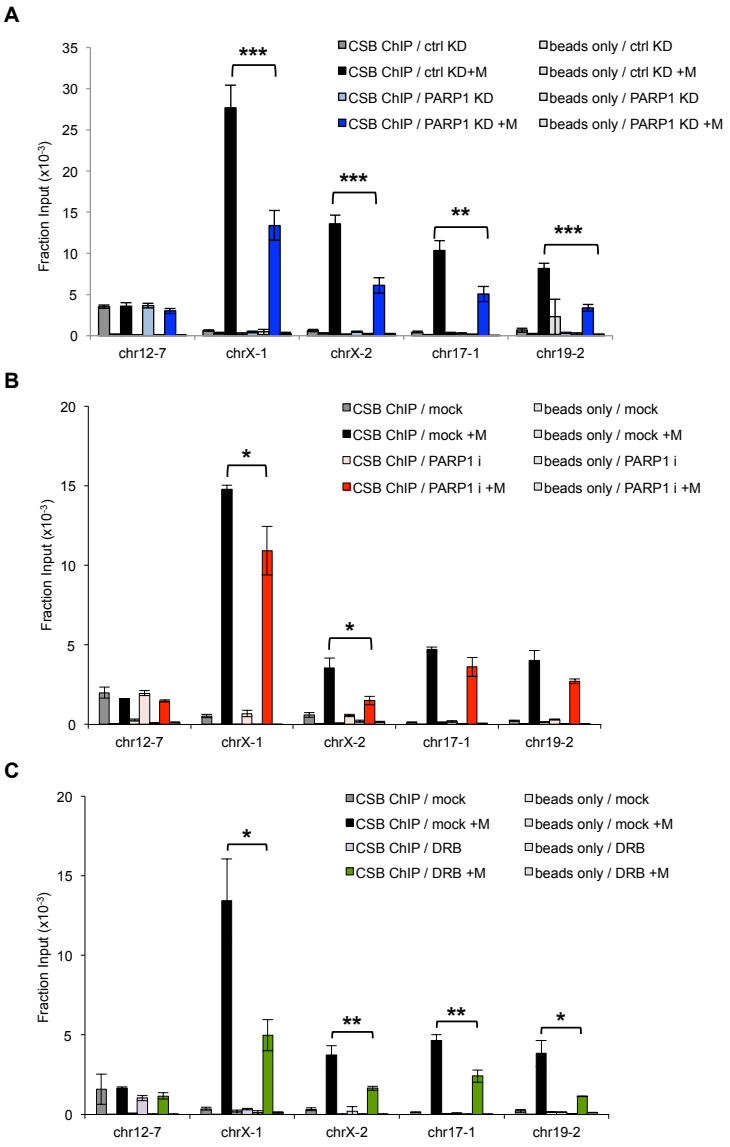

Figure 6. PARP1 and elongating RNA pol II contribute to menadione-induced CSB occupancy at specific genomic loci. ChIP-qPCR analyses of CSB recruitment to specific genomic loci in response to menadione treatment. Shown are four loci where CSB occupancy is significantly enhanced by oxidative stress (chrX-1, chrX-2, chr17-1, and chr19-2) and a control locus where CSB occupancy is not changed by oxidative stress (chr12-7). (**A**) CSB ChIP-qPCR analyses of CS1AN-CSB^{WT} cells expressing a control (ctrl) or PARP1 shRNA. Shown are means ± SEM. Paired *t*-test comparing CSB enrichment (*n*=3, ** $p \leq 0.01$, *** $p \leq 0.001$). (**B**) CSB ChIP-qPCR analyses as above except that cells were exposed to KU-0058948 (PARP i) or DMSO for 1 hour prior to menadione treatment. Shown are means ± SEM. Paired *t*-test comparing CSB enrichment (*n*=2, * $p \leq 0.05$). (**C**) CSB ChIP-qPCR analyses of cells exposed to DRB or DMSO for 1 hour prior to menadione treatment. Shown are means ± SEM. Paired *t*-test comparing CSB enrichment (*n*=2, * $p \leq 0.05$, ** $p \leq 0.01$).

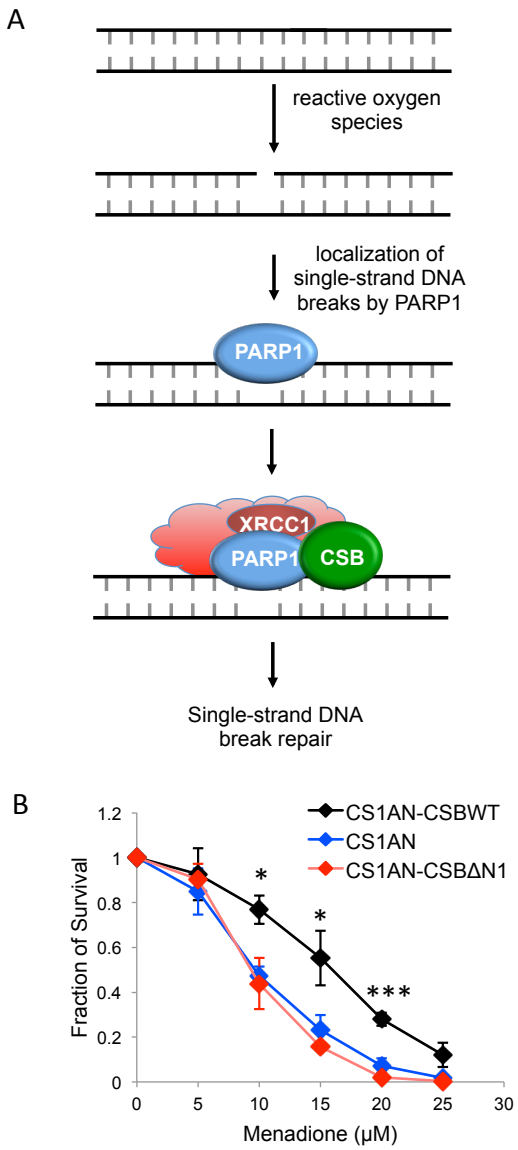

Figure 7. A model for CSB function during oxidative stress-induced DNA damage. (**A**) Single strand DNA breaks directly generated by reactive oxygen species are recognized by PARP1. Localization of PARP1 to single-strand breaks facilitates the recruitment of CSB, the scaffolding protein XRCC1 and other factors needed for repair. CSB may function to make the chromatin landscape more permissible for DNA repair and/or regulate protein factor retention at sites of repair. (**B**) Menadione sensitivity assays. The chromatin remodeling deficient CSBΔN1 does not complement the menadione sensitivity of the CS1AN-sv cells. Paired *t*-test comparing CS1AN-CSB^{WT} to CS1AN-CSBΔN1 (n=5, * $p \leq 0.05$, *** $p \leq 0.001$).

3.8. Supporting Information

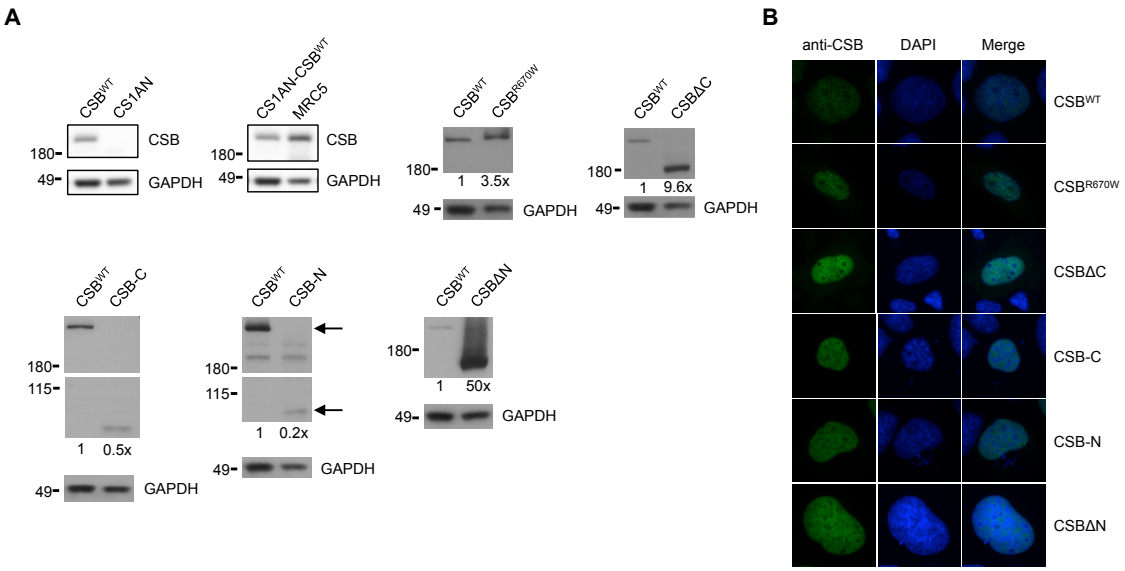

Figure S1. CSB derivatives in CS1AN cell lines. (**A**) Western blots showing CSB^{WT} and mutant CSB expression levels. Values report GAPDH normalized mutant protein levels relative to GAPDH normalized CSB^{WT} level. The second western blot shows CSB^{WT} level in CS1AN fibroblasts relative to endogenous CSB in the MRC5 fibroblast cell line. An arrow indicates CSB-N, while other bands are antibody background signal present in both cell lines. (**B**) Immunofluorescence images demonstrating CSB^{WT} and all CSB mutant constructs localize to the nucleus of CS1AN cells (CSB green, DAPI blue).

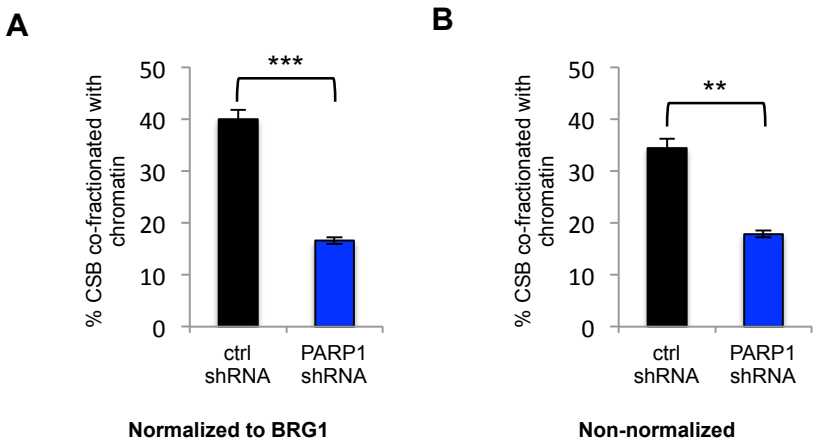

Figure S2. PARP1 decreases CSB-chromatin co-fractionation regardless of BRG1 normalization. Eleven replicate experiments are depicted comparing the impact of control shRNA (ctrl) and PARP1 shRNA on CSB-chromatin co-fractionation following treatment with 100 μM menadione for 20 minutes. (**A**) Values were normalized to BRG1. (**B**) Values are not normalized. Shown are means ± SEM. Paired *t*-test compares enrichment in cells with control vs PARP1 shRNA (n=11, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Table S1: Primers used in ChIP-qPCR		
Name of primer set	Primer name	Sequence (5' to 3')
chr12-7	chr12-7F	CGG TGG AAT TCA GGA GTC AG
	chr12-7R	ACC ATT CCA GCA TTC CCA TT
chrX-1	chrX-1F	GTC TTG ACC ACA TGT GAC TGG
	chrX-1R	AGC TGG TCT AAG CCG ATC AA
chrX-2	chrX-2F	GAC TAA CCT GGA CCA GCT GT
	chrX-2R	AGG ATG GGT CAA AGC CAG TC
chr17-1	chr17-1F	TGC GAA TTT CCA TGG GTT AT
	chr17-1R	GTC AAC ATT GGC TGA AGC TG
chr19-2	chr19-2F	AAC TGA CTG TGA CCG GCT TT
	chr19-2R	TAG CCA GAC ACC ACT GGT CA

CHAPTER 4: CONCLUDING REMARKS

4.1. Summary of Major Conclusions

The overall goal of this dissertation research was to work towards understanding the role of CSB in the relief of oxidative stress by characterizing the mechanisms by which CSB associates with chromatin upon oxidative stress. CSB's interaction with chromatin is highly dynamic. Under replicative cell growth conditions, only ~10% of CSB in cells is stably associated with chromatin while the remaining 90% remains soluble. UV irradiation stabilizes CSB's interaction with chromatin where it is required for transcription-coupled nucleotide excision repair (TC-NER) and, likely, regulating transcription. Similarly, we demonstrate treating cells with menadione, which induces oxidative stress by producing reactive oxygen species, also induces stable association of CSB with chromatin. Importantly, we demonstrate that CS1AN cells, which are deficient in functional CSB, are more sensitive to menadione treatment than those reconstituted with wild type CSB.

We then sought to characterize CSB's genomic occupancy genome-wide upon oxidative stress by ChIP-seq. Compared to our previously published untreated ChIP-seq data set, CSB's occupancy in response to oxidative stress revealed common occupancy sites as well as sites induced or repressed by menadione treatment. Furthermore, oxidative stress resulted in a significant increase in CSB's promoter occupancy from 2% in untreated cells to 11% in menadione treated cells, suggesting CSB participates in transcription regulation in response to oxidative stress. We previously demonstrated CSB occupies c-Jun/AP1 binding sites under non-stress conditions, and the percentage of these sites occupied by CSB was not changed by menadione treatment. Interestingly, we found CSB was significantly enriched at sites containing the binding motif for CTCF. We demonstrated loss of CTCF resulted in a significant decrease in CSB occupancy at several stress-induced loci, including loci with and without CTCF binding motifs. We then demonstrated a novel, direct interaction between CSB and CTCF and that this interaction is enhanced by oxidative stress. CSB also positively regulates CTCF's interactions

with DNA *in vitro*. We then examined CTCF occupancy at several genomic loci containing CTCF binding motifs where CSB occupancy is enhanced by oxidative stress. We observed a significant increase in CTCF occupancy upon oxidative stress at these loci, and, strikingly, the menadione-induced occupancy does not occur in the absence of CSB. Together these findings show CSB and CTCF interact and cooperate upon oxidative stress by regulating each other's interactions with chromatin. We hypothesize CSB functions in regulating transcription in response to oxidative stress, perhaps through cooperation with CTCF in 3-dimensional genome organization.

To further characterize CSB's oxidative stress-induced chromatin association, we examined several CSB mutants to define the critical determinants of CSB required for this to occur. In stark contrast to CSB's UV-induced chromatin association, CSB's ATP hydrolysis activity is not required for CSB to stably associate with chromatin in response to oxidative stress. Furthermore, CSB's ATPase and C-terminal domains mediate this association. As CSB is hypothesized to participate in BER, we examined two CSB-interacting BER proteins, OGG1 and APE1, however neither was required for CSB-chromatin association upon oxidative stress. We then examined PARP1 and demonstrated PARP1, but not its enzymatic activity, is required for global CSB-chromatin association upon oxidative stress. As OGG1 and APE1 knockdown did not impact CSB's global oxidative stress-induced chromatin targeting, we hypothesize targeting by PARP1 is largely representative of targeting to single strand DNA breaks directly resulting from menadione-induced SSB production. CSB may facilitate DNA repair by removing PARP1 from the lesion or by opening chromatin structure as the remodeling-deficient CSB derivative CSBΔN1 cannot complement the sensitivity of CSB-deficient cells to oxidative stress. We also examined several genomic loci at which CSB's occupancy is oxidative stress-induced and found PARP1 also targets CSB to chromatin in a locus-specific manner in response to oxidative stress and, furthermore, that PARP1's enzymatic activity was required at a subset of these loci. CSB's targeting to specific genomic loci by PARP1 also supports a role of CSB in transcription regulation, as PARP1 functions in this process. While active RNA polymerase II transcription inhibition did not significantly impact CSB's global chromatin association, it did affect CSB's

locus-specific targeting, suggesting CSB's role in DNA repair is not transcription dependent while supporting the hypothesis that CSB functions in transcription regulation during oxidative stress.

4.2. Compare Targeting by UV Irradiation to that of Oxidative Stress

Work presented in this dissertation suggests that CSB's targeting to chromatin in response to oxidative stress is unique from its targeting following UV irradiation. Upon UV irradiation, CSB targets RNA polymerase II stalled at a bulky DNA lesion, which is recognized by a substrate recognition domain within the N-terminus of CSB. Stable chromatin association requires CSB undergo an ATP-dependent confirmation to relieve N-terminal autorepression, opening CSB and exposing contact surfaces along the protein. Furthermore, this requires active RNA polymerase II transcription as inhibition of RNA polymerase II elongation abolishes UV irradiation-induced CSB-chromatin association. Here, we show that stable chromatin association following oxidative stress occurs by a different mechanism. This does not require an ATP-dependent confirmation change nor does it require active RNA polymerase II transcription. Furthermore, CSB's oxidative stress-induced chromatin association is mediated by the ATPase and C-terminal domains, which alone do not response to UV irradiation. This raises the question of why these targeting paradigms so different for one protein responding to two DNA damage-inducing stress conditions. One factor underlying these differences is likely the types of lesions created. The major type of damage created by UV irradiation is bulky, transcription-stalling lesions such as cyclobutane pyrimidine dimers (CPD) and (6,4)-photoproducts, which are repaired by nucleotide excision repair pathways. In contrast, oxidative stress creates smaller lesions, such as single base modifications, and single strand breaks, which are repaired by base excision repair and single strand break repair, respectively. Therefore, inherent differences in the damage itself as well as the repair pathway initiated by the damage by underlie the apparent differences in CSB's recruitment to chromatin. While it is largely reported that oxidative DNA damage does not impair transcription by RNA polymerase II, some groups report varying degrees of RNA polymerase II stalling. Therefore, it is possible that a fraction of total cellular CSB may be

engaged in TC-NER in response to oxidative stress, however, the finding that neither CSB's ATP hydrolysis activity nor active RNA polymerase II transcription is required for stable CSB-chromatin association upon oxidative stress suggests this is not the major activity. We would also like to introduce the hypothesis that while CSB's ATPase activity is not required for chromatin association in response to oxidative stress, it is still possible that CSB is undergoing an ATP-independent confirmation change. For example, this may be induced by binding to or modification of CSB by PARP1.

Although it appears targeting following oxidative stress is different from UV irradiation and may be do to inherent differences in the types of DNA damage induced during these different stress conditions, it is still possible that CSB's major role in responding to DNA damage is similar. In response to UV irradiation, CSB is required to recruit downstream repair factors, which may also be the case for oxidative DNA damage repair. Furthermore, CSB's remodeling facilitates TC-NER, which is hypothesized to be the result of CSB opening chromatin and allowing efficient repair or repair factor binding or remodeling may allow RNA polymerase II to resume transcription post-repair. CSB's remodeling activity is also required for CSB to relieve oxidative stress, as cells containing a remodeling-deficient CSB are more sensitive to the killing effects of the oxidative stress-inducing drug menadione. Again, CSB's remodeling activity may allow efficient DNA repair by making the chromatin structure amenable to repair. Lastly, there are likely similarities and differences in how CSB is targeted to specific genomic loci upon UV irradiation and oxidative stress. In response to oxidative stress, we demonstrate that CSB is targeted to specific genomic loci by CTCF and PARP1 and hypothesize CSB regulates transcription, allowing cells to respond to stress. Upon UV irradiation, CSB is hypothesized to relieve widespread transcription inhibition by ATF3. It is possible, however, that CSB is also targeted by UV irradiation to similar sites as it is for oxidative stress where this allows cells to respond to these stress conditions.

4.3. Perspectives

In agreement with previous studies, this work supports the hypothesis that CSB partially relieves oxidative stress by regulating transcription, allowing cells to respond. This also supports the hypothesis that CSB's role in transcription regulation may partially underlie the Cockayne syndrome phenotype, as its role in TC-NER alone cannot account for all deficiencies associated with the devastating disorder, such as the neurological defects. CSB is targeted to specific genomic loci by PARP1 and CTCF and some of these sites overlap, therefore, it is possible that these proteins cooperate in transcription regulation by altering 3-D genome organization.

While several previous studies have suggested CSB functions in the major repair pathway for oxidative DNA damage, base excision repair (BER), direct evidence supporting this hypothesis has been limited and exactly how CSB functions in this process has been unknown. We observed neither OGG1 nor APE1 knockdown impacted CSB's oxidative stress-induced chromatin association, suggesting CSB functions upstream of these proteins. As PARP1, which is downstream of OGG1 and APE1, does participate in CSB-chromatin association in response to oxidative stress, we hypothesize the major role for CSB following menadione treatment may be in PARP1-dependent single strand break repair. Taken together with the discovery that CTCF targets CSB to chromatin in response to oxidative stress, it is also possible that CSB and CTCF promote the formation of 3-D repair hubs to facilitate efficient PARP1-dependent DNA repair. Furthermore, CSB's chromatin remodeling activity may facilitate this process.

Determining the role of CSB in the relief of oxidative stress is of particular interest for its contribution to the Cockayne syndrome phenotype. As CSB's role in response to UV irradiation cannot fully account for the severity of this disorder, understanding how CSB functions in response to oxidative stress may shed light into how this contributes to the disease pathology, in particular the neurological and early aging phenotype. In the future this may lead to treatments for this incurable disease as well as further our understanding of these processes in humans.

4.4. Future Directions

As CTCF is a major regulator of 3-dimensional genome architecture and we found CSB and CTCF cooperate in response to oxidative stress, a key experiment to further characterize these interactions is performing chromatin confirmation capture studies. This would determine how 3-D genome architecture is regulated in response to oxidative stress, and, critically whether this depends on CSB.

Previous work as well as the work presented in this dissertation suggests CSB regulates transcription in response to oxidative stress. Genes occupied by CSB in response to oxidative stress include those involved in gene expression, cell cycle control, spliceosome, and protein metabolism. RNA sequencing in cells with and without CSB as well as with and without oxidative stress will reveal exactly what genes are regulated by CSB in response to oxidative stress and further characterize the role CSB plays in this process. It would be of particular interest to conduct RNA sequencing in disease relevant tissues collected from patients to provide additional information into how CSB's role in transcription regulation contributes to the severity of the Cockayne syndrome phenotype.

Although we have identified two proteins, CTCF and PARP1, which mediate CSB's interaction with chromatin in response to oxidative stress, there are likely other proteins which regulate CSB's chromatin targeting. For example, CSB only occupies a subset of CTCF binding sites and how CSB distinguishes one site from another is unclear. Furthermore, as CTCF stabilizes CSB's occupancy at specific genomic loci devoid of CTCF binding sites, CSB's initial targeting to these sites may rely upon an additional, yet to be identified protein. This could be done by purifying CSB in complex following menadione treatment to identify additional proteins interacting with CSB, with a particular interest in interactions that occur specifically upon oxidative stress.

A critical element to all of the above studies is examining the role of CSB's remodeling activity. We demonstrated CSB's remodeling activity is required for CSB to protect cells from oxidative stress, as survival of the remodeling deficient CSBΔN1 mutant was also more sensitive to menadione treatment than cells with wild type CSB. However, we still do not know the exact

function of CSB's remodeling activity in the relief of oxidative stress. It is likely that CSB's remodeling activity is required if it is determined that CSB functions in transcription regulation upon oxidative stress, however, similar to non-stress conditions, it is likely that CSB has remodeling-independent functions as well. CSB's remodeling activity may also facilitate oxidative DNA repair and future studies may utilize the remodeling deficient mutant to determine how this activity participates, such as opening chromatin to allow efficient downstream repair.

BIBLIOGRAPHY

Abbotts, R., & Wilson, D. M. (2017). Coordination of DNA single strand break repair. *Free Radical Biology & Medicine, 107*, 228-244. S0891-5849(16)31073-5.

Ahel, D., Horejsi, Z., Wiechens, N., Polo, S. E., Garcia-Wilson, E., Ahel, I., . . . Boulton, S. J. (2009). Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. *Science (New York, N.Y.), 325*(5945), 1240-1243. 10.1126/science.1177321.

Ahel, I., Ahel, D., Matsusaka, T., Clark, A. J., Pines, J., Boulton, S. J., & West, S. C. (2008). Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. *Nature, 451*(7174), 81-85. 10.1038/nature06420.

Aherne, S. A., & O'Brien, N. M. (2000). Mechanism of protection by the flavonoids, quercetin and rutin, against tert-butylhydroperoxide- and menadione-induced DNA single strand breaks in caco-2 cells. *Free Radical Biology & Medicine, 29*(6), 507-514. S0891-5849(00)00360-9.

Allinson, S. L., Dianova, I. I., & Dianov, G. L. (2003). Poly(ADP-ribose) polymerase in base excision repair: Always engaged, but not essential for DNA damage processing. *Acta Biochimica Polonica, 50*(1), 169-179. 035001169.

Althaus, F. R., Kleczkowska, H. E., Malanga, M., Muntener, C. R., Pleschke, J. M., Ebner, M., & Auer, B. (1999). Poly ADP-ribosylation: A DNA break signal mechanism. *Molecular and Cellular Biochemistry, 193*(1-2), 5-11.

Andrade, L. N., Nathanson, J. L., Yeo, G. W., Menck, C. F., & Muotri, A. R. (2012). Evidence for premature aging due to oxidative stress in iPSCs from cockayne syndrome. *Human Molecular Genetics, 21*(17), 3825-3834. 10.1093/hmg/dds211.

Andrade-Lima, L. C., Veloso, A., Paulsen, M. T., Menck, C. F., & Ljungman, M. (2015). DNA repair and recovery of RNA synthesis following exposure to ultraviolet light are delayed in long genes. *Nucleic Acids Research, 43*(5), 2744-2756. 10.1093/nar/gkv148.

Anindya, R., Mari, P. O., Kristensen, U., Kool, H., Giglia-Mari, G., Mullenders, L. H., . . . Svejstrup, J. Q. (2010). A ubiquitin-binding domain in cockayne syndrome B required for transcription-coupled nucleotide excision repair. *Molecular Cell, 38*(5), 637-648. 10.1016/j.molcel.2010.04.017.

Bailey, A. D., Gray, L. T., Pavelitz, T., Newman, J. C., Horibata, K., Tanaka, K., & Weiner, A. M. (2012). The conserved cockayne syndrome B-piggyBac fusion protein (CSB-PGBD3) affects DNA repair and induces both interferon-like and innate antiviral responses in CSB-null cells. *DNA Repair, 11*(5), 488-501. 10.1016/j.dnarep.2012.02.004.

Balajee, A. S., May, A., Dianov, G. L., Friedberg, E. C., & Bohr, V. A. (1997). Reduced RNA polymerase II transcription in intact and permeabilized cockayne syndrome group B cells. *Proceedings of the National Academy of Sciences of the United States of America, 94*(9), 4306-4311.

Bartkova, J., Horejsi, Z., Koed, K., Kramer, A., Tort, F., Zieger, K., . . . Bartek, J. (2005). DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature, 434*(7035), 864-870. nature03482.

Beerens, N., Hoeijmakers, J. H., Kanaar, R., Vermeulen, W., & Wyman, C. (2005). The CSB protein actively wraps DNA. *The Journal of Biological Chemistry, 280*(6), 4722-4729. M409147200.

Berquist, B. R., & Wilson, D. M.,3rd. (2009). Nucleic acid binding activity of human cockayne syndrome B protein and identification of ca(2+) as a novel metal cofactor. *Journal of Molecular Biology, 391*(5), 820-832. 10.1016/j.jmb.2009.06.078.

Blosser, T. R., Yang, J. G., Stone, M. D., Narlikar, G. J., & Zhuang, X. (2009). Dynamics of nucleosome remodelling by individual ACF complexes. *Nature, 462*(7276), 1022-1027. 10.1038/nature08627.

Bohr, V. A., Smith, C. A., Okumoto, D. S., & Hanawalt, P. C. (1985). DNA repair in an active gene: Removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell, 40*(2), 359-369. 0092-8674(85)90150-3.

Bootsma, D., & Hoeijmakers, J. H. (1994). The molecular basis of nucleotide excision repair syndromes. *Mutation Research, 307*(1), 15-23. 0027-5107(94)90273-9.

Bork, P., Hofmann, K., Bucher, P., Neuwald, A. F., Altschul, S. F., & Koonin, E. V. (1997). A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology, 11*(1), 68-76.

Brooks, P. J. (2013). Blinded by the UV light: How the focus on transcription-coupled NER has distracted from understanding the mechanisms of cockayne syndrome neurologic disease. *DNA Repair, 12*(8), 656-671. 10.1016/j.dnarep.2013.04.018; 10.1016/j.dnarep.2013.04.018

Brosh, R. M., Jr, Balajee, A. S., Selzer, R. R., Sunesen, M., Proietti De Santis, L., & Bohr, V. A. (1999). The ATPase domain but not the acidic region of cockayne syndrome group B gene product is essential for DNA repair. *Molecular Biology of the Cell, 10*(11), 3583-3594.

Cadet, J., & Davies, K. J. A. (2017). Oxidative DNA damage & repair: An introduction. *Free Radical Biology & Medicine, 107*, 2-12. S0891-5849(17)30184-3.

Caldecott, K. W. (2008). Single-strand break repair and genetic disease. *Nature Reviews.Genetics, 9*(8), 619-631. 10.1038/nrg2380.

Caldecott, K. W., McKeown, C. K., Tucker, J. D., Ljungquist, S., & Thompson, L. H. (1994). An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. *Molecular and Cellular Biology, 14*(1), 68-76.

Cappelli, E., Taylor, R., Cevasco, M., Abbondandolo, A., Caldecott, K., & Frosina, G. (1997). Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. *The Journal of Biological Chemistry, 272*(38), 23970-23975.

Chalut, C., Moncollin, V., & Egly, J. M. (1994). Transcription by RNA polymerase II: A process linked to DNA repair. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology, 16*(9), 651-655. 10.1002/bies.950160910.

Chernukhin, I. V., Shamsuddin, S., Robinson, A. F., Carne, A. F., Paul, A., El-Kady, A. I., . . . Klenova, E. M. (2000). Physical and functional interaction between two pluripotent proteins, the Y-box DNA/RNA-binding factor, YB-1, and the multivalent zinc finger factor, CTCF. *The Journal of Biological Chemistry, 275*(38), 29915-29921. 10.1074/jbc.M001538200.

Chiou, Y. Y., Hu, J., Sancar, A., & Selby, C. P. (2018). RNA polymerase II is released from the DNA template during transcription-coupled repair in mammalian cells. *The Journal of Biological Chemistry, 293*(7), 2476-2486. 10.1074/jbc.RA117.000971.

Cho, I., Tsai, P., Lake, R. J., Basheer, A., & Fan, H. (2013). ATP-dependent chromatin remodeling by cockayne syndrome protein B and NAP1-like histone chaperones is required for efficient transcription-coupled DNA repair. *Plos Genetics, 9*(4), e1003407. 10.1371/journal.pgen.1003407.

Ciaffardini, F., Nicolai, S., Caputo, M., Canu, G., Paccosi, E., Costantino, M., . . . Proietti-De-Santis, L. (2014). The cockayne syndrome B protein is essential for neuronal differentiation and neuritogenesis. *Cell Death & Disease, 5*, e1268. 10.1038/cddis.2014.228.

Citterio, E., Rademakers, S., van der Horst, G., van Gool, A. J., Hoeijmakers, J., & Vermeulen, W. (1998). Biochemical and biological characterization of wild-type and ATPase-deficient cockayne syndrome B repair protein. *Journal of Biological Chemistry, 273*(19), 11844-11851. 10.1074/jbc.273.19.11844.

Citterio, E., Van Den Boom, V., Schnitzler, G., Kanaar, R., Bonte, E., Kingston, R. E., . . . Vermeulen, W. (2000). ATP-dependent chromatin remodeling by the cockayne syndrome B DNA repair-transcription-coupling factor. *Molecular and Cellular Biology, 20*(20), 7643-7653. 10.1128/MCB.20.20.7643-7653.2000.

Clapier, C. R., & Cairns, B. R. (2009). The biology of chromatin remodeling complexes. *Annual Review of Biochemistry, 78*, 273-304. 10.1146/annurev.biochem.77.062706.153223.

Cleaver, J. E., Bezrookove, V., Revet, I., & Huang, E. J. (2013). Conceptual developments in the causes of cockayne syndrome. *Mechanisms of Ageing and Development, 134*(5-6), 284-290. 10.1016/j.mad.2013.02.005.

Cleaver, J. E., Brennan-Minnella, A. M., Swanson, R. A., Fong, K. W., Chen, J., Chou, K. M., . . . Bezrookove, V. (2014). Mitochondrial reactive oxygen species are scavenged by cockayne syndrome B protein in human fibroblasts without nuclear DNA damage. *Proceedings of the National Academy of Sciences of the United States of America, 111*(37), 13487-13492. 10.1073/pnas.1414135111.

Cleaver, J. E., & Kraemer, K. H. (1989). The metabolic basis of inherited disease. *Scriver, CR, Beaudet.AL, Sly, VS and Valle, D., Editors, McGraw-Hill, New York,* , 2949-2971.

Cockayne, E. A. (1936). Dwarfism with retinal atrophy and deafness. *Archives of Disease in Childhood, 11*(61), 1-8.

D'Amours, D., Desnoyers, S., D'Silva, I., & Poirier, G. G. (1999). Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *The Biochemical Journal, 342 (Pt 2)*(Pt 2), 249-268.

Dantzer, F., de La Rubia, G., Menissier-De Murcia, J., Hostomsky, Z., de Murcia, G., & Schreiber, V. (2000). Base excision repair is impaired in mammalian cells lacking poly(ADP-ribose) polymerase-1. *Biochemistry, 39*(25), 7559-7569. bi0003442.

Dantzer, F., Schreiber, V., Niedergang, C., Trucco, C., Flatter, E., De La Rubia, G., . . . de Murcia, G. (1999). Involvement of poly(ADP-ribose) polymerase in base excision repair. *Biochimie, 81*(1-2), 69-75. S0300-9084(99)80040-6.

de Murcia, G., & Menissier de Murcia, J. (1994). Poly(ADP-ribose) polymerase: A molecular nick-sensor. *Trends in Biochemical Sciences, 19*(4), 172-176. 0968-0004(94)90280-1.

de Waard, H., de Wit, J., Andressoo, J. O., van Oostrom, C. T., Riis, B., Weimann, A., . . . van der Horst, G T. (2004). Different effects of CSA and CSB deficiency on sensitivity to oxidative DNA damage. *Molecular and Cellular Biology, 24*(18), 7941-7948. 10.1128/MCB.24.18.7941-7948.2004.

de Waard, H., de Wit, J., Gorgels, T., van den Aardweg, G., Andressoo, J. O., Vermeij, M., . . . van der Horst, G. (2003). Cell type-specific hypersensitivity to oxidative damage in CSB and XPA mice. *Dna Repair, 2*(1), 13-25. 10.1016/S1568-7864(02)00188-X.

D'Errico, M., Pascucci, B., Iorio, E., Van Houten, B., & Dogliotti, E. (2013). The role of CSA and CSB protein in the oxidative stress response. *Mechanisms of Ageing and Development, 134*(5-6), 261-269. 10.1016/j.mad.2013.03.006.

Dianov, G. L., Houle, J. F., Iyer, N., Bohr, V. A., & Friedberg, E. C. (1997). Reduced RNA polymerase II transcription in extracts of cockayne syndrome and xeroderma pigmentosum/cockayne syndrome cells. *Nucleic Acids Research, 25*(18), 3636-3642. gka581.

Dianov, G. L., & Hubscher, U. (2013). Mammalian base excision repair: The forgotten archangel. *Nucleic Acids Research, 41*(6), 3483-3490. 10.1093/nar/gkt076.

Dianov, G. L., Prasad, R., Wilson, S. H., & Bohr, V. A. (1999). Role of DNA polymerase beta in the excision step of long patch mammalian base excision repair. *The Journal of Biological Chemistry, 274*(20), 13741-13743.

Dianov, G. L., Thybo, T., Dianova, I. I., Lipinski, L. J., & Bohr, V. A. (2000). Single nucleotide patch base excision repair is the major pathway for removal of thymine glycol from DNA in human cell extracts. *The Journal of Biological Chemistry, 275*(16), 11809-11813.

Dianov, G., Bischoff, C., Piotrowski, J., & Bohr, V. A. (1998). Repair pathways for processing of 8-oxoguanine in DNA by mammalian cell extracts. *The Journal of Biological Chemistry, 273*(50), 33811-33816.

Dianov, G., Bischoff, C., Sunesen, M., & Bohr, V. A. (1999). Repair of 8-oxoguanine in DNA is deficient in cockayne syndrome group B cells. *Nucleic Acids Research, 27*(5), 1365-1368. gkc238.

Dianov, G., Price, A., & Lindahl, T. (1992). Generation of single-nucleotide repair patches following excision of uracil residues from DNA. *Molecular and Cellular Biology, 12*(4), 1605-1612.

Dizdaroglu, M. (1992). Oxidative damage to DNA in mammalian chromatin. *Mutation Research, 275*(3-6), 331-342. 0921-8734(92)90036-O.

Eisen, J. A., Sweder, K. S., & Hanawalt, P. C. (1995). Evolution of the SNF2 family of proteins: Subfamilies with distinct sequences and functions. *Nucleic Acids Research, 23*(14), 2715-2723. 5a0167.

El-Khamisy, S. F., Masutani, M., Suzuki, H., & Caldecott, K. W. (2003). A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. *Nucleic Acids Research, 31*(19), 5526-5533.

Enoiu, M., Jiricny, J., & Scharer, O. D. (2012). Repair of cisplatin-induced DNA interstrand crosslinks by a replication-independent pathway involving transcription-coupled repair and translesion synthesis. *Nucleic Acids Research, 40*(18), 8953-8964. 10.1093/nar/gks670.

Epanchintsev, A., Costanzo, F., Rauschendorf, M. A., Caputo, M., Ye, T., Donnio, L. M., . . . Egly, J. M. (2017). Cockayne's syndrome A and B proteins regulate transcription arrest after genotoxic stress by promoting ATF3 degradation. *Molecular Cell, 68*(6), 1066.e6. S1097-2765(17)30844-4.

Fan, H. Y., Narlikar, G. J., & Kingston, R. E. (2004). Noncovalent modification of chromatin: Different remodeled products with different ATPase domains. *Cold Spring Harbor Symposia on Quantitative Biology, 69*, 183-192. 10.1101/sqb.2004.69.183.

Fan, H. Y., Trotter, K. W., Archer, T. K., & Kingston, R. E. (2005). Swapping function of two chromatin remodeling complexes. *Molecular Cell, 17*(6), 805-815. S1097-2765(05)01123-8.

Filippova, G. N., Fagerlie, S., Klenova, E. M., Myers, C., Dehner, Y., Goodwin, G., . . . Lobanenkov, V. V. (1996). An exceptionally conserved transcriptional repressor, CTCF, employs different combinations of zinc fingers to bind diverged promoter sequences of avian and mammalian c-myc oncogenes. *Molecular and Cellular Biology, 16*(6), 2802-2813.

Fisher, A. E., Hochegger, H., Takeda, S., & Caldecott, K. W. (2007). Poly(ADP-ribose) polymerase 1 accelerates single-strand break repair in concert with poly(ADP-ribose) glycohydrolase. *Molecular and Cellular Biology, 27*(15), 5597-5605. MCB.02248-06.

Flaus, A., Martin, D. M., Barton, G. J., & Owen-Hughes, T. (2006). Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. *Nucleic Acids Research, 34*(10), 2887-2905. 34/10/2887.

Fousteri, M., Vermeulen, W., van Zeeland, A. A., & Mullenders, L. H. F. (2006). Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. *Molecular Cell, 23*(4), 471-482. 10.1016/j.molcel.2006.06.029.

Friedberg, E. C., Bardwell, A. J., Bardwell, L., Wang, Z., & Dianov, G. (1994). Transcription and nucleotide excision repair--reflections, considerations and recent biochemical insights. *Mutation Research, 307*(1), 5-14. 0027-5107(94)90272-0.

Frosina, G., Fortini, P., Rossi, O., Carrozzino, F., Raspaglio, G., Cox, L. S., . . . Dogliotti, E. (1996). Two pathways for base excision repair in mammalian cells. *The Journal of Biological Chemistry, 271*(16), 9573-9578.

Furuta, T., Ueda, T., Aune, G., Sarasin, A., Kraemer, K. H., & Pommier, Y. (2002). Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. *Cancer Research, 62*(17), 4899-4902.

Gibson, B. A., Zhang, Y., Jiang, H., Hussey, K. M., Shrimp, J. H., Lin, H., . . . Kraus, W. L. (2016). Chemical genetic discovery of PARP targets reveals a role for PARP-1 in transcription elongation. *Science (New York, N.Y.), 353*(6294), 45-50. 10.1126/science.aaf7865.

Giorno, R., & Sauerbier, W. (1976). A radiological analysis of the transcription units for heterogeneous nuclear RNA in cultured murine cells. *Cell, 9*(4 PT 2), 775-783. 0092-8674(76)90140-9.

Gottschalk, A. J., Timinszky, G., Kong, S. E., Jin, J., Cai, Y., Swanson, S. K., . . . Conaway, R. C. (2009). Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. *Proceedings of the National Academy of Sciences of the United States of America, 106*(33), 13770-13774. 10.1073/pnas.0906920106.

Gray, L. T., Fong, K. K., Pavelitz, T., & Weiner, A. M. (2012). Tethering of the conserved piggyBac transposase fusion protein CSB-PGBD3 to chromosomal AP-1 proteins regulates expression of nearby genes in humans. *PLoS Genetics, 8*(9), e1002972. 10.1371/journal.pgen.1002972.

Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., . . . Nakatani, Y. (2003). The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell, 113*(3), 357-367. S0092867403003167.

Guo, Y., Xu, Q., Canzio, D., Shou, J., Li, J., Gorkin, D. U., . . . Wu, Q. (2015). CRISPR inversion of CTCF sites alters genome topology and enhancer/promoter function. *Cell, 162*(4), 900-910. 10.1016/j.cell.2015.07.038.

Hai, T., & Hartman, M. G. (2001). The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: Activating transcription factor proteins and homeostasis. *Gene, 273*(1), 1-11. S0378-1119(01)00551-0.

Hai, T., Wolfgang, C. D., Marsee, D. K., Allen, A. E., & Sivaprasad, U. (1999). ATF3 and stress responses. *Gene Expression, 7*(4-6), 321-335.

Hanawalt, P. C. (1994). Transcription-coupled repair and human disease. *Science (New York, N.Y.), 266*(5193), 1957-1958.

Hanawalt, P. C., & Spivak, G. (2008). Transcription-coupled DNA repair: Two decades of progress and surprises. *Nature Reviews Molecular Cell Biology, 9*(12), 958-970. 10.1038/nrm2549.

Hanzlikova, H., Gittens, W., Krejcikova, K., Zeng, Z., & Caldecott, K. W. (2017). Overlapping roles for PARP1 and PARP2 in the recruitment of endogenous XRCC1 and PNKP into oxidized chromatin. *Nucleic Acids Research, 45*(5), 2546-2557. 10.1093/nar/gkw1246.

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y. C., Laslo, P., . . . Glass, C. K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Molecular Cell, 38*(4), 576-589. 10.1016/j.molcel.2010.05.004.

Hopfner, K. P., Gerhold, C. B., Lakomek, K., & Wollmann, P. (2012). Swi2/Snf2 remodelers: Hybrid views on hybrid molecular machines. *Current Opinion in Structural Biology, 22*(2), 225-233. 10.1016/j.sbi.2012.02.007.

Huang da, W., Sherman, B. T., & Lempicki, R. A. (2009a). Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Research, 37*(1), 1-13. 10.1093/nar/gkn923.

Huang da, W., Sherman, B. T., & Lempicki, R. A. (2009b). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols, 4*(1), 44-57. 10.1038/nprot.2008.211.

Hwang, H., & Myong, S. (2014). Protein induced fluorescence enhancement (PIFE) for probing protein-nucleic acid interactions. *Chemical Society Reviews, 43*(4), 1221-1229. 10.1039/c3cs60201j.

Imam, S. Z., Indig, F. E., Cheng, W. H., Saxena, S. P., Stevnsner, T., Kufe, D., & Bohr, V. A. (2007). Cockayne syndrome protein B interacts with and is phosphorylated by c-abl tyrosine kinase. *Nucleic Acids Research, 35*(15), 4941-4951. gkm386.

Iyama, T., Lee, S. Y., Berquist, B. R., Gileadi, O., Bohr, V. A., Seidman, M. M., . . . Wilson, D. M.,3rd. (2015). CSB interacts with SNM1A and promotes DNA interstrand crosslink processing. *Nucleic Acids Research, 43*(1), 247-258. 10.1093/nar/gku1279 [doi]

Iyama, T., & Wilson, D. M.,3rd. (2016). Elements that regulate the DNA damage response of proteins defective in cockayne syndrome. *Journal of Molecular Biology, 428*(1), 62-78. 10.1016/j.jmb.2015.11.020.

Jaruga, P., Birincioglu, M., Rosenquist, T. A., & Dizdaroglu, M. (2004). Mouse NEIL1 protein is specific for excision of 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine from oxidatively damaged DNA. *Biochemistry, 43*(50), 15909-15914. 10.1021/bi048162l.

Karikkineth, A. C., Scheibye-Knudsen, M., Fivenson, E., Croteau, D. L., & Bohr, V. A. (2017). Cockayne syndrome: Clinical features, model systems and pathways. *Ageing Research Reviews, 33*, 3-17. S1568-1637(16)30177-5.

Khobta, A., & Epe, B. (2013). Repair of oxidatively generated DNA damage in cockayne syndrome. *Mechanisms of Ageing and Development, 134*(5-6), 253-260. 10.1016/j.mad.2013.03.001.

Kipling, D., & Faragher, R. G. (1997). Progeroid syndromes: Probing the molecular basis of aging? *Molecular Pathology : MP, 50*(5), 234-241.

Kirkali, G., de Souza-Pinto, N. C., Jaruga, P., Bohr, V. A., & Dizdaroglu, M. (2009). Accumulation of (5'S)-8,5'-cyclo-2'-deoxyadenosine in organs of cockayne syndrome complementation group B gene knockout mice. *DNA Repair, 8*(2), 274-278. 10.1016/j.dnarep.2008.09.009.

Klungland, A., & Bjelland, S. (2007). Oxidative damage to purines in DNA: Role of mammalian Ogg1. *DNA Repair, 6*(4), 481-488. S1568-7864(06)00321-1.

Klungland, A., & Lindahl, T. (1997). Second pathway for completion of human DNA base excision-repair: Reconstitution with purified proteins and requirement for DNase IV (FEN1). *The EMBO Journal, 16*(11), 3341-3348. 10.1093/emboj/16.11.3341.

Kraus, W. L., & Lis, J. T. (2003). PARP goes transcription. *Cell, 113*(6), 677-683. S0092867403004331.

Kristensen, U., Epanchintsev, A., Rauschendorf, M. A., Laugel, V., Stevnsner, T., Bohr, V. A., . . . Egly, J. M. (2013). Regulatory interplay of cockayne syndrome B ATPase and stress-

response gene ATF3 following genotoxic stress. *Proceedings of the National Academy of Sciences of the United States of America, 110*(25), 2261. 10.1073/pnas.1220071110.

Krokan, H. E., Standal, R., & Slupphaug, G. (1997). DNA glycosylases in the base excision repair of DNA. *The Biochemical Journal, 325* (Pt 1), 1-16.

Kubota, Y., Nash, R. A., Klungland, A., Schar, P., Barnes, D. E., & Lindahl, T. (1996). Reconstitution of DNA base excision-repair with purified human proteins: Interaction between DNA polymerase beta and the XRCC1 protein. *The EMBO Journal, 15*(23), 6662-6670.

Kyng, K. J., May, A., Brosh, R. M., Jr, Cheng, W. H., Chen, C., Becker, K. G., & Bohr, V. A. (2003). The transcriptional response after oxidative stress is defective in cockayne syndrome group B cells. *Oncogene, 22*(8), 1135-1149. 10.1038/sj.onc.1206187.

Lake, R. J., Boetefuer, E. L., Tsai, P. F., Jeong, J., Choi, I., Won, K. J., & Fan, H. Y. (2014). The sequence-specific transcription factor c-jun targets cockayne syndrome protein B to regulate transcription and chromatin structure. *PLoS Genetics, 10*(4), e1004284. 10.1371/journal.pgen.1004284.

Lake, R. J., Boetefuer, E. L., Won, K. J., & Fan, H. Y. (2016). The CSB chromatin remodeler and CTCF architectural protein cooperate in response to oxidative stress. *Nucleic Acids Research, 44*(5), 2125-2135. gkv1219.

Lake, R. J., & Fan, H. (2013). Structure, function and regulation of CSB: A multi-talented gymnast. *Mechanisms of Ageing and Development, 134*(5-6), 202-211. 10.1016/j.mad.2013.02.004.

Lake, R. J., Geyko, A., Hemashettar, G., Zhao, Y., & Fan, H. (2010). UV-induced association of the CSB remodeling protein with chromatin requires ATP-dependent relief of N-terminal autorepression. *Molecular Cell, 37*(2), 235-246. 10.1016/j.molcel.2009.10.027.

Lee, B. K., & Iyer, V. R. (2012). Genome-wide studies of CCCTC-binding factor (CTCF) and cohesin provide insight into chromatin structure and regulation. *The Journal of Biological Chemistry, 287*(37), 30906-30913. 10.1074/jbc.R111.324962.

Lee, J. Y., Lake, R. J., Kirk, J., Bohr, V. A., Fan, H. Y., & Hohng, S. (2017). NAP1L1 accelerates activation and decreases pausing to enhance nucleosome remodeling by CSB. *Nucleic Acids Research, 45*(8), 4696-4707. 10.1093/nar/gkx188.

Lehmann, A. R. (1982). Three complementation groups in cockayne syndrome. *Mutation Research, 106*(2), 347-356. 0027-5107(82)90115-4.

Li, L., Lyu, X., Hou, C., Takenaka, N., Nguyen, H. Q., Ong, C. T., . . . Corces, V. G. (2015). Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. *Molecular Cell, 58*(2), 216-231. 10.1016/j.molcel.2015.02.023.

Li, M. D., Burns, T. C., Morgan, A. A., & Khatri, P. (2014). Integrated multi-cohort transcriptional meta-analysis of neurodegenerative diseases. *Acta Neuropathologica Communications, 2*, y. 10.1186/s40478-014-0093-y.

Li, S. (2015). Transcription coupled nucleotide excision repair in the yeast saccharomyces cerevisiae: The ambiguous role of Rad26. *DNA Repair, 36*, 43-48. 10.1016/j.dnarep.2015.09.006.

Licht, C. L., Stevnsner, T., & Bohr, V. A. (2003). Cockayne syndrome group B cellular and biochemical functions. *American Journal of Human Genetics, 73*(6), 1217-1239. S0002-9297(07)63976-7.

Lindahl, T. (1974). An N-glycosidase from escherichia coli that releases free uracil from DNA containing deaminated cytosine residues. *Proceedings of the National Academy of Sciences of the United States of America, 71*(9), 3649-3653.

Lindahl, T. (1979). DNA glycosylases, endonucleases for apurinic/apyrimidinic sites, and base excision-repair. *Progress in Nucleic Acid Research and Molecular Biology, 22*, 135-192.

Lindahl, T. (1980). Uracil-DNA glycosylase from escherichia coli. *Methods in Enzymology, 65*(1), 284-290.

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. *Nature, 362*(6422), 709-715. 10.1038/362709a0.

Lombard, D. B., Chua, K. F., Mostoslavsky, R., Franco, S., Gostissa, M., & Alt, F. W. (2005). DNA repair, genome stability, and aging. *Cell, 120*(4), 497-512. S0092-8674(05)00102-9.

Luijsterburg, M. S., de Krijger, I., Wiegant, W. W., Shah, R. G., Smeenk, G., de Groot, A. J., . . . van Attikum, H. (2016). PARP1 links CHD2-mediated chromatin expansion and H3.3 deposition to DNA repair by non-homologous end-joining. *Molecular Cell, 61*(4), 547-562. 10.1016/j.molcel.2016.01.019.

Lutz, M., Burke, L. J., Barreto, G., Goeman, F., Greb, H., Arnold, R., . . . Renkawitz, R. (2000). Transcriptional repression by the insulator protein CTCF involves histone deacetylases. *Nucleic Acids Research, 28*(8), 1707-1713. gkd296.

Ma, W., Halweg, C. J., Menendez, D., & Resnick, M. A. (2012). Differential effects of poly(ADP-ribose) polymerase inhibition on DNA break repair in human cells are revealed with epstein-barr virus. *Proceedings of the National Academy of Sciences of the United States of America, 109*(17), 6590-6595. 10.1073/pnas.1118078109.

Mallery, D. L., Tanganelli, B., Colella, S., Steingrimsdottir, H., van Gool, A. J., Troelstra, C., . . . Lehmann, A. R. (1998). Molecular analysis of mutations in the CSB (ERCC6) gene in patients with cockayne syndrome. *American Journal of Human Genetics, 62*(1), 77-85. S0002-9297(07)60125-6.

Matsumoto, Y., & Kim, K. (1995). Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. *Science (New York, N.Y.), 269*(5224), 699-702.

Matsumoto, Y., Kim, K., & Bogenhagen, D. F. (1994). Proliferating cell nuclear antigen-dependent abasic site repair in xenopus laevis oocytes: An alternative pathway of base excision DNA repair. *Molecular and Cellular Biology, 14*(9), 6187-6197.

Mayne, L. V., & Lehmann, A. R. (1982). Failure of RNA-synthesis to recover after UV irradiation - an early defect in cells from individuals with cockaynes syndrome and xeroderma pigmentosum. *Cancer Research, 42*(4), 1473-1478.

McLean, C. Y., Bristor, D., Hiller, M., Clarke, S. L., Schaar, B. T., Lowe, C. B., . . . Bejerano, G. (2010). GREAT improves functional interpretation of cis-regulatory regions. *Nature Biotechnology, 28*(5), 495-501. 10.1038/nbt.1630.

Mellon, I., Spivak, G., & Hanawalt, P. C. (1987). Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell, 51*(2), 241-249. 0092-8674(87)90151-6.

Menoni, H., Hoeijmakers, J. H. J., & Vermeulen, W. (2012). Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions in vivo. *Journal of Cell Biology, 199*(7), 1037-1046. 10.1083/jcb.201205149.

Muftuoglu, M., de Souza-Pinto, N. C., Dogan, A., Aamann, M., Stevnsner, T., Rybanska, I., . . . Bohr, V. A. (2009). Cockayne syndrome group B protein stimulates repair of formamidopyrimidines by NEIL1 DNA glycosylase. *The Journal of Biological Chemistry, 284*(14), 9270-9279. 10.1074/jbc.M807006200.

Muftuoglu, M., Sharma, S., Thorslund, T., Stevnsner, T., Soerensen, M. M., Brosh, R. M.,Jr, & Bohr, V. A. (2006). Cockayne syndrome group B protein has novel strand annealing and exchange activities. *Nucleic Acids Research, 34*(1), 295-304. 34/1/295.

Nance, M. A., & Berry, S. A. (1992). Cockayne syndrome - review of 140 cases. *American Journal of Medical Genetics, 42*(1), 68-84. 10.1002/ajmg.1320420115.

Nash, R. A., Caldecott, K. W., Barnes, D. E., & Lindahl, T. (1997). XRCC1 protein interacts with one of two distinct forms of DNA ligase III. *Biochemistry, 36*(17), 5207-5211. 10.1021/bi962281m.

Newman, J. C., Bailey, A. D., Fan, H. Y., Pavelitz, T., & Weiner, A. M. (2008). An abundant evolutionarily conserved CSB-PiggyBac fusion protein expressed in cockayne syndrome. *PLoS Genetics, 4*(3), e1000031. 10.1371/journal.pgen.1000031.

Newman, J. C., Bailey, A. D., & Weiner, A. M. (2006). Cockayne syndrome group B protein (CSB) plays a general role in chromatin maintenance and remodeling. *Proceedings of the National Academy of Sciences of the United States of America, 103*(25), 9613-9618. 10.1073/pnas.0510909103.

Nicolai, S., Filippi, S., Caputo, M., Cipak, L., Gregan, J., Ammerer, G., . . . Proietti-De-Santis, L. (2015). Identification of novel proteins co-purifying with cockayne syndrome group B (CSB) reveals potential roles for CSB in RNA metabolism and chromatin dynamics. *PloS One, 10*(6), e0128558. 10.1371/journal.pone.0128558.

Ong, C. T., & Corces, V. G. (2014). CTCF: An architectural protein bridging genome topology and function. *Nature Reviews.Genetics, 15*(4), 234-246. 10.1038/nrg3663.

Orta, M. L., Hoglund, A., Calderon-Montano, J. M., Dominguez, I., Burgos-Moron, E., Visnes, T., . . . Helleday, T. (2014). The PARP inhibitor olaparib disrupts base excision repair of 5-aza-2'-deoxycytidine lesions. *Nucleic Acids Research, 42*(14), 9108-9120. 10.1093/nar/gku63.8.

Osenbroch, P. O., Auk-Emblem, P., Halsne, R., Strand, J., Forstrom, R. J., van der Pluijm, I., & Eide, L. (2009). Accumulation of mitochondrial DNA damage and bioenergetic dysfunction in CSB defective cells. *The FEBS Journal, 276*(10), 2811-2821. 10.1111/j.1742-4658.2009.07004.x.

Osterod, M., Larsen, E., Le Page, F., Hengstler, J. G., Van Der Horst, G T, Boiteux, S., . . . Epe, B. (2002). A global DNA repair mechanism involving the cockayne syndrome B (CSB) gene product can prevent the in vivo accumulation of endogenous oxidative DNA base damage. *Oncogene, 21*(54), 8232-8239. 10.1038/sj.onc.1206027.

Pachkowski, B. F., Tano, K., Afonin, V., Elder, R. H., Takeda, S., Watanabe, M., . . . Nakamura, J. (2009). Cells deficient in PARP-1 show an accelerated accumulation of DNA single strand breaks, but not AP sites, over the PARP-1-proficient cells exposed to MMS. *Mutation Research, 671*(1-2), 93-99. 10.1016/j.mrfmmm.2009.09.006.

Parsons, J. L., Dianova, I. I., Allinson, S. L., & Dianov, G. L. (2005). Poly(ADP-ribose) polymerase-1 protects excessive DNA strand breaks from deterioration during repair in human cell extracts. *The FEBS Journal, 272*(8), 2012-2021. EJB4628.

Pascucci, B., Lemma, T., Iorio, E., Giovannini, S., Vaz, B., Iavarone, I., . . . D'Errico, M. (2012). An altered redox balance mediates the hypersensitivity of cockayne syndrome primary fibroblasts to oxidative stress. *Aging Cell, 11*(3), 520-529. 10.1111/j.1474-9726.2012.00815.x.

Plasschaert, R. N., Vigneau, S., Tempera, I., Gupta, R., Maksimoska, J., Everett, L., . . . Bartolomei, M. S. (2014). CTCF binding site sequence differences are associated with unique regulatory and functional trends during embryonic stem cell differentiation. *Nucleic Acids Research, 42*(2), 774-789. 10.1093/nar/gkt910.

Pleschke, J. M., Kleczkowska, H. E., Strohm, M., & Althaus, F. R. (2000). Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins. *The Journal of Biological Chemistry, 275*(52), 40974-40980. 10.1074/jbc.M006520200.

Podlutsky, A. J., Dianova, I. I., Podust, V. N., Bohr, V. A., & Dianov, G. L. (2001). Human DNA polymerase beta initiates DNA synthesis during long-patch repair of reduced AP sites in DNA. *The EMBO Journal, 20*(6), 1477-1482. 10.1093/emboj/20.6.1477.

Proietti-De-Santis, L., Drane, P., & Egly, J. M. (2006). Cockayne syndrome B protein regulates the transcriptional program after UV irradiation. *The EMBO Journal, 25*(9), 1915-1923. 7601071.

Pyle, A. M. (2008). Translocation and unwinding mechanisms of RNA and DNA helicases. *Annual Review of Biophysics, 37*, 317-336. 10.1146/annurev.biophys.37.032807.125908.

Ranes, M., Boeing, S., Wang, Y., Wienholz, F., Menoni, H., Walker, J., . . . Svejstrup, J. Q. (2016). A ubiquitylation site in cockayne syndrome B required for repair of oxidative DNA damage, but not for transcription-coupled nucleotide excision repair. *Nucleic Acids Research, 44*(11), 5246-5255. 10.1093/nar/gkw216.

Ray Chaudhuri, A., & Nussenzweig, A. (2017). The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nature Reviews.Molecular Cell Biology, 18*(10), 610-621. 10.1038/nrm.2017.53.

Reynolds, P., Cooper, S., Lomax, M., & O'Neill, P. (2015). Disruption of PARP1 function inhibits base excision repair of a sub-set of DNA lesions. *Nucleic Acids Research, 43*(8), 4028-4038. 10.1093/nar/gkv250.

Rockx, D. A., Mason, R., van Hoffen, A., Barton, M. C., Citterio, E., Bregman, D. B., . . . Mullenders, L. H. (2000). UV-induced inhibition of transcription involves repression of transcription initiation and phosphorylation of RNA polymerase II. *Proceedings of the National Academy of Sciences of the United States of America, 97*(19), 10503-10508. 10.1073/pnas.180169797.

Satoh, M. S., & Lindahl, T. (1992). Role of poly(ADP-ribose) formation in DNA repair. *Nature, 356*(6367), 356-358. 10.1038/356356a0.

Scheibye-Knudsen, M., Croteau, D. L., & Bohr, V. A. (2013). Mitochondrial deficiency in cockayne syndrome. *Mechanisms of Ageing and Development, 134*(5-6), 275-283. 10.1016/j.mad.2013.02.007.

Scheibye-Knudsen, M., Mitchell, S. J., Fang, E. F., Iyama, T., Ward, T., Wang, J., . . . Bohr, V. A. (2014). A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. *Cell Metabolism, 20*(5), 840-855. 10.1016/j.cmet.2014.10.005.

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols, 3*(6), 1101-1108.

Selby, C. P., Drapkin, R., Reinberg, D., & Sancar, A. (1997). RNA polymerase II stalled at a thymine dimer: Footprint and effect on excision repair. *Nucleic Acids Research, 25*(4), 787-793. gka137.

Selby, C. P., & Sancar, A. (1997a). Cockayne syndrome group B protein enhances elongation by RNA polymerase II. *Proceedings of the National Academy of Sciences of the United States of America, 94*(21), 11205-11209.

Selby, C. P., & Sancar, A. (1997b). Human transcription-repair coupling factor CSB/ERCC6 is a DNA-stimulated ATPase but is not a helicase and does not disrupt the ternary transcription complex of stalled RNA polymerase II. *The Journal of Biological Chemistry, 272*(3), 1885-1890.

Selzer, R. R., Nyaga, S., Tuo, J. S., May, A., Muftuoglu, M., Christiansen, M., . . . Bohr, V. A. (2002). Differential requirement for the ATPase domain of the cockayne syndrome group B gene in the processing of UV-induced DNA damage and 8-oxoguanine lesions in human cells. *Nucleic Acids Research, 30*(3), 782-793. 10.1093/nar/30.3.782

Sena, L. A., & Chandel, N. S. (2012). Physiological roles of mitochondrial reactive oxygen species. *Molecular Cell, 48*(2), 158-167. 10.1016/j.molcel.2012.09.025.

Singhal, R. K., & Wilson, S. H. (1993). Short gap-filling synthesis by DNA polymerase beta is processive. *The Journal of Biological Chemistry, 268*(21), 15906-15911.

Smeenk, G., Wiegant, W. W., Marteijn, J. A., Luijsterburg, M. S., Sroczynski, N., Costelloe, T., . . . van Attikum, H. (2013). Poly(ADP-ribosyl)ation links the chromatin remodeler SMARCA5/SNF2H to RNF168-dependent DNA damage signaling. *Journal of Cell Science, 126*(Pt 4), 889-903. 10.1242/jcs.109413.

Sobol, R. W., Horton, J. K., Kuhn, R., Gu, H., Singhal, R. K., Prasad, R., . . . Wilson, S. H. (1996). Requirement of mammalian DNA polymerase-beta in base-excision repair. *Nature, 379*(6561), 183-186. 10.1038/379183a0.

Stevnsner, T., Muftuoglu, M., Aamann, M. D., & Bohr, V. A. (2008). The role of cockayne syndrome group B (CSB) protein in base excision repair and aging. *Mechanisms of Ageing and Development, 129*(7-8), 441-448. 10.1016/j.mad.2008.04.009.

Strom, C. E., Johansson, F., Uhlen, M., Szigyarto, C. A., Erixon, K., & Helleday, T. (2011). Poly (ADP-ribose) polymerase (PARP) is not involved in base excision repair but PARP

inhibition traps a single-strand intermediate. *Nucleic Acids Research, 39*(8), 3166-3175. 10.1093/nar/gkq1241.

Svejstrup, J. Q. (2002). Mechanisms of transcription-coupled DNA repair. *Nature Reviews.Molecular Cell Biology, 3*(1), 21-29. 10.1038/nrm703.

Tanaka, K., Kawai, K., Kumahara, Y., Ikenaga, M., & Okada, Y. (1981). Genetic complementation groups in cockayne syndrome. *Somatic Cell Genetics, 7*(4), 445-455.

Tantin, D., Kansal, A., & Carey, M. (1997). Recruitment of the putative transcription-repair coupling factor CSB/ERCC6 to RNA polymerase II elongation complexes. *Molecular and Cellular Biology, 17*(12), 6803-6814.

Thorslund, T., von Kobbe, C., Harrigan, J. A., Indig, F. E., Christiansen, M., Stevnsner, T., & Bohr, V. A. (2005). Cooperation of the cockayne syndrome group B protein and poly(ADP-ribose) polymerase 1 in the response to oxidative stress. *Molecular and Cellular Biology, 25*(17), 7625-7636. 25/17/7625.

Troelstra, C., Odijk, H., de Wit, J., Westerveld, A., Thompson, L. H., Bootsma, D., & Hoeijmakers, J. H. (1990). Molecular cloning of the human DNA excision repair gene ERCC-6. *Molecular and Cellular Biology, 10*(11), 5806-5813.

Troelstra, C., van Gool, A., de Wit, J., Vermeulen, W., Bootsma, D., & Hoeijmakers, J. (1992). ERCC6, a member of a subfamily of putative helicases, is involved in cockaynes-syndrome and preferential repair of active genes. *Cell, 71*(6), 939-953. 10.1016/0092-8674(92)90390-X.

Tuo, J., Chen, C., Zeng, X., Christiansen, M., & Bohr, V. A. (2002). Functional crosstalk between hOgg1 and the helicase domain of cockayne syndrome group B protein. *DNA Repair, 1*(11), 913-927. S1568786402001167.

Tuo, J., Jaruga, P., Rodriguez, H., Bohr, V. A., & Dizdaroglu, M. (2003). Primary fibroblasts of cockayne syndrome patients are defective in cellular repair of 8-hydroxyguanine and 8-hydroxyadenine resulting from oxidative stress. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology, 17*(6), 668-674. 10.1096/fj.02-0851com.

Tuo, J., Jaruga, P., Rodriguez, H., Dizdaroglu, M., & Bohr, V. A. (2002). The cockayne syndrome group B gene product is involved in cellular repair of 8-hydroxyadenine in DNA. *The Journal of Biological Chemistry, 277*(34), 30832-30837. 10.1074/jbc.M204814200.

Tuo, J., Muftuoglu, M., Chen, C., Jaruga, P., Selzer, R. R., Brosh, R. M.,Jr, . . . Bohr, V. A. (2001). The cockayne syndrome group B gene product is involved in general genome base excision repair of 8-hydroxyguanine in DNA. *The Journal of Biological Chemistry, 276*(49), 45772-45779. 10.1074/jbc.M107888200.

van den Boom, V., Citterio, E., Hoogstraten, D., Zotter, A., Egly, J. M., van Cappellen, W. A., . . . Vermeulen, W. (2004). DNA damage stabilizes interaction of CSB with the transcription elongation machinery. *The Journal of Cell Biology, 166*(1), 27-36. 10.1083/jcb.200401056.

van Gool, A. J., Citterio, E., Rademakers, S., van Os, R., Vermeulen, W., Constantinou, A., . . . Hoeijmakers, J. H. (1997). The cockayne syndrome B protein, involved in transcription-coupled DNA repair, resides in an RNA polymerase II-containing complex. *The EMBO Journal, 16*(19), 5955-5965. 10.1093/emboj/16.19.5955.

Venema, J., Mullenders, L., Natarajan, A. T., Vanzeeland, A. A., & Mayne, L. V. (1990). The genetic-defect in cockayne syndrome is associated with a defect in repair of uv-induced dna damage in transcriptionally active dna. *Proceedings of the National Academy of Sciences of the United States of America, 87*(12), 4707-4711. 10.1073/pnas.87.12.4707.

Venema, J., van Hoffen, A., Natarajan, A. T., van Zeeland, A. A., & Mullenders, L. H. (1990). The residual repair capacity of xeroderma pigmentosum complementation group C fibroblasts is highly specific for transcriptionally active DNA. *Nucleic Acids Research, 18*(3), 443-448.

Vodenicharov, M. D., Sallmann, F. R., Satoh, M. S., & Poirier, G. G. (2000). Base excision repair is efficient in cells lacking poly(ADP-ribose) polymerase 1. *Nucleic Acids Research, 28*(20), 3887-3896.

Wang, J., Wang, Y., & Lu, L. (2012). De-SUMOylation of CCCTC binding factor (CTCF) in hypoxic stress-induced human corneal epithelial cells. *The Journal of Biological Chemistry, 287*(15), 12469-12479. 10.1074/jbc.M111.286641.

Wang, L., Limbo, O., Fei, J., Chen, L., Kim, B., Luo, J., . . . Wang, D. (2014). Regulation of the Rhp26 ERCC6/CSB chromatin remodeler by a novel conserved leucine latch motif. *Proceedings of the National Academy of Sciences of the United States of America, 111*(52), 18566-18571. 10.1073/pnas.1420227112.

Wang, Y., Chakravarty, P., Ranes, M., Kelly, G., Brooks, P. J., Neilan, E., . . . Svejstrup, J. Q. (2014). Dysregulation of gene expression as a cause of cockayne syndrome neurological disease. *Proceedings of the National Academy of Sciences of the United States of America, 111*(40), 14454-14459. 10.1073/pnas.1412569111.

Wang, Y., Jones-Tabah, J., Chakravarty, P., Stewart, A., Muotri, A., Laposa, R. R., & Svejstrup, J. Q. (2016). Pharmacological bypass of cockayne syndrome B function in neuronal differentiation. *Cell Reports, 14*(11), 2554-2561. 10.1016/j.celrep.2016.02.051.

Wang, Z. Q., Stingl, L., Morrison, C., Jantsch, M., Los, M., Schulze-Osthoff, K., & Wagner, E. F. (1997). PARP is important for genomic stability but dispensable in apoptosis. *Genes & Development, 11*(18), 2347-2358.

Watanabe, N., Dickinson, D. A., Liu, R. M., & Forman, H. J. (2004). Quinones and glutathione metabolism. *Methods in Enzymology, 378*, 319-340. 10.1016/S0076-6879(04)78024-6.

Wei, L., Nakajima, S., Bohm, S., Bernstein, K. A., Shen, Z., Tsang, M., . . . Lan, L. (2015). DNA damage during the G0/G1 phase triggers RNA-templated, cockayne syndrome B-dependent homologous recombination. *Proceedings of the National Academy of Sciences of the United States of America, 112*(27), 3495. 10.1073/pnas.1507105112.

Wei, Y. F., Robins, P., Carter, K., Caldecott, K., Pappin, D. J., Yu, G. L., . . . Schar, P. (1995). Molecular cloning and expression of human cDNAs encoding a novel DNA ligase IV and DNA ligase III, an enzyme active in DNA repair and recombination. *Molecular and Cellular Biology, 15*(6), 3206-3216.

Wilson, D. M., & Barsky, D. (2001). The major human abasic endonuclease: Formation, consequences and repair of abasic lesions in DNA. *Mutation Research, 485*(4), 283-307. S0921-8777(01)00063-5.

Wong, H. K., Muftuoglu, M., Beck, G., Imam, S. Z., Bohr, V. A., & Wilson, D. M.,3rd. (2007). Cockayne syndrome B protein stimulates apurinic endonuclease 1 activity and protects

against agents that introduce base excision repair intermediates. *Nucleic Acids Research, 35*(12), 4103-4113. gkm404.

Woodhouse, B. C., Dianova, I. I., Parsons, J. L., & Dianov, G. L. (2008). Poly(ADP-ribose) polymerase-1 modulates DNA repair capacity and prevents formation of DNA double strand breaks. *DNA Repair, 7*(6), 932-940. 10.1016/j.dnarep.2008.03.017.

Wu, P. K., Wang, J. Y., Chen, C. F., Chao, K. Y., Chang, M. C., Chen, W. M., & Hung, S. C. (2017). Early passage mesenchymal stem cells display decreased radiosensitivity and increased DNA repair activity. *Stem Cells Translational Medicine, 6*(6), 1504-1514. 10.1002/sctm.15-0394.

Xu, J., Lahiri, I., Wang, W., Wier, A., Cianfrocco, M. A., Chong, J., . . . Wang, D. (2017). Structural basis for the initiation of eukaryotic transcription-coupled DNA repair. *Nature, 551*(7682), 653-657. 10.1038/nature24658.

Yu, A., Fan, H. Y., Liao, D., Bailey, A. D., & Weiner, A. M. (2000). Activation of p53 or loss of the cockayne syndrome group B repair protein causes metaphase fragility of human U1, U2, and 5S genes. *Molecular Cell, 5*(5), 801-810. S1097-2765(00)80320-2.

Yusufzai, T., & Kadonaga, J. T. (2008). HARP is an ATP-driven annealing helicase. *Science (New York, N.Y.), 322*(5902), 748-750. 10.1126/science.1161233.

Zhou, H. X., & Wang, G. (2001). Predicted structures of two proteins involved in human diseases. *Cell Biochemistry and Biophysics, 35*(1), 35-47. 35.