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Review
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Perspectives in the investigation of Cockayne syndrome group B neurological disease: the utility of patient-derived brain organoid models

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Abstract: Cockayne syndrome (CS) group B (CSB), which results from mutations in the excision repair cross-complementation group 6 (*ERCC6*) genes, which produce CSB protein, is an autosomal recessive disease characterized by multiple progressive disorders including growth failure, microcephaly, skin photosensitivity, and premature aging. Clinical data show that brain atrophy, demyelination, and calcification are the main neurological manifestations of CS, which progress with time. Neuronal loss and calcification occur in various brain areas, particularly the cerebellum and basal ganglia, resulting in dyskinesia, ataxia, and limb tremors in CSB patients. However, the understanding of neurodevelopmental defects in CS has been constrained by the lack of significant neurodevelopmental and functional abnormalities observed in CSB-deficient mice. In this review, we focus on elucidating the protein structure and distribution of CSB and delve into the impact of CSB mutations on the development and function of the nervous system. In addition, we provide an overview of research models that have been instrumental in exploring CS disorders, with a forward-looking perspective on the substantial contributions that brain organoids are poised to further advance this field.

Key words: Cockayne syndrome; Cockayne syndrome group B (CSB); Neurological function; Cerebellum; Organoids

1 Introduction

Cockayne syndrome (CS), initially documented by Dr. Cockayne in 1936, is a genetic disorder with a shared array of symptoms but exhibiting significant phenotypic heterogeneity. Individuals with CS typically manifest with distinctive features, including microcephaly, progeroid facial characteristics, ocular anomalies, and varying degrees of hearing impairment. Neurologically, CS patients face a wide spectrum of complications, including developmental delay and regression, intellectual disability, movement disorders, tigroid leukodystrophy, basal ganglia calcifications, seizures, and tremors (Wilson et al., 2016; Karikkineth et al., 2017). Rarely, they also experience hemiplegic migraines and hemiplegic strokes (Atalay et al., 2021; Carroll et al., 2023). Since the clinical manifestations of CS cover a wide range of subtypes, Nance and Berry (1992) conducted a thorough examination of 140 cases of CS and categorized them into three distinct types. It is noteworthy that various subtypes of CS partially overlap and constitute a continuous spectrum (Laugel, 2013). Patients with type I typically exhibit normal features at birth and begin to develop clinical features between 1–2 years of age. These symptoms progressively worsen and often lead to mortality between 20–30 years of age. Conversely, type II patients are characterized by more severe symptoms,

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often apparent at birth or even earlier. These individuals usually have a survival rate of no more than ten years. Type III, the mildest form, manifests later in childhood, and those affected may have a lifespan extending to several decades (Karikkineth et al., 2017; Calmels et al., 2018). The accurate diagnosis of CS has been historically challenging due to the diverse array of clinical symptoms, with genome sequencing widely utilized only recently to uncover the potential links between genomic alterations and phenotypic manifestations.

Approximately two-thirds of CS cases present excision repair cross-complementation group 6 (*ERCC6*) mutations, and the rest have mutations in the *ERCC8* gene, which produces the protein Cockayne syndrome protein A (CSA) (Karikkineth et al., 2017). Patients with Cockayne syndrome group B (CSB) and CSA mutations often have similar clinical symptoms, with a slight tendency of more severe symptoms in CSB patients (Calmels et al., 2018). Therefore, studies on CS have mainly addressed the CSB protein function, yet the heterogeneity of disease pathogenesis-associated links between CSB activity and CS symptoms remains partially obscure. In this review, we mainly focus on describing the structure and distribution of the CSB protein, as well as the effects of CSB mutations on neurological development and functions. In addition, we discuss the research models that have been developed so far to investigate CS disorders and the promising contribution that brain organoids are expected to make in this field.

2 CSB protein structure and localization

The full length of human CSB is 1493 amino acids (about 168 kDa). The N-terminal region of CSB is followed by acidic domain, central helicase domain, and nuclear localization signal (NLS) sequences. The helicase region of CSB comprises seven highly conserved canonical modules of ATPase motifs. The C-terminus of the protein includes ubiquitin-binding domain (UBD) and winged helix domain (WHD) (Fig. 1a). The Switch/sucrose non-fermentable (SWI2/SNF2)-family protein, CSB, exhibits adenosine triphosphate (ATP)-dependent chromatin remodeling activity but lacks helicase activity (Citterio et al., 2000). The CSB domain functions are listed in Table 1. CSB functions as a homodimer (Tiwari et al., 2021). The N-terminus intramolecularly interacts with the ATPase motif to inhibit ATPase activity (Cho et al., 2013; Batenburg et al., 2017). Furthermore, the C-terminus stimulates the CSB ATPase and chromatin remodeling activities (Wang LF et al., 2014). CSB recruits CSA via its CSA-interaction motif (CIM, upstream of the UBD) to DNA damage-stalled RNA polymerase II (Pol II) (van der Weegen et al., 2020). The WHD domain in its turn binds to ubiquitin, participates in DNA repair (Takahashi et al., 2019), and modulates transcription elongation (Batenburg et al., 2021).

The major CSB pool is found in the nucleus (Fig. 1b), where it is involved in a number of nuclear processes such as transcription and DNA repair (Scheibye-Knudsen et al., 2012; van den Heuvel et al., 2021). It is noteworthy that CSB has a dynamic localization during cell cycle progression. It is present in the cytoplasm at the beginning of mitosis and eventually accumulates in the intercellular bridge between two daughter cells, where it binds ubiquitin ligases and the proteasome to degrade the intercellular bridge (Paccosi et al., 2020). Under oxidative stress, CSB localizes in the mitochondria, acts as a sensor of DNA damage, and regulates mitophagy (Aamann et al., 2010; Scheibye-Knudsen et al., 2012; Kamenisch and Berneburg, 2013) (Fig. 1b). Although there is no evidence that CSB is localized in the endoplasmic reticulum, CSB deletion enhances the endoplasmic reticulum stress response to proteins that are not properly folded or conformed (Caputo et al., 2017). As for cell and tissue localization, the specificity of CSB is low, as may be seen in the *CSB* messenger RNA (mRNA) expression levels from the Human Protein Atlas (HPA) databases (Fig. 1c). The cerebellum, in particular, exhibits significantly higher levels of *CSB* mRNA expression, highlighting the crucial roles of CSB in the central nervous system (Fig. 1c).

Unlike other species such as mice, chickens, frogs, zebrafish, and worms, cells of primates such as humans and marmosets specifically express another CSB chimeric protein called CSB-PiggyBac transposable element-derived 3 (CSB-PGBD3) (International Human Genome Sequencing Consortium, 2001; Sarkar et al., 2003; Newman et al., 2008; Weiner and Gray, 2013). *PGBD3* is situated within intron 5 of the *ERCC6* gene (Fig. 1a). As a result, in primate cells, the *ERCC6* gene encodes the full-length CSB protein as well as CSB-PGBD3 fusion protein containing the initial 465 amino acids of the CSB protein (Newman

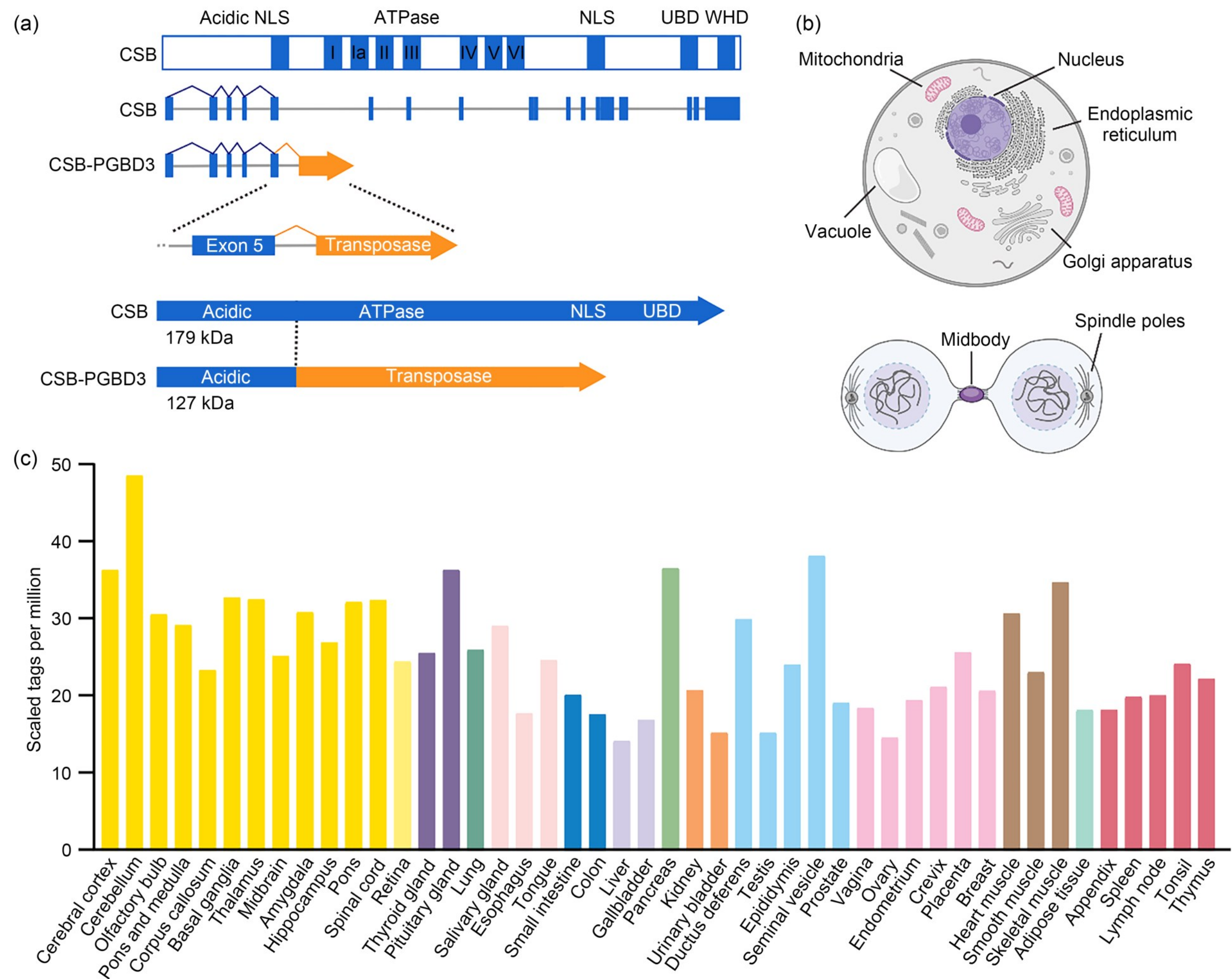

Fig. 1 Cockayne syndrome group B (CSB) protein structure and distribution. (a) Domain organization of CSB and CSB-PiggyBac transposable element-derived 3 (CSB-PGBD3). (b) Cellular localization of CSB (created with BioRender.com). (c) *CSB* messenger RNA (mRNA) expression levels in human tissues. The tissue data for mRNA expression from the functional annotation of mammalian genome 5 (FANTOM5) project using cap analysis of gene expression (CAGE) are presented as scaled tags per million. The color scheme is based on tissue groupings (Kawaji et al., 2017). NLS: nuclear localization signal; UBD: ubiquitin-binding domain; WHD: winged helix domain.

et al., 2008). The CSB-PGBD3 is generally four times more abundant than CSB (Newman et al., 2008; Weiner and Gray, 2013). Moreover, research revealed that CSB-PGBD3 is primarily located in the nuclei of oocytes from primordial, primary, secondary, and antral follicles (Qin et al., 2015). Importantly, sustained expression of CSB-PGBD3 has the potential to modify the transcriptome in the UVSS1KO cell line (Gray et al., 2012; Weiner and Gray, 2013). In conclusion, CSB-PGBD3 fusion proteins may exert an influence on gene transcription pathways that regulate embryonic developmental processes.

3 Genetic variants of CSB

The human gene mutation database (HGMD) provides information on CSB-causing mutations in the *CSB* exons, including about 50 missense/nonsense mutants, 22 deletion mutants, and 14 insertion mutants (Table S1). We mapped the mutant site distribution on the *CSB* exon and intron (Fig. 2a). The *CSB* exon and intron sequences were obtained from Universal Mutation Database (UMD). Most of these mutation sites are located in exons 5–10 and exons 13–15 that encode the NLS and ATPase domains (Fig. 2b). Intron sequence mutations typically affect pre-mRNA splicing,

Table 1 Cockayne syndrome group B (CSB) domain functions

Domain	Amino acids	Domain functions
CSB-N-terminal	1–510	Repression of CSB ATPase activity. Phosphorylation at Ser-10 and Ser-158 increases the N-terminal intramolecular interaction with ATP-binding domain, thereby inhibiting ATPase activity (Cho et al., 2013; Batenburg et al., 2017).
NLS3	285–354	CSB nuclear localization (NLS3) was predicted via computational analysis (Iyama et al., 2018).
NLS1	466–481	CSB nuclear localization (Iyama et al., 2018).
CSB-ATPase	510–960	Structural domain consisting of seven conserved helicase motifs with DNA-dependent ATPase activity but no helicase activity (Selby and Sancar, 1997).
NLS2	1038–1055	CSB nuclear localization (Iyama et al., 2018).
CSB-C-terminal	972–1493	Stimulation of the CSB ATPase activity and the chromatin-remodeling activity (Wang LF et al., 2014). Amino acid residues (1463–1493) are essential for interaction with RNA Pol II and chromatin, and CSA translocation to the nuclear matrix (Sin et al., 2016).
CIM	1385–1399	CSB–CSA interaction motif (van der Weegen et al., 2020).
UBD	1400–1428	Binding to ubiquitin and TC-NER (Lake et al., 2013).
WHD	1429–1493	Mediation of the CSB interaction with other proteins including MRE11/RAD50/NBS1 (Batenburg et al., 2019), RIF1 (Batenburg et al., 2017), RNA Pol II (Sin et al., 2016), and ubiquitin, which allow CSB to regulate transcription elongation and DNA repair (Takahashi et al., 2019; Batenburg et al., 2021).

NLS: nuclear localization signal; CIM: CSA-interaction motif; UBD: ubiquitin-binding domain; ATP: adenosine triphosphate; WHD: winged helix domain; TC-NER: transcription-coupled nucleotide excision repair; Pol II: polymerase II.

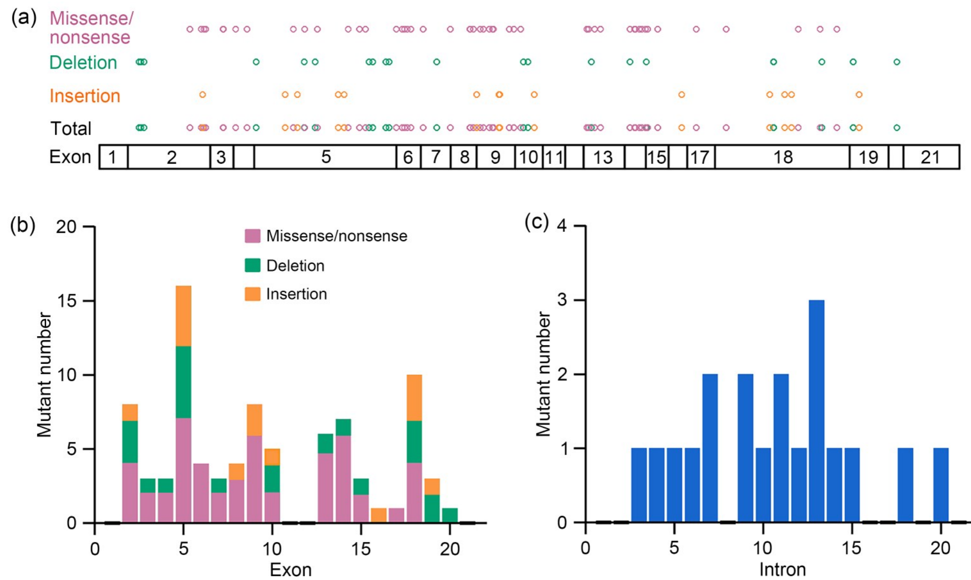

Fig. 2 Cockayne syndrome group B (*CSB*)-related mutations. (a) Genetic variants of *CSB* in each exon from clinical reports. Sourced from the human gene mutation database (HGMD). (b) Number of *CSB*-causing mutations in each exon from clinical reports. (c) Number of *CSB*-causing mutations in each intron from clinical reports.

which impacts protein expression (Anna and Monika, 2018). Various mutants of the *CSB* intron were also included on the HGMD, and these mutant sites are mainly located in introns 3–15 (Fig. 2c). Above all, based on the distribution of CSB mutation sites in each exon, the majority of CSB mutations are found in or affect the NLS and ATPase regions. This could imply that alterations in nuclear localization and DNA-dependent ATPase activity are frequent causes of the CSB symptoms.

Evolutional bioinformatics approaches have revealed that amino acid sequences from diverse species frequently share a common ancestry. Accordingly, the amino acid homology analysis is useful in understanding gene functions shared by homologous sequences and interspecies translational research (Fang et al.,

2010). We compared the homologs in CSB amino acid sequences from five species including *Homo sapiens*, *Mus musculus*, *Gallus gallus*, *Xenopus laevis*, and *Danio rerio* by using Clustal Omega analysis (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). According to the protein sequence alignment, the N-terminal, ATPase, CIM, and WHD domains of CSB are evolutionarily conserved (Fig. 3a). This suggests that DNA-dependent ATPase activity, DNA repair, and transcription elongation are inter-specifically the uniform functions of CSB. We then examined the proportion of clinical CSB mutation sites on conserved sequences and found that 90% of CS missense mutation sites are annotated to the conserved sites (Fig. 3b), which are predominantly located on exons 8–10 and 13–15 (encoding the ATPase domain) (Fig. 3c). Overall, this may suggest that CSB altering DNA-dependent ATPase activity is the most common cause of CS. The pathophysiological meaning of CSB dysfunction in the context of neurological activity and CS is discussed further in the next section.

Due to the wide spectrum of CSB clinical manifestations, studies have focused on analyzing the genotype–phenotype correlation. It was shown that mutations downstream of intron 5 may result in more severe forms than mutations upstream of the *PGBD3* transposon insertion (Newman et al., 2008; Weiner and Gray, 2013; Sin et al., 2018; Damaj-Fourcade et al.,

Fig. 3 Cockayne syndrome group B (*CSB*) mutations in homologous sequences associated with CSB. (a) Amino acid homology analysis between *Homo sapiens* (Hom), *Mus musculus* (Mus), *Gallus gallus* (Gal), *Xenopus laevis* (Xen), and *Danio rerio* (Dan) by Clustal Omega analysis. (b) Proportion of *CSB* missense mutation sites on conserved and unconserved sequences. (c) Number of *CSB* missense mutation sites on conserved and unconserved sequences per exon.

2022). Mutations in exons 2–5 affect both full-length CSB and CSB-PGBD3, whereas mutations in exons 6–21 impact the complete CSB but not CSB-PGBD3. Therefore, it is speculated that CSB-PGBD3 appears to exert adverse effects in the context of the deficiency of full-length CSB.

4 CSB neurological functions

Brain atrophy, demyelination, and calcification in specific brain regions represent the fundamental neurological abnormalities observed in CSB cases. The majority of patients undergo motor dysfunction, ataxia, and tremor of the extremities as a result of significant cerebellar atrophy. Atrophy is the result of substantial loss of Purkinje cells and granule cells, accompanied by Purkinje cells dendritic branching reduction, axonal degeneration, and cerebellar demyelination (Karikkineth et al., 2017). Cerebellar Bergmann glial cells and microglia are abnormally increased in the cerebellum of patients with CS (Koob et al., 2010), and it is unclear whether this gliosis is a response to cerebellar neuronal loss and demyelination or the result of the abnormal directed differentiation of neural stem cells. Most patients with CS also suffer from tremor, which is typically intention tremor, consistent with cerebellar etiology (Wilson et al., 2016). In CS, the cerebellum, cerebral cortex, basal ganglia, and thalamus are the brain regions with the most pronounced calcification sites (Karikkineth et al., 2017). Patients' social ability is maintained through the later stages mostly due to the forebrain cortex remaining physiologically unaltered (Koob et al., 2010). Given that CSB is extensively expressed in the cerebellar region and that most CS patients exhibit cerebellar retardation/impaired development and dysfunction, the cerebellum is the primary affected brain region in CSB due to CSB mutations. CSB deficiency affects neuronal synapse development and functions. Vessoni et al. (2016) generated induced pluripotent stem cells (iPSCs) from CSB patients and discovered that patient-derived neurons featured fewer synapses, lower frequency of spontaneous firing and synchronous neuronal activity, and aberrant insulin-like growth factor-1 (IGF-1) pathways. In addition, CSB loss-of-function mutation led to mitochondrial dysfunction and consequently affected axonal degeneration in *Caenorhabditis elegans* (Lopes et al., 2020), suggesting the existence of a similar mechanism in mammalian neurons.

CSB is initially found to be a core protein for the transcription-coupled nucleotide excision repair (TC-NER) process. When DNA damage leads to Pol II arrest and the termination of transcription, CSB recognizes the binding of stalled Pol II, and then its ATPase structure promotes the forward movement of Pol II (Kokic et al., 2021) and facilitates the recovery of transcription (van den Heuvel et al., 2021). In addition to the TC-NER, CSB also participates in other multiple cellular events such as interstrand cross-link (ICL) repair, DNA double-strand break (DSB) repair, and chromatin remodeling. CSB recognizes ICL and triggers the activity of the nucleic acid exonuclease SNM1A, which facilitates ICL repair (Iyama and Wilson, 2016). CSB scavenges histone proteins onto the damaged chromatin and promotes efficient homologous recombination-dependent DSB repair (Batenburg et al., 2017). CSB hydrolyzes ATP to uncouple DNA–histone interactions in nucleosomes, thereby altering chromatin composition and structure (Tiwari et al., 2021). It is worth noting that xeroderma pigmentosum (XP) is also completely devoid of nucleotide excision repair (Vélez-Cruz and Egly, 2013). XP patients are prone to ultraviolet (UV)-induced skin and eye damage, as well as an elevated risk of developing skin tumors, yet they do not exhibit severe neurodevelopmental deficits like CS patients (Piccione et al., 2021). Therefore, although CSB has a significant role in DNA repair, it is certainly not a central factor contributing to the neurological development and dysfunction of CS (Vélez-Cruz and Egly, 2013). Studies have increasingly shown that CSB plays a more important function than expected in the transcription and cellular homeostasis of neuronal cells (Vélez-Cruz and Egly, 2013; Wang et al., 2016; Boetefuer et al., 2018; Xu et al., 2019; Weems et al., 2021).

Normal neurodevelopment is controlled by precise transcriptional and cellular homeostatic processes. Multiple studies have shown that, in addition to DNA damage repair, CSB is crucial for transcription regulation (Weiner and Gray, 2013; Wang YM et al., 2014; van den Heuvel et al., 2021; Kokic et al., 2021). Interestingly, the CSB ATPase domain was discovered to serve a key function in transcription regulation (Kyng et al., 2003). Besides, as discussed earlier, mutations in the ATPase and NLS domains are the most common causes of CS (Fig. 2). Therefore, it is speculated

that abnormal transcriptional regulation in the nucleus is likely to be one of the important pathological mechanisms in CS. Accordingly, transcriptome analysis revealed that CSB is required for the transcription of a large number of genes involved in neuronal development and/or survival (Wang YM et al., 2014). CSB binds to the promoter and participates in the transcriptional activation of microtubule-associated protein 2 (*MAP2*) and neurogenic differentiation 1 (*NeuroD1*), which are essential for neurogenesis and differentiation (Ciaffardini et al., 2014). CSB also acts as a transcriptional repressor of *p21* and *Necdin*, which are critical for the proliferation and differentiation of neural progenitor cells (NPCs) (Li and Wong, 2018; Liang et al., 2023) (Fig. 4a). Besides, CSB UBD promotes the binding of nucleolin to ribosomal DNA (rDNA) to regulate rDNA transcription (Okur et al., 2020).

Furthermore, CSB plays a role in ubiquitin/proteasome-directed protein degradation. It is a component of the E3 ubiquitin ligase murine double minute 2 (MDM2) complex and involved in the proteasome recruitment on ubiquitinated target proteins (Paccosi and Proietti-De-Santis, 2021) (Fig. 4b). CSB promotes the recruitment of proteasome for activating transcription factor 3 (ATF3), a cyclic adenosine monophosphate (cAMP)-dependent transcription factor causing transcription arrest upon exposure UV irradiation. Following ATF3 degradation by the proteasome, Pol II is recruited and RNA synthesis restarts (Epanchintsev et al., 2017). During cell division, CSB migrates to the intercellular bridge and recruits the proteasome to degrade protein regulator of cytokinesis 1 (PRC1), a main component of the intercellular bridge (Paccosi et al., 2020). More importantly, CSB modulates the activity of p53. CSB and CSA promote p53 ubiquitination and degradation (Latini et al., 2011; Proietti-De-Santis et al., 2018). Substantial evidence has indicated that p53 is involved in neural stem cell proliferation, neuronal death, axon degeneration, and transcriptional regulation (Maor-Nof et al., 2021). Given that $CSB^{-/-}$ neural precursors possess a reduced capacity for self-renewal (Sacco et al., 2013), it is predicted that altered p53 signaling in the nervous system might have an essential part in CSB neurological dysfunction.

Despite the above knowledge, the precise function of CSB-PGBD3 proteins in neurological disorders associated with CS remains elusive. CSB-PGBD3 primarily exerts its transcriptional regulation via binding to the chromosomal protein c-Jun (Weiner and Gray, 2013). CSB-PGBD3 overexpression in CSB-deficient cells triggers an interferon response (Weiner and Gray, 2013). Notably, the interferon signaling pathway, activated by CSB-PGBD3, plays a role in regulating the differentiation process of neural progenitors (Warre-Cornish et al., 2020). Therefore, it is suggested that CSB-PGBD3 fusion proteins may be intricately involved in the differentiation process of neural stem cells.

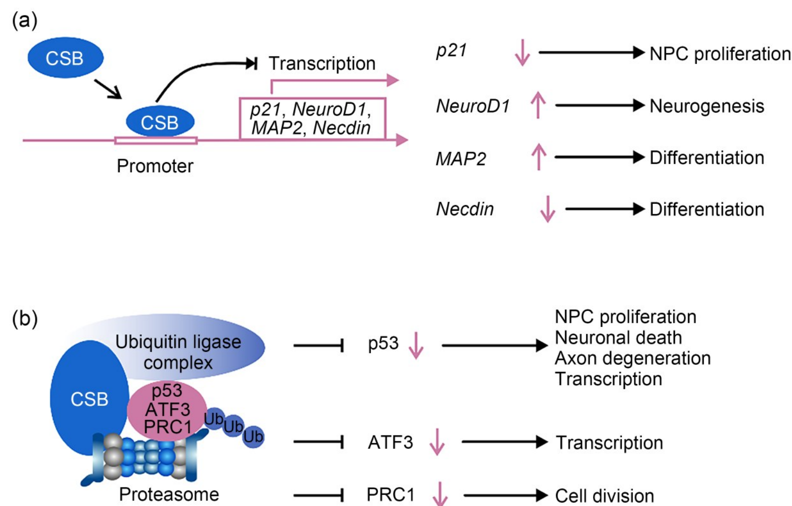

Fig. 4 Cockayne syndrome group B (CSB)'s role in transcription and ubiquitin/proteasome-directed protein degradation. (a) CSB is a transcription inhibitor of *p21* and *Necdin* genes and a transcription promotor of neurogenic differentiation 1 (*NeuroD1*) and microtubule-associated protein 2 (*MAP2*) genes. (b) CSB is a component of the E3 ubiquitin ligase murine double minute 2 (MDM2) complex that promotes p53, activating transcription factor 3 (ATF3), and protein regulator of cytokinesis 1 (PRC1) ubiquitin/proteasome-directed degradation. NPC: neural progenitor cell; Ub: ubiquitin.

5 Research/experimental models of CSB

Animal models have been widely utilized in the study of molecular pathways underlying human diseases. To investigate how cellular pathology translates into CS symptoms, *CSB*-mutant mice have been created (Laposa et al., 2007; Jaarsma et al., 2013). Both *CSB* knockout and *CSB* mutation knockin mice exhibit the TC-NER deficiency, including UV-induced skin, eye damage, and photoreceptor loss, but fail to develop severe neurological disorders (van der Horst et al., 1997; Laposa et al., 2007). Besides, aberrant gene expression has been identified in postmortem cerebella from CS patients, but this was not observed in $CSB^{-/-}$ mice (Wang YM et al., 2014, 2016). The CSB-PGBD3 fusion protein and potentially the murine transcriptional machinery, particularly the Pol II-dependent one, are different from those of humans and may not necessarily require CSB-Elongin involvement, which can also explain the differences in disease phenotype between species. This may suggest that the severe neurological symptoms of CS are caused by transcriptional abnormalities rather than DNA repair dysfunction (Wang YM et al., 2014, 2016; Xu et al., 2019). Remarkably, no myelin deficits have been documented in CS knockout mice to date, in stark contrast to the severe myelination defects consistently observed in CS patients (Revet et al., 2012; Jaarsma et al., 2013). Consequently, the existing mouse model proves inadequate for the comprehensive study of neurological lesions in CS, and it does not lend itself well to therapeutic testing for the associated neurological disorders. An intriguing alternative was presented by Xu et al. (2019), who described a rat model of CS (CSB^{R571X}), in which the animals displayed several neurologic abnormalities including cerebellar atrophy, thinner cerebellar cortex, and Purkinje cell degeneration. However, it is worth noting that despite these observations, CSB^{R571X} rats exhibited normal appearance and behaviors, presenting a disparity in the clinical symptoms observed in CS patients.

Lopes et al. (2020) reported progressive neurodegeneration in adult *C. elegans* specimens with *csb-1* gene mutation. The *csb-1*-mutant *C. elegans* exhibited sensory neuronal function loss and mitochondrial abnormalities. Moreover, knockdown *csb-1* gene heightened the sensitivity of *C. elegans* to UV irradiation, evidenced by higher levels of germ cell development arrest, apoptosis, and increased embryonic lethality (Lee et al., 2002). Of note, CSB regulates the developmental growth of *C. elegans* related to decay-accelerating factor-16 (DAF-16) and insulin/insulin-like signaling (Bianco and Schumacher, 2018), with IGF1 signaling also impaired in CS patient-derived neurons (Vessoni et al., 2016).

iPSC culture and differentiation methods are becoming increasingly popular in the investigation of neurological disorders. (Engle et al., 2018). iPSCs derived from CS patients (CS-iPSCs) exhibit higher cell death rates and elevated oxidative stress (de Sousa Andrade et al., 2012). Neural stem cells derived from CS-iPSCs are more vulnerable to DNA damage stress (Wang et al., 2020). Vessoni et al. (2016) reconstructed functional neural networks from human CS-iPSCs, which exhibited dysregulated grouth hormone (GH)/IGF-1 pathway, synapse formation, neuronal homeostasis, and differentiation pathways in CSB neurons. Notably, CS individuals have been documented who had the same mutations but distinct symptoms (Colella et al., 2000), which could be attributed to individual genetic heterogeneity or various degrees of transcriptional deficiency possibly linked to gender and family history (Zhao and Usdin, 2014). The genetic background of the CS donor and the pathogenic mutation are both reproduced in iPSC-derived neurons (Vessoni et al., 2016), which therefore present as a useful tool to facilitate studying CS neurological disease.

6 Research prospects

Both animal models and CS-iPSC-derived neurons exhibit substantial limitation due to challenges in interspecies translational research or the lack of brain microenvironment context. In recent years, cerebral organoids have been widely used to study human brain development, neurological disease, and aspects of precision medicine. Patient-derived iPSC-induced organoids can recapitulate the developmental process of neurological diseases in multiple dimensions, such as developmental timeline, three-dimensional (3D) structure, and molecular signaling pathways, providing important disease models for the study of neurological disorders, such as Alzheimer's disease, Down's syndrome, and major depressive disorder (Park et al., 2018; Tang et al., 2021; Lu et al., 2023). Existing

animal-based research is unsuitable for studying neurodevelopment and dysfunction from the perspective of CS neurological disorders. Two significant factors warrant attention in this context. (1) Human cells uniquely express CSB-PGBD3 fusion proteins distinguishing them from other animals. CSB-PGBD3 fusion proteins potentially play a role in transcription and the neural stem cell differentiation process. Notably, compared to mutations located upstream of the *PGBD3* insertion, those positioned downstream of the *PGBD3* insertion might result in more severe manifestations (Newman et al., 2008; Weiner and Gray, 2013; Calmels et al., 2018). (2) The protein sequence of human CSB is different from other animals and thus may have other critical biological functions. Consequently, utilizing somatic cells from patients, which are reprogrammed into iPSC and further differentiated into cerebral organoids, is a promising path to investigate neurological development and functional disorders of CS.

One of the primary brain regions affected by CS is the cerebellum. Cerebellar atrophy produced by a decrease in the number of cerebellar granule cells and Purkinje cells as well as demyelination is the common cause of dyskinesia, ataxia, and limb tremor. Muguruma (2018) and Muguruma et al. (2015) developed a method to generate cerebellar neurons using a 3D culture of human iPSC with growth factors, including fibroblast growth factor 2 (FGF2), FGF19, and stromal cell-derived factor-1 (SDF-1). Chen et al. (2023) generated a more mature and complex cerebellar organoid tissue, in which several types of differentiated neurons were established, including Purkinje cells, granule cells, and interneurons. Implementing the key types of cerebellar neurons and addressing the personalized genomic profiles, patient-derived cerebellar organoids are useful to investigate cerebellar diseases. One example is spinocerebellar ataxia type 6 (SCA6), which is a genetic disorder characterized by the absence of Purkinje cells. Produced from patient-derived cerebellar organoids, it has been revealed that SCA6 iPSC-derived Purkinje cells exhibit significant degeneration induced by thyroid hormone depletion (Ishida et al., 2016). Tuberous sclerosis complex (TSC) is an autosomal dominant disease that is often comorbid with autism disorder. TSC iPSC-derived Purkinje cells exhibit hyperexcitability and synaptic defects, which are reversed by rapamycin (Sundberg et al., 2018), suggesting that cerebellar organoids comprise an essential model for investigating the molecular and cellular mechanisms of cerebellar disorders and act as a platform for therapeutic screening including drug-based and gene-editing approaches.

Overall, the above accomplishments make us wonder whether CS patient-derived cerebellar organoids are capable of reproducing cerebellar atrophy, with specific reduction in granule cell and Purkinje cell numbers. In the future, CS cerebellar organoids can assist in further investigating the effects of CSB deficiency on early cerebellar development and the regulatory mechanisms of transcriptional regulation and DNA repair, which may provide new ideas for understanding the neurological lesions of CS and identifying potential therapeutic targets.

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Author contributions

Xintai WANG, Rui ZHENG, Ying SHEN, and Zhijie LIN designed the manuscript. Xintai WANG, Rui ZHENG, and Marina DUKHINOVA wrote the manuscript. Luxi WANG, Ying SHEN, and Zhijie LIN provided opinions. Xintai WANG, Rui ZHENG, Marina DUKHINOVA, Ying SHEN, and Zhijie LIN were involved in the discussion. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Xintai WANG, Rui ZHENG, Marina DUKHINOVA, Luxi WANG, Ying SHEN and Zhijie LIN declare that they have no conflict of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

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Supplementary information

Table S1