

RESEARCH HIGHLIGHT

# UVSSA and USP7: new players regulating transcription-coupled nucleotide excision repair in human cells

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## Abstract

Transcription-coupled nucleotide excision repair (TC-NER) specifically removes DNA damage located in actively transcribed genes. Defects in TC-NER are associated with several human disorders, including Cockayne syndrome (CS) and ultraviolet (UV)-sensitive syndrome (UV<sup>S</sup>S). Using exome sequencing, and genetic and proteomic approaches, three recent studies have identified mutations in the *UVSSA* gene as being responsible for UV<sup>S</sup>S-A. These findings suggest a new mechanistic model involving UV-stimulated scaffold protein A (UVSSA) and the ubiquitin-specific protease 7 (USP7) in the fate of stalled RNA polymerase II during TC-NER, and provide insights into the diverse clinical features of CS and UV<sup>S</sup>S.

**Keywords** DNA repair-deficient diseases, transcription-coupled repair, stalled RNA polymerase, UV sensitivity

## Disorders of nucleotide excision repair

Nucleotide excision repair (NER) is a versatile DNA repair system that removes a wide range of structurally unrelated lesions, including ultraviolet (UV) photo-products. NER operates through two sub-pathways, depending on whether the damage is located anywhere in the genome (global genome repair, GG-NER) or in an actively transcribed gene (transcription-coupled repair, TC-NER) [1,2]. The current model for TC-NER postulates that the pathway is initiated by the arrest of RNA polymerase IIo at a lesion on the transcribed strand of an active gene - a process requiring several factors, including CSA, CSB and XAB2 proteins [1]. To ensure full repair, the coordinated action of other factors and complexes, including the repair/transcription complex TFIIH, and

the repair enzymes XPA, XPG and ERCC1-XPF (in addition to those required for repair replication and ligation), is necessary [1,2].

Defects in NER are associated with major autosomal, recessive disorders, such as xeroderma pigmentosum (XP) and Cockayne syndrome (CS). XP is characterized by a highly increased incidence of tumors in sun-exposed areas of the skin. In contrast, CS patients are cancer-free, displaying developmental and neurological abnormalities, and premature ageing (reviewed by Lehmann [3]). Two genes, *CSA/ERCC8* and *CSB/ERCC6*, have been implicated in CS (Table 1), and these are specifically involved in TC-NER.

NER defects have also been reported in UV-sensitive syndrome (UV<sup>S</sup>S), initially described in 1994 by Itoh *et al.* [4]. Patients with this syndrome exhibit photosensitivity and only mild skin abnormalities; their growth, mental development and lifespan are normal, and no skin or internal cancers have been reported to date. UV<sup>S</sup>S and CS cells exhibit similar responses to UV irradiation: increased UV sensitivity, reduced recovery of RNA synthesis (RRS) after UV irradiation, and normal GG-NER but deficient TC-NER of UV-induced cyclobutane pyrimidine dimers [5,6]. Three complementation groups have been identified among UV<sup>S</sup>S patients, defined by mutations in (1) the *CSA* gene, (2) the *CSB* gene, and (3) a previously unidentified gene (UV<sup>S</sup>S-A complementation group) [5] (Table 1).

## Genetic characterization of the third complementation group of UV<sup>S</sup>S patients

Three papers published recently in *Nature Genetics* have reported the isolation of the gene responsible for the UV<sup>S</sup>S-A complementation group, *UVSSA* (encoding UV-stimulated scaffold protein A), using three different approaches: exome sequencing of cell lines derived from patients with UV<sup>S</sup>S-A [7], complementation of the repair defect of UV<sup>S</sup>S-A cells [8] and stable isotope labeling by amino acids in cell culture (SILAC)-based proteomic isolation of differentially ubiquitinated proteins following UV irradiation [9].

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**Table 1. Clinical, genetic and biochemical comparisons of XP, CS and UV<sup>S</sup>S**

	XP	CS	UV <sup>S</sup> S
Sun-sensitivity	Yes	Yes	Yes
Skin cancers	Yes	No	No
Progressive neurological degeneration	Yes/no <sup>a</sup>	Yes	No
Developmental abnormalities	No	Yes	No
Ageing	Yes/no <sup>a</sup>	Yes	No
UV-DNA repair deficiency	Yes	Yes	Yes
ROS-DNA repair deficiency	Yes/no <sup>a</sup>	Yes	No
Complementation groups	7	2	3
Involved genes	XPA to XPG <sup>b</sup>	CSA and CSB	CSA, CSB and UVSSA

<sup>a</sup>Some XP patients exhibit XP and CS (mutated on XPA, XPB, XPD and XPG). <sup>b</sup>Seven genes are involved in XP. CS, Cockayne syndrome; ROS, reactive oxygen species; UV, ultraviolet; UV<sup>S</sup>S, UV-sensitivity syndrome; XP, xeroderma pigmentosum.

Using cell lines derived from two UV<sup>S</sup>S-A patients, Nakazawa *et al.* [7] identified homozygous mutations (c.367A>T) leading to a premature stop codon in the *KIAA1530* (renamed *UVSSA*) gene. Interestingly, a homozygous missense mutation (p.Cys32Arg) in this gene was found in an individual previously mis-diagnosed with mild XP. Zhang *et al.* [8] carried out microcell-mediated chromosome transfer of mouse DNA in order to complement the repair deficiency of UV<sup>S</sup>S-A human cells, and isolated the mouse homologue of *KIAA1530* as the gene responsible for UV<sup>S</sup>S-A. Sequencing of this gene from several UV<sup>S</sup>S-A cells also revealed mutations leading to premature termination of the UVSSA protein [8]. Schwertman *et al.* [9] identified several differentially ubiquitinated proteins following UV irradiation of HeLa cells. The most prominent factors were repair proteins involved in NER (XPC, DDB2, RNA RNA polymerase (RNA Pol) II and CSB) as well as UVSSA. Using mass spectrometry, it was demonstrated that UVSSA interacts with the deubiquitinating enzyme ubiquitin carboxyl-terminal hydrolase 7 (also known as ubiquitin-specific protease 7, USP7) [8,9]. Transfection of wild-type tagged *UVSSA* cDNA restored normal RRS in UV<sup>S</sup>S-A cells, while small interfering RNA (siRNA)-based depletion of *UVSSA* transcripts caused a marked reduction of RRS in normal cells, illustrating that the UVSSA-USP7 complex is crucial for restoration of gene expression following UV irradiation. Taken together these data indicate that *UVSSA* is the causal gene in UV<sup>S</sup>S-A, and that the UVSSA-USP7 complex is involved in TC-NER.

### UVSSA interactions and the role of the ubiquitin proteasome pathway

Using three-dimensional structure prediction of UVSSA, Nakazawa *et al.* [7] identified two domains of unknown

function: a VHS domain (homology with the Vps-27, Hrs and STAM domain) near the amino terminus and a DUF2043 domain (EMBL-EBI IPR018610) near the carboxyl terminus. The VHS domain has been implicated in ubiquitin binding and in interaction with the carboxy-terminal part of RNA Pol II; it has also been suggested that the UV<sup>S</sup>S-A mutation Cys32Arg might obstruct interactions between the VHS domain and ubiquitinated proteins. *UVSSA* truncated mutants lacking either VHS or DUF2043 domains failed to complement the UV<sup>S</sup>S-A deficiency, indicating that these two domains are necessary for TC-NER activity.

One of the major players in TC-NER is the ten-protein complex TFIIH, involved in both NER and transcription initiation [1]. Nakazawa *et al.* [7] demonstrated that UVSSA interacts with ERCC2, ERCC3, p62 and the CAK subcomplex (all part of TFIIH). Furthermore, Zhang *et al.* [8] showed that UVSSA interacts with CSA in the absence of UV, and with CSB and RNA Pol II after UV irradiation, and that both UVSSA and CSB are necessary for full completion of TC-NER. Thus, interaction between UVSSA and the major proteins involved in TC-NER suggests that UVSSA may play a pivotal role in this process.

USP7 has several NER and DNA damage response (DDR) proteins as substrates. Schwertman *et al.* [9] showed that USP7 resided in chromatin-immunoprecipitated TC-NER complexes in a UV- and UVSSA-dependent manner. In UV<sup>S</sup>S-A cells, the absence of UVSSA correlated with the instability of CSB, probably due to the lack of USP7 recruitment in the TC-NER complex. Indeed, depletion of USP7 by siRNA caused a similar RRS deficiency and decreased levels of CSB. These data indicate that UVSSA and USP7 cooperate to protect CSB from UV-induced degradation in TC-NER via the ubiquitin-proteasome pathway. Because USP7 has multiple roles in the DDR, an important role of UVSSA might also be to deliver the deubiquitinating enzyme to the vicinity of TC-NER factors, allowing smooth regulation of ubiquitination and deubiquitination. Zhang *et al.* [8] also showed that this recruitment is CSA-dependent.

Using local UV damage, it has also been shown that tagged-UVSSA accumulated *in vivo* at UV-induced DNA lesions (with kinetics similar to CSB), and interacted with the elongating form of RNA Pol II (Pol IIo) [9]. Following UV irradiation, transcription is rapidly inhibited and fast repair of lesions is necessary to ensure survival of damaged cells. Pol IIo is stalled at UV-induced DNA lesions and needs first to be displaced by backtracking or degradation to allow access to repair factors. During this step, Pol IIo is ubiquitinated, and CSA and CSB proteins are necessary for this process. In UV<sup>S</sup>S-A cells, the ubiquitinated Pol IIo was almost undetectable and the normal Pol IIo form disappeared over a 6-h period

following UV irradiation. During the same period, CSB protein was degraded in a proteasome- and UV-dependent manner, indicating that UVSSA contributes to the stabilization of the CSB complex during TC-NER [7,9]. The absence of UVSSA or USP7, or mutations in the VHS domain, destabilizes Pol Ilo [8]. These anomalies were corrected by wild-type *UVSSA* cDNA transfection, but not with mutants in the VHS domain. During TC-NER, Pol Ilo can be dephosphorylated and recycled to Pol Ila, ready to start another round of transcription. In UV<sup>S</sup>-A cells, dephosphorylation of Pol Ilo is inhibited, as previously found in CS cells [8], and the absence of dephosphorylation does not allow the recycling of Pol II for transcription initiation [7]. Taken together, these findings suggest a new model for UV-induced TC-NER in which UVSSA and USP7 are crucial for Pol Ilo ubiquitination and the resumption of normal transcription.

### A new model for UV-induced TC-NER

Patients with UV<sup>S</sup> exhibit photosensitivity and mild skin abnormalities; in contrast, patients with CS show major growth retardation, abnormal mental development and neurological anomalies, usually leading to early death [3,10] (Table 1). Although both classes of patients are deficient in UV-induced TC-NER, we have previously shown that CS cells are deficient in repair of oxidative DNA lesions [6]. In a patient with *CSA*-mutated UV<sup>S</sup>1VI, a missense mutation found in the carboxy-terminal part of the gene, suggested that this mutation does not allow UV-induced TC-NER, but does allow repair of reactive oxygen species (ROS)-induced damage, explaining the mild symptoms [6].

In the new model where the UVSSA and USP7 proteins are taken into account, ubiquitination of stalled RNA Pol Ilo at DNA lesions is a necessary step to allow repair [1]. The model proposed by Nakazawa *et al.* [7] suggests that UVSSA recruits an E3 ubiquitin ligase allowing efficient ubiquitination of Pol Ilo. In UV<sup>S</sup>-A cells, stalled RNA Pol Ilo can still be ubiquitinated by a CSB- and CSA-dependent pathway, but cannot be deubiquitinated due to the absence of the UVSSA-USP7 complex [8], so that transcription resumption does not occur and the cells are RRS deficient. In CS cells (mutated in *CSA* or *CSB*) neither of these two processes occurs and stalled Pol Ilo is arrested for a longer time, leading to apoptosis and to more severe clinical features as described in Table 1. However, no explanation has been proposed for the paradoxical group of patients with *CSB*-mutated UV<sup>S</sup> [4,5], where the complete absence of the CSB protein gave rise to a mild UV<sup>S</sup> phenotype [10]. The defective processing of oxidative DNA damage characteristic of CS patients does not occur in patients with *CSB*-mutated UV<sup>S</sup> for reasons still not explained by this new model of TC-NER.

In conclusion, these results suggest a new model for TC-NER involving UVSSA and USP7, and provide new insights into the mechanisms underlying the differences in severity of CS and UV<sup>S</sup>. UVSSA and USP7 are identified as two new key factors controlling the fate of stalled RNA Pol II, the steady-state level of CSB, the efficiency of TC-NER and cell survival following DNA damage. The data reported indicate that differences between severe CS syndromes and mild UV<sup>S</sup> are due to differences in transcription and/or repair of oxidative DNA damage in transcribed strands. Further investigation of the repair of oxidative damage at the transcriptional level should help us to understand how this process might be involved in progressive neurological deterioration associated with ageing in CS.

### Abbreviations

CS, Cockayne syndrome; DDR, DNA damage response; GG, global genome; NER, nucleotide excision repair; Pol Ilo, elongating form of RNA Pol II; RNA Pol, RNA polymerase; RRS, recovery of RNA synthesis; siRNA, small interfering RNA; TC, transcription-coupled; USP7, ubiquitin-specific protease 7; UV, ultraviolet; UV<sup>S</sup>, UV-sensitive syndrome; UVSSA, UV-stimulated scaffold protein A; XP, xeroderma pigmentosum.

### Competing interests

The author declares that he has no competing interests.

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