Unravelling molecular mechanisms in transcription-coupled nucleotide excision repair

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Summary

The cellular response to transcription-blocking DNA lesions, such as UV light-induced photolesions, involves the stalling of elongating RNA Polymerase II (RNAPIIo) at the lesion as well as a global shutdown of transcription. The stalling of RNAPIIo at such lesions initiates the transcription-coupled nucleotide excision repair pathway (TCR) to efficiently remove the damage and restore transcription. Mutations in genes that selectively impair TCR give rise to Cockayne syndrome (CS), which is a severe neurodegenerative disorder, and UV-sensitive syndrome ($UV^{S}S$), which is characterized by mild UV-sensitivity with no neurological symptoms. In **chapter 1**, we review recent literature on TCR and propose a model that provides an explanation for the strikingly different clinical features associated with TCR deficiency disorders.

It has been known for quite some time that the TCR proteins, CSB, CSA, and UVSSA, are essential for the repair of transcription-blocking DNA lesions, but how the interplay between these proteins targets the core repair machinery to lesion-stalled RNAPIIo remained enigmatic. In **chapter 2**, we undertook a systematic approach to establish the molecular mechanism of TCR complex assembly in human cells. We demonstrate that CSB contains a C-terminal CSA-interaction motif (CIM) that directly interacts with CSA to facilitate its recruitment to lesion-stalled RNAPIIo. Once recruited, CSA mediates UVSSA recruitment by interacting with the CSA-interaction region (CIR) located within UVSSA, after which, the transcription factor IIH (TFIIH) complex is targeted to RNAPIIo via direct protein-protein contacts with the TFIIH-interaction region (TIR) in UVSSA. Importantly, the TCR proteins are assembled in a highly cooperative manner in which the interaction between CSA and UVSSA is stabilized by CSB, and the interaction between UVSSA and the TFIIH complex is stabilized by CSA.

In addition to direct protein-protein contacts, recent studies have demonstrated that ubiquitylation of the RPB1 subunit of RNAPII at a single lysine residue (RPB1-K1268) is essential for the assembly of the TCR complex and the subsequent repair of transcription-blocking lesions. RPB1-K1268 ubiquitylation is not required for association of CSB and CSA with DNA damage-stalled RNAPIIo, but K1268 ubiquitylation does enhance the interaction between UVSSA and lesion-stalled RNAPIIo, since UVSSA preferentially interacts with ubiquitylated RNAPII. In addition to RNAPII, UVSSA is also ubiquitylated in response to DNA damage and it has been suggested that ubiquitylation of UVSSA is required for the transfer of TFIIH from UVSSA to RNAPIIo. CSA is part of a DDB1-CUL4A-RBX1 ubiquitin ligase complex ($CRL4^{CSA}$) that is one of the key regulators of RNAPII. CSB binds to DNA upstream of RNAPII and recruits the $CRL4^{CSA}$ complex through an evolutionary conserved motif in its C-terminus. However, how the $CRL4^{CSA}$ ubiquitin ligase activity is specifically directed toward the K1268 site remains to be elucidated. In **chapter 3**, we identified the previously uncharacterized ELOF1 gene as a core TCR factor that facilitates RNAPII ubiquitylation by directing the ubiquitin ligase activity of $CRL4^{CSA}$ towards the K1268 site. ELOF1 is a transcrip-

tion elongation factor that constitutively interacts with RNAPII close to the K1268 ubiquitylation site. When RNAPIIo stalls at a transcription-blocking DNA lesion, CSB and the $CRL4^{CSA}$ complex are recruited in a manner that is independent of ELOF1. Although ELOF1 is not required for $CRL4^{CSA}$ recruitment, ELOF1 does interact directly with the $CRL4^{CSA}$ complex and brings the ubiquitin ligase activity of $CRL4^{CSA}$ in close proximity to the K1268 site. Furthermore, we demonstrate that ELOF1 is not only a core TCR factor, but also functions in a CSB-independent repair pathway that deals with DNA damage during replication. Even though the precise nature of this pathway remains to be elucidated, our data suggests that cells become more dependent on this pathway when canonical TCR fails.

Despite the fact that the DNA damage-induced ubiquitylation of TCR proteins by the $CRL4^{CSA}$ complex is essential for efficient TCR, we do not have a complete picture of the relevant substrates of the $CRL4^{CSA}$ complex. A complication in their identification is that substrates often transiently associate with ubiquitin ligase complexes, while regular immunoprecipitation approaches rely on relatively stable protein-protein interactions, which are often not suitable to detect transient interactions. In **chapter 4**, we established a proximity-dependent biotin identification (BioID) method to capture DNA damage-induced CSA-proximal proteins, including transient interactors such as potential substrates of the $CRL4^{CSA}$ complex. With this method we were able to capture known CSA-interaction proteins, including UVSSA and the TFIIH complex, as well as the previously unknown CSA-associated proteins, chromodomain Y-like protein (CDYL) and HIV Tat-specific factor 1 (HTATSF1).

In **chapter 5**, which is the final chapter of this dissertation, we give an overview of the cellular responses to different types of collisions between the transcription and replication machinery, we discuss the replication stress response in CSB- and ELOF1-deficient cells and we speculate on the CSB-independent function of ELOF1. Furthermore, we provide suggestions for future research aimed to gain a better understanding on the link between transcription stress and replication stress and elucidate the second ELOF1 pathway.