



Universiteit
Leiden
The Netherlands

Regulation of DNA damage and immune response pathways by post-translational protein modification

Dijk, M.

Citation

Dijk, M. (2020, September 9). *Regulation of DNA damage and immune response pathways by post-translational protein modification*. Retrieved from <https://hdl.handle.net/1887/136522>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/136522>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/136522> holds various files of this Leiden University dissertation.

Author: Dijk, M.

Title: Regulation of DNA damage and immune response pathways by post-translational protein modification

Issue Date: 2020-09-09



Summary

Samenvatting

Curriculum vitae

Publications

Acknowledgements

Summary

Our cells are continuously challenged by numerous external as well as internal hazards that, if not dealt with in an appropriate manner, may interfere with critical processes and underlie disease. Therefore, they heavily rely on essential protective mechanisms that recognize and counteract potential risks. For example, the physical barriers and biochemical cascades of the immune system provide a first line of defense against pathogens, which attempt to invade host cells and could cause serious illness. In addition, the well-coordinated networks of the DNA damage response (DDR) detect and remove damaged nucleotides that could drive mutagenesis and thereby provoke inherited disorders or ageing-related diseases. Evidently, the correct activation, execution and completion of the implicated pathways, as well as their crosstalk, is of key importance. This necessitates the well-timed and -positioned presence of proteins with the desired functionality. Apart from regulation of the overall availability of such proteins by adjusting transcription or translation, this is to a great extent established by post-translational modifications (PTMs). A variety of chemical alterations is able to fine-tune protein activity, localization and interactions. The research described in this thesis addresses the role and importance of PTMs in the mechanisms that safeguard our cells. The background for this study is provided by Chapter 1.

An important strategy to coordinate the proteins of the DDR involves the reversible, covalent addition of functional groups. As combining multiple (types of) PTMs can work either supportive or antagonistically, their introduction is perfectly balanced to guarantee optimal protein and pathway functioning.

A frequently applied modification is the attachment of a small regulatory protein called ubiquitin. This reaction, referred to as ubiquitination, is in many cases catalyzed by a protein complex from the family of cullin-RING ligases (CRLs). Two of such CRLs play important roles during nucleotide excision repair (NER) – a mechanism that protects our cells against certain types of DNA damage and, in humans, is the only pathway capable of removing the covalent linkages between adjacent pyrimidines in the DNA that are inflicted by sunlight. In the subpathway of NER that acts on the DNA damage throughout the entire genome, the CRL that is built with DDB2 (CRL^{DDB2}) significantly contributes to DNA damage detection. Fascinatingly, the activity of CRLs is in turn regulated through another PTM, that is the attachment of the ubiquitin-like protein NEDD8, known as NEDDylation. When not required, the ligase functionality of CRL^{DDB2} is indeed kept inactivated through the removal of NEDD8. However, in response to DNA damage CRL^{DDB2} activation leads to the ubiquitination of many target proteins, thereby altering their performances in a manner that contributes to efficient DNA damage repair. Interestingly, the architecturally similar CRL^{CSA}, which is built with CSA instead of DDB2, is indispensable for the NER subpathway that specifically removes damage from active genes. When RNA polymerase II (RNAPII) gets stalled at such damage during transcription, CRL^{CSA} is one of the first complexes that is recruited. As described in Chapter 2, NEDDylation not only regulates the activation of the CRL, but also appears to modulate the interaction with RNAPII. Inhibition of NEDD8 activation leads to increased association of CRL^{CSA} with RNAPII. Furthermore, both NEDDylation in general and the specific presence of

CSA appear to be important for the UV-dependent degradation of RNAPII that can result from UV irradiation. This method to remove stalled RNAPII presumably avoids cell death when NER is compromised. Possibly, this last resort mechanism plays a role in the prevention of the neurodevelopmental problems that characterize Cockayne syndrome, which can be caused by *CSA* gene mutations that result in CSA deficiency.

To warrant sufficient levels of functional CSA, and thus CRL^{CSA}, not only gene integrity but also protein stability is essential. In Chapter 3, we uncover a crucial role for the TRiC chaperonin in regulating CSA stability and localization, as well as the assembly of CRL^{CSA}. As a part of the proteostasis network that serves to maintain protein homeostasis, TRiC facilitates the folding of many proteins. Accordingly, TRiC also appears to stabilize CSA. This seems critical for the correct functioning of CRL^{CSA} in transcription-coupled NER and possibly other protective responses. Importantly, disease-associated missense mutations in the *CSA* gene lead to increased binding of the mutated protein to TRiC, which at least in part may explain the consequences of such mutations for the development of Cockayne syndrome.

&

In addition to protein modification by ubiquitination or NEDDylation, also the conjugation of the ubiquitin-like protein SUMO (SUMOylation) plays an important role in the detection and repair of DNA damage and during the required signaling responses. The PIAS proteins, through their highly conserved catalytic SP-RING domains, act as SUMO ligases in many different (DDR) pathways. In Chapter 4, we demonstrate that the SP-RING-like domain of Zimp7, which resembles the catalytic domain of the PIAS proteins, confers true SUMOylating activity as well. Since Zimp7 appears to be recruited to sites of DNA damage, this newly identified SUMO ligase may be an important DDR factor. Furthermore, Zimp7 might play a role in DNA replication, as indicated by its interaction with the sliding clamp PCNA. Its exact biological functions are yet to be determined and seem to not only depend on its SUMOylating activity but also on its intrinsic transcriptional activity. However, current knowledge suggests a broad implication of Zimp7 in securing proper cellular functioning.

Whereas the connection and disconnection of functional groups is a leading example of pathway regulation in the DDR, PTM-mediated regulation of many immune response pathways involves the activation of proteins through peptide cleavage. Sequential cleavage of a protease that in turn catalyzes the activation of the next enzyme can quickly amplify a signal and evoke a massive response. Although this enables rapid pathogen elimination, these cascades should be tightly controlled as both defective as well as disproportional activation may have devastating effects. C1-inhibitor regulates several pathways of the immune system by trapping the initiating proteases into a conformation with a disrupted active site, thereby preventing their spontaneous activations. Interestingly, the activity of this inhibitor itself can be potentiated by the interaction with long linear polysaccharides, called glycosaminoglycans (GAGs). Our model, shown in Chapter 5, explains why this potentiation can only be observed towards specific target proteases. Since most likely the polysaccharide neutralizes the repulsive forces between C1-inhibitor and the protease's autolysis loop, GAG binding is only helpful in case this loop is positively charged. Fine-tuning C1-inhibitor's activity against its different targets may be a good strategy to optimize replacement therapy

in the treatment of hereditary angioedema – a disorder that is characterized by recurrent attacks of potentially life-threatening swelling. This uncontrolled immune response results from C1-inhibitor deficiency, showing once more the importance of a regulatory protein in health and disease.

The methods of protein regulation described in this thesis are just a fine selection of the broad repertoire of modifications that can be applied by our cells. Nevertheless, they one by one are indispensable to the resilience against internal and external threats. Chapter 6 outlines how our research provides valuable insights in this area, as well as a base for further research and discussion. Together, these findings improve our understanding, and ultimately contribute to the treatment or prevention, of the numerous disorders that are associated with loss of protein regulation in the implicated cell-protecting pathways.

