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

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6

Perspectives

The genomic DNA provides all the instructions for correct development and functioning of organisms. Damage to this DNA may interfere with critical cellular processes such as transcription and replication, and has the potential to drive mutagenesis, which in turn may underlie inherited disorders and accelerate progression of cancer and ageing-related diseases[1,2]. The protection of cells and organisms against these potentially devastating effects of DNA damage largely relies on the DNA damage response (DDR), which comprises a network of signaling and repair pathways that coordinate lesion removal and accommodate suitable adjustments in for instance transcription and cell cycle status[3].

Although essential in the protection against DNA damage, the DDR is not sufficient to protect us against all conceivably life-threatening hazards. For example, a first line of defense against pathogens, which upon host invasion can cause serious diseases, is provided by the immune system. Activation of the immune response encompasses multiple mechanisms, including physical barriers and biochemical cascades that are specialized in the neutralization of potentially harmful germs[4,5]. Importantly, by recognizing and facilitating the clearance of non-self cells or molecules, the immune system also contributes to the prevention of cancer[6].

Evidently, it is crucial that all proteins that are involved in the pathways that protect our cells, be it via the DDR, the immune system or other defense mechanisms, function correctly. Plain (upregulation of) transcription to produce the required proteins is not sufficient to guarantee their performances in the respective stress responses. Additionally, it is essential that protein functionality is tweaked by post-translational modifications (PTMs) that fine-tune activity, localization and interactions. Such modifications range from the stabilization of a protein by creating disulfide bonds, to its activation by cleavage of a small peptide or the reversible introduction of a functional group to spatiotemporally coordinate the different steps of a pathway. Clearly, extension of our knowledge regarding these post-translational control mechanisms could significantly contribute to our understanding of the development, and potentially the treatment, of the numerous disorders that are associated with loss of protein regulation in protective responses. The research described in this thesis has already improved our insights into how the availability of functional proteins is maintained, as well as how their activities are further modulated by PTMs, yet has raised some additional questions that provide a base for further research and discussion.

The importance of chaperonins in the prevention of disease

Upon translation, folding of a nascent polypeptide into a stable and potentially active protein can be considered the first critical step in guaranteeing its subsequent functioning. Protein homeostasis is maintained by a network of chaperones and protein degradation machineries, collectively referred to as the proteostasis network (PN)[7]. Assistance in the folding of fast-folding proteins is usually provided by the heat shock protein families, which mainly act as monomers or homodimers that stabilize hydrophobic regions of nascent polypeptides until the desired conformation has been established. Proteins that are more difficult to fold are transferred to specialized chaperone systems that apply a method of non-native protein encapsulation in a central cavity, as displayed by the chaperonin TRiC[8-10]. We uncovered a crucial role for TRiC in ensuring the stability of the CSA protein that is essential in DNA damage recognition and subsequent DNA damage repair via transcription-coupled

nucleotide excision repair (TC-NER) (described in Chapter 3). When not folded or stabilized correctly, CSA is degraded instead of being incorporated into the cullin-RING E3 ubiquitin ligase complex CRL^{CSA}, leading to a deficiency in functional CSA/CRL^{CSA}. Importantly, we discovered that patient mutations in CSA's WD40 domain cause increased binding of CSA to TRiC, indicating the protein's instability and/or folding complications[11]. While the mechanism behind the cause of Cockayne syndrome by these mutations had thus far remained elusive, this finding may provide an important explanation for how they affect the functioning of CSA and could ultimately underlie disease. Most likely, the examined mutations inhibit proper folding and thereby the release of CSA by TRiC. Alternatively, CSA harboring these mutations is incapable of adopting the correct conformation, even despite the assistance of TRiC, making incorporation into CRL^{CSA} impossible and resulting in re-binding of the unstable CSA protein by TRiC.

By regulating the functioning of CSA, TRiC likely contributes to preserving genome stability in response to transcription-stalling DNA damage. Given that TRiC's interactome has been estimated to comprise at least 5-10% of all cytoplasmic proteins, it is conceivable that it facilitates the folding and/or stabilization of additional, yet to be identified DDR factors, and thus protects genome stability in an even broader manner than currently anticipated[12]. The frequently applied method of protein precipitation followed by mass spectrometry to analyze coprecipitated factors could be a first step in identifying TRiC-bound proteins. However, if aiming to tag the TRiC complex to ease its purification, the subunit should be carefully chosen to prevent interference with complex build-up, as well as masking of the tag inside the chaperonin complex. Furthermore, tagging of the endogenous protein via CRISPR-Cas9-mediated gene editing is preferred over ectopic (over)expression of a tagged subunit to minimize overshadowing of TRiC by tagged protein that has not been incorporated into the complex.

Ultimately, TRiC's involvement in the DDR may also imply a role for this chaperonin in suppressing cancer development. Accordingly, TRiC was shown to stabilize several proteins that are implicated in genome stability maintenance and cancer progression, including the tumor suppressors VHL and p53 and oncoproteins cyclin B and cyclin E, which have all been shown to function in the DDR as well[13-21].

Evidently, given its role as a chaperonin, TRiC may also be crucial in the prevention of (ageing-related) neurodegenerative diseases that are hallmarked by protein aggregation. In agreement, TRiC-interacting proteins were shown to be enriched for aggregation-prone polypeptides. Importantly, its substrates include the Huntingtin protein, which in its aggregated form has been linked to Huntington's disease[12,22,23]. A possible association between the chaperone system and abnormal protein deposits in the brain is furthermore supported by a decline in the expression of heat shock proteins in Alzheimer's disease patients[24]. In contrast, cancer cells display upregulated levels of TRiC, as well as other molecular chaperones, resulting from de novo protein synthesis[19,24]. Thus, regulation of the levels of PN components seems to be crucial in the prevention of disease. Interestingly, however, elevated chaperone protein levels do not necessarily correlate with increased activity, as this is likely modulated by the functional interplay with other chaperones. For example, overall TRiC folding activity was shown to be reduced when levels of co-chaperones

Hsc/p70 and prefoldin are high and vice versa[19]. Potentially this implies the precise fine-tuning of specific chaperone activities, and consequently protein homeostasis, by modulating co-chaperone as well as substrate protein availabilities. Likewise, we speculate that disease-causing mutations that lead to high levels of protein persistently bound to TRiC could also have an impact on pathways in which the mutated protein is not (in)directly involved. By lowering the amount of free chaperone that is available to facilitate the folding/stabilization of other proteins, even seemingly unrelated cellular processes may be negatively affected.

The contribution of Zimp7 to cellular functioning

Further expanding our knowledge on protein-modifying enzymes, we uncovered the PIAS-like protein Zimp7 as a conceivable new SUMO E3 ligase. Its Siz/PIAS-RING (SP-RING) domain, which resembles that in PIAS ligases, appeared to confer bona fide SUMO-conjugating activity. Evidently, the ligase activity of full-length Zimp7, which may adopt a conformation that differs from the truncated protein that we studied, remains to be established. Furthermore, its actual behavior in vivo may be precisely regulated and needs to be examined as well. Mass spectrometry using cells that express either wildtype or SP-RING-mutated Zimp7 would be a straightforward first approach to reveal its contribution to overall SUMOylation under various conditions. In addition, this may expose specific potential targets, which subsequently could be validated in an in vitro set-up. Although the catalytic activity of Zimp7's SP-RING domain already is a valuable finding in terms of our general understanding of the repertoire of PTM-catalyzing factors, the true challenge lies in elucidating Zimp7's specific biological functions. Our observations, which are supported by those described in literature, indicate potential roles for Zimp7 in normal DNA replication and transcription, as well as in the DDR (described in Chapter 4).

In agreement with the colocalization of Zimp7 with newly synthesized DNA and PCNA at replication foci in S-phase, we established a clear interaction between Zimp7 and PCNA in unperturbed conditions, strengthening its possible involvement in DNA replication[25]. Furthermore, the described enrichment of Zimp7 at hydroxyurea-stalled replication forks, as detected in an iPOND study, argues for an additional role in the response to replication stress[26]. In yeast, adjustment of the PCNA SUMOylation status comprises an important mechanism to coordinate normal DNA replication as well as pathway choice upon replication stress. SUMOylation of PCNA influences its interaction with the anti-recombinogenic protein Srs2 that prevents (undesired) RAD51 filament formations that could facilitate homologous recombination between the newly formed sister chromatids[27-29]. Likewise, the human helicase PARI interacts with PCNA and may function analogously to Srs2[30,31]. Investigating potential functions of Zimp7 in regulating the SUMOylation of PCNA and other replication (stress) factors and/or the spatiotemporal modulation of the PARI-PCNA interaction could shed significant light on Zimp7's contribution to DNA replication and associated stress response pathways.

In addition, Zimp7's function may extend to (other pathways of) the DDR, which is demonstrated by its recruitment to sites of laser-induced DNA damage. The importance of the SUMO conjugation system for various aspects of the DDR is underscored by for example roles of PIAS1 and PIAS4 in the SUMOylation and/or recruitment of numerous factors in

response to double-strand DNA breaks (such as BRCA1, RAP80, 53BP1, RNF168 and RNF4), the enhancement of both homologous recombination and non-homologous end-joining by PIAS3, and the SUMOylation of proteins during BER (for instance TDG) or NER (including GG-NER factors XPC and DDB2, and TC-NER factor CSB)[32-41]. Exploring Zimp7's interaction partners and SUMOylation targets will gain more insight into the DNA damage response(s) that Zimp7 is involved in. Given that Zimp7 interacts with the BRG1 and BAF57 components of the SWI/SNF-like chromatin remodeling complexes, which have been implicated in several DDR pathways, it is interesting to speculate that Zimp7 assists in making structural adjustments in the chromatin that contribute to lesion accessibility during repair[25,42-44]. Furthermore, this may constitute a mechanism by which Zimp7 modulates regular transcription, during which chromatin remodeling is essential as well. Since Zimp7 was originally identified as an enhancer of androgen receptor-mediated transcription, studies related to its function have consequently focused on its transcription-regulating capacities[25,45,46]. Accordingly, augmentation of transcription that is controlled by a number of other nuclear hormone receptors, as well as the coactivation of the Wnt/β-catenin signaling pathway have been functionally ascribed to Zimp7[25,47]. RNA-seq experiments that examine the varying transcriptome in the presence or absence of Zimp7 would comprise a meaningful extension to this research, which has solely been based on reporter assays, and could enlighten a role in regular transcription. Importantly, the ability to regulate transcription would further justify Zimp7's classification as a PIAS-like protein, as numerous cases of either transcriptional activation or repression by the PIAS proteins have been reported[48]. Interestingly, whereas most of these were shown to depend on the SUMO-conjugating function of the involved PIAS protein, implying a functional link between its SUMOylating and transcription-regulating activities, this catalytic activity appeared to be dispensable for other cases of PIAS-dependent transcriptional regulation. For example, gene activation that is mediated by the DNA-binding protein SATB2 was shown to be decreased by PIAS1-induced SUMOylation[49]. On the contrary, although PIAS1 and PIAS4 were shown to SUMOylate the transcription factors Msx1 and LEF1, respectively, their effects on transcription via modulating the subnuclear localization of these proteins may occur through SUMO-independent mechanisms[48,50,51]. Regulation of hormone receptor-mediated transcription by Zimp7 is most likely at least in part facilitated by the intrinsic transcriptional activity that is provided by a C-terminal transactivation domain (TAD)[25]. The identification of a specific mutation that renders Zimp7 incapable of promoting SUMO conjugation, that is C616A, will significantly aid in determining to what extent Zimp7's functions, including those in transcription, can (additionally) be explained by its SUMOylating activity.

Given Zimp7's potential implications in the correct functioning of several crucial cellular processes such as transcription and DNA replication, as well as the protection against the hazardous effects of DNA damage, resolving its specific functions may also prove Zimp7's importance in the prevention of ageing-related diseases and cancer development. For example, Zimp7 was shown to be highly expressed in prostate cells, which require correct functioning of the androgen receptor (AR) for normal development as well as the prevention of tumorigenesis[45,52]. In addition to Zimp7, all the PIAS proteins have been shown to affect AR-mediated transcription in prostate cancer cells and presumably play important roles in

tumor initiation and progression[53-57]. Interestingly, again both coactivating and co-repressing effects on AR-controlled genes have been reported. Regulation of AR target genes by the PIAS proteins has consequentially been suggested to be target specific[54,57]. Furthermore, a delicate interplay between the AR, transcription factors and the PIAS proteins seems to regulate transcriptional activity[58]. Since we detected a clear interaction between Zimp7 and PIAS3, and interactions with other PIAS proteins have been described as well, it is tempting to hypothesize that Zimp7 may contribute to (physical) protein-protein interaction competitions and thereby provide an additional regulatory layer to AR-mediated transcription[59]. As the TAD in Zimp7 is (partly) responsible for Zimp7-induced stimulation of transcription that is controlled by the AR, the engagement of this domain in the involved interactions would be interesting to investigate[25]. Evidently, the requirement of Zimp7's SUMOylating catalytic activity for such mechanisms and regulation of AR-coordinated transcription in general should be assessed as well.

Most likely, future studies will not only give insight into Zimp7's role in the protection against tumorigenesis of prostate cells, but instead reveal general mechanisms that extend to the prevention of other types of cancer and ageing-related diseases. Although we are just at the beginning of unraveling its exact biological functions, Zimp7's broad implications in the processes that ensure correct cellular development and functioning, including genome stability maintenance, already seem to predict its significance in health and disease.

The association between NER defects and varying human disorders

The biological relevance of accurate spatiotemporal coordination of TC-NER stages and signaling cascades is illustrated by the broadly varying clinical consequences that are associated with inherited NER defects. The observation that not only different mutations in the same gene/protein, but even identical genetic alterations can have multiple clinical outcomes adds even more complexity to explaining the multifaceted disease manifestations[3,60]. Overall, defects in one of the XP (xeroderma pigmentosum) proteins that function in GG-NER seem to predominantly render patients hypersensitive to sunlight and increase cancer susceptibility[61]. This may be explained by the accumulation of DNA lesions throughout the genome, leading to mutagenesis in case of impaired GG-NER and bypass by translesion DNA polymerases during DNA replication[62]. Mutations in genes encoding the TC-NER proteins CSA or CSB primarily cause neurodevelopmental problems and accelerated ageing, while not augmenting cancer predisposition[63,64]. These clinical hallmarks of Cockayne syndrome may at least in part be accounted for by the accelerated cell death that is induced by persistently stalled transcription complexes[2]. This simplified explanation of the clinical consequences of defective NER also elegantly provides an interpretation of the combined XP/CS phenotypes that can be observed for (certain) mutations in proteins that function in the core NER machinery, that is XPA, XPB, XPD, XPF or XPG[61,65-67].

The hypothesis that the ability of cells to degrade lesion-stalled RNA polymerase and repair complexes when NER is compromised may contribute to the clinical outcome of NER gene mutations has remained an important matter of debate. The rationale behind this theory is not only that avoidance of persistently stalled RNA polymerase is crucial in the prevention of a p53-mediated signaling response that leads to premature cell death, but also involves the

accessibility of lesions to other repair pathways that could be generated by displacement of incompetent repair complexes[62,68-70]. If valid, this would imply that the more severe (neurological) phenotypes are to be expected when DNA damage-induced RNAPII degradation is disabled. Interestingly, while wildtype fibroblasts showed an overall decrease in the levels of the serine 5-phosphorylated RPB1 subunit of RNAPII (p-S5-RPB1) upon UV irradiation, we indeed observed that CS-A and CS-B patient cells were incapable of degrading p-S5-RPB1 after DNA damage induction. In contrast, cells derived from UVsS patients displayed slightly faster UV-induced p-S5-RPB1 degradation as compared to wildtype, which is in agreement with the relatively mild clinical phenotype of photosensitivity[3]. Furthermore, cells derived from an XP patient with a defect in XPA, for whom no neurological problems have been described, displayed a decrease in p-S5-RPB1 levels upon UV irradiation as well. Surprisingly, however, when we examined cells that were derived from two different patients that both harbored a mutation in the *XPD* gene, yet suffered from either XP or combined XP/CS, UV-induced p-S5-RPB1 degradation was detectable in (and comparable between) both cell lines. Thus, although seemingly a valid explanation for the difference between CS and UVsS, the ability to remove stalled RNAPII or repair complexes does not necessarily preclude the development of neurological problems. Evidently, the impact of a single cellular event, in this case the removal of stalled RNA polymerase, on the clinical outcome of a genetic defect is difficult to evaluate. In our efforts to explain patient phenotypes, potential implications of the associated defective protein in cellular processes other than NER, or even outside the DDR, should be taken into account as well. This is illustrated by mutations in XPB or XPD that not only cause CS features, but owing to their functions as subunits of the transcription factor TFIIH, additionally result in brittle hair and nails – a disorder known as trichothiodystrophy[72]. Similarly, roles for the CS proteins in transcription, maintenance of mitochondrial DNA stability and oxidative damage repair have been described[73-78]. For example, CSB may contribute to BER by facilitating the recruitment of XRCC1 to transcription-stalled RNAPII that is trapped at repair intermediates[79]. Removal of oxidative damage may be of particular importance in the brain, where the relatively high metabolic activity generates high levels of reactive oxygen species. Accordingly, defective oxidative damage repair in neurons has been linked to neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease[80,81]. Defects in CS proteins that, next to TC-NER impairment, cause additional defects in oxidative damage repair, could therefore (theoretically) lead to even more severe neurological problems.

Altogether, the complexity of NER-associated diseases and the clinical heterogeneity between patients, argue for research that not only studies the changes in cellular responses in the complete absence of a NER factor, but also examines the effect of specific patient mutations. As described previously, such an approach has already provided us with some important insights regarding the TRiC-mediated stability of CSA, by explaining how specific missense mutations can cause an overall decrease in protein availability[11]. Notably, the absolute absence of a protein may in some cases even be less detrimental than the presence of a defective one, as has been demonstrated for a case of UVsS that is associated with a mutation in the *CSB* gene causing a complete deficiency in CSB protein[82]. Although studying

individual patient mutations may at first complicate disease explanations even further, this will eventually improve our understanding of (the heterogeneity in) NER-related disorders.

Potentiation of C1-inhibitor activity by polysaccharides

The consequences of protein defects that negatively interfere with pathway progression and restrict its proper and complete execution have been described extensively. Yet, inappropriate or uncontrolled activation of protective responses may be equally harmful. Good examples are provided by the numerous autoimmune disorders that are characterized by abnormal activation of self-reactive immune responses, resulting in the attack of healthy body tissue. As a part of the immune system, cascades of proteolytic cleavages in short time can amplify a signal to evoke a massive response[83]. The serine protease inhibitor (serpin) C1-inhibitor regulates several of these pathways by trapping the initiating proteases into a conformation with a disrupted active site, thereby preventing their spontaneous activations[84]. Fundamental to C1-inhibitor's function is its reactive center loop (RCL), which contains the P1-P1' residues that appear as a substrate to the target protease. Attempts of the protease to process this bond result in an irreversible conformational change that transfers the protease to the other side of the serpin[85-87]. Importantly, deficiency of C1-inhibitor has been shown to underlie hereditary angioedema (HAE), which is hallmarked by recurrent attacks of swellings that are potentially life-threatening when occurring in the upper airways[88,89]. Disproportional activation of the kallikrein-kinin cascade of the contact system, which is under control of C1-inhibitor, results in the release of bradykinin that increases vascular permeability. Since its development in the 1970s, replacement therapy using C1-inhibitor isolated from human blood plasma has been used as a treatment for HAE[90,91]. Despite its proven effectiveness in the treatment of acute attacks, the use of plasma-derived protein has its downsides, including its availability and the risk of contamination. A significant step towards improving the therapy was made by the production of recombinant human C1-inhibitor secreted in the milk of transgenic rabbits, which for instance eliminates the risk of contamination with blood-borne viruses, but has its own limitations[92]. We suggest that further optimization can be sought in increasing the activity and/or effectiveness of the medicated serpin. The reported potentiation by glycosaminoglycans (GAGs), of which heparin and the synthetic dextran sulfate have been studied the most in this context, therefore seems a promising observation[93-95]. It would be interesting to explore the possibility to administer a pre-incubated GAG-C1-inhibitor complex or a combination of C1-inhibitor and a specifically designed molecule that enhances its activity. Evidently, a good understanding of how this potentiation is established is a prerequisite for such design. Our model, which is based on the structure of active serpin crystallized in the presence of dextran sulfate, provides some essential insights into the binding of the ligand to C1-inhibitor. Dextran sulfate binds to C1-inhibitor's F1 helix without inducing a conformational change.

Importantly, our docking studies also explain why potentiation of C1-inhibitor's activity is different towards its various target proteins – a finding that can be relevant in developing a molecule that specifically enhances the inhibition of a certain protease. Most likely, negatively charged polysaccharides neutralize the repulsive forces between the serpin's positively charged F1 helix and the protease's autolysis loop[96]. To validate these models (described in

Chapter 5), we propose that crystallization and structure solving of C1-inhibitor and dextran sulfate together with the studied proteases are performed.

Clearly, in view of therapy optimization, formation of single C1-inhibitor-GAG-protease complexes is preferred over the binding of either multiple C1-inhibitor or protease molecules. Although binding of GAGs can potentiate the activity of C1-inhibitor against some of its target proteases, the molecule to be used should therefore be carefully designed to prevent polymerization. Accordingly, we observed that multiple C1-inhibitor molecules bind to increasing sizes of dextran sulfate[96]. Isothermal calorimetry (ITC) experiments in the presence of a protease could show whether this would interfere with serpin-protease complex formation and which relative concentrations would be a suitable starting point for further research. Complex formation and stoichiometries could additionally be studied by native PAGE or (another method of) size exclusion chromatography, followed by immunoblotting.

Overall, exploring the possibilities for implementation of C1-inhibitor potentiation in disease treatment involves multiple complicated considerations that should guarantee therapy improvement as opposed to for example inducing multimerization or affecting other cellular processes. Furthermore, the desired specific promotion of kallikrein inhibition by a GAG(-like molecule) seems challenging, as our models indicate that the introduction of dextran sulfate mainly affects the inactivation of proteases with a positively charged autolysis loop. Nevertheless, we consider dextran sulfate a good foundation for further research, as it does not seem to interfere with antithrombin functioning (in contrast to heparin) and doses required for potentiation might be non-toxic, as suggested by some of our valuable insights into its binding and potentiating behavior[96,97].

References

1. Bartek, J., Bartkova, J. & Lukas, J. DNA damage signalling guards against activated oncogenes and tumour progression. *Oncogene* 26, 7773-9 (2007).
2. Hoeijmakers, J.H. DNA damage, aging, and cancer. *N Engl J Med* 361, 1475-85 (2009).
3. Dijk, M., Typas, D., Mullenders, L. & Pines, A. Insight in the multilevel regulation of NER. *Exp Cell Res* 329, 116-23 (2014).
4. Parkin, J. & Cohen, B. An overview of the immune system. *Lancet* 357, 1777-89 (2001).
5. Marshall, J.S., Warrington, R., Watson, W. & Kim, H.L. An introduction to immunology and immunopathology. *Allergy Asthma Clin Immunol* 14, 49 (2018).
6. Candeias, S.M. & Gaipl, U.S. The Immune System in Cancer Prevention, Development and Therapy. *Anticancer Agents Med Chem* 16, 101-7 (2016).
7. Balchin, D., Hayer-Hartl, M. & Hartl, F.U. In vivo aspects of protein folding and quality control. *Science* 353, aac4354 (2016).
8. Freund, A. et al. Proteostatic Control of Telomerase Function through TRiC-Mediated Folding of TCAB1. *Cell* 159, 1389-1403 (2014).
9. Horwich, A.L., Fenton, W.A., Chapman, E. & Farr, G.W. Two families of chaperonin: physiology and mechanism. *Annu Rev Cell Dev Biol* 23, 115-45 (2007).
10. Leitner, A. et al. The molecular architecture of the eukaryotic chaperonin TRiC/CCT. *Structure* 20, 814-25 (2012).
11. Pines, A. et al. TRiC controls transcription resumption after UV damage by regulating Cockayne syndrome protein A. *Nat Commun* 9, 1040 (2018).
12. Yam, A.Y. et al. Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. *Nat Struct Mol Biol* 15, 1255-62 (2008).
13. Espana-Agusti, J., Warren, A., Chew, S.K., Adams, D.J. & Matakidou, A. Loss of PBRM1 rescues VHL dependent replication stress to promote renal carcinogenesis. *Nat Commun* 8, 2026 (2017).
14. Feldman, D.E., Spiess, C., Howard, D.E. & Frydman, J. Tumorigenic mutations in VHL disrupt folding in vivo by interfering with chaperonin binding. *Mol Cell* 12, 1213-24 (2003).
15. Melville, M.W., McClellan, A.J., Meyer, A.S., Darveau, A. & Frydman, J. The Hsp70 and TRiC/CCT chaperone systems cooperate in vivo to assemble the von Hippel-Lindau tumor suppressor complex. *Mol Cell Biol* 23, 3141-51 (2003).
16. Metcalf, J.L. et al. K63-ubiquitylation of VHL by SOCS1 mediates DNA double-strand break repair. *Oncogene* 33, 1055-65 (2014).
17. Trinidad, A.G. et al. Interaction of p53 with the CCT complex promotes protein folding and wild-type p53 activity. *Mol Cell* 50, 805-17 (2013).
18. Reinhardt, H.C. & Schumacher, B. The p53 network: cellular and systemic DNA damage responses in aging and cancer. *Trends Genet* 28, 128-36 (2012).
19. Boudiaf-Benmammar, C., Cresteil, T. & Melki, R. The cytosolic chaperonin CCT/TRiC and cancer cell proliferation. *PLoS One* 8, e60895 (2013).
20. Won, K.A., Schumacher, R.J., Farr, G.W., Horwich, A.L. & Reed, S.I. Maturation of human cyclin E requires the function of eukaryotic chaperonin CCT. *Mol Cell Biol* 18, 7584-9 (1998).
21. Nakayama, Y. & Yamaguchi, N. Role of cyclin B1 levels in DNA damage and DNA damage-induced senescence. *Int Rev Cell Mol Biol* 305, 303-37 (2013).
22. Shahmoradian, S.H. et al. TRiC's tricks inhibit huntingtin aggregation. *Elife* 2, e00710 (2013).
23. Tam, S. et al. The chaperonin TRiC blocks a huntingtin sequence element that promotes the conformational switch to aggregation. *Nat Struct Mol Biol* 16, 1279-85 (2009).
24. Calderwood, S.K. & Murshid, A. Molecular Chaperone Accumulation in Cancer and Decrease in Alzheimer's Disease: The Potential Roles of HSF1. *Front Neurosci* 11, 192 (2017).
25. Huang, C.Y. et al. hZimp7, a novel PIAS-like protein, enhances androgen receptor-mediated transcription and interacts with SWI/SNF-like BAF complexes. *Mol Endocrinol* 19, 2915-29 (2005).
26. Dungrawala, H. et al. The Replication Checkpoint Prevents Two Types of Fork Collapse without Regulating Replisome Stability. *Mol Cell* 59, 998-1010 (2015).

27. Papouli, E. et al. Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol Cell* 19, 123-33 (2005).
28. Pfander, B., Moldovan, G.L., Sacher, M., Hoege, C. & Jentsch, S. SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature* 436, 428-33 (2005).
29. Watts, F.Z. Sumoylation of PCNA: Wrestling with recombination at stalled replication forks. *DNA Repair (Amst)* 5, 399-403 (2006).
30. Garcia-Rodriguez, N., Wong, R.P. & Ulrich, H.D. Functions of Ubiquitin and SUMO in DNA Replication and Replication Stress. *Front Genet* 7, 87 (2016).
31. Moldovan, G.L. et al. Inhibition of homologous recombination by the PCNA-interacting protein PARI. *Mol Cell* 45, 75-86 (2012).
32. Morris, J.R. et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. *Nature* 462, 886-90 (2009).
33. Hu, X., Paul, A. & Wang, B. Rap80 protein recruitment to DNA double-strand breaks requires binding to both small ubiquitin-like modifier (SUMO) and ubiquitin conjugates. *J Biol Chem* 287, 25510-9 (2012).
34. Galanty, Y., Belotserkovskaya, R., Coates, J. & Jackson, S.P. RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev* 26, 1179-95 (2012).
35. Bekker-Jensen, S. & Mailand, N. The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks. *FEBS Lett* 585, 2914-9 (2011).
36. Liu, S. et al. PIAS3 promotes homology-directed repair and distal non-homologous end joining. *Oncol Lett* 6, 1045-1048 (2013).
37. Hardeland, U., Steinacher, R., Jiricny, J. & Schar, P. Modification of the human thymine-DNA glycosylase by ubiquitin-like proteins facilitates enzymatic turnover. *EMBO J* 21, 1456-64 (2002).
38. Steinacher, R. & Schar, P. Functionality of human thymine DNA glycosylase requires SUMO-regulated changes in protein conformation. *Curr Biol* 15, 616-23 (2005).
39. Poulsen, S.L. et al. RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J Cell Biol* 201, 797-807 (2013).
40. van Cuijk, L. et al. SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair. *Nat Commun* 6, 7499 (2015).
41. Sin, Y., Tanaka, K. & Saijo, M. The C-terminal Region and SUMOylation of Cockayne Syndrome Group B Protein Play Critical Roles in Transcription-coupled Nucleotide Excision Repair. *J Biol Chem* 291, 1387-97 (2016).
42. Hara, R. & Sancar, A. The SWI/SNF chromatin-remodeling factor stimulates repair by human excision nuclease in the mononucleosome core particle. *Mol Cell Biol* 22, 6779-87 (2002).
43. Ogiwara, H. et al. Histone acetylation by CBP and p300 at double-strand break sites facilitates SWI/SNF chromatin remodeling and the recruitment of non-homologous end joining factors. *Oncogene* 30, 2135-46 (2011).
44. Park, J.H. et al. Mammalian SWI/SNF complexes facilitate DNA double-strand break repair by promoting gamma-H2AX induction. *EMBO J* 25, 3986-97 (2006).
45. Beliakoff, J. & Sun, Z. Zimp7 and Zimp10, two novel PIAS-like proteins, function as androgen receptor coregulators. *Nucl Recept Signal* 4, e017 (2006).
46. Li, X. et al. ZMIZ1 preferably enhances the transcriptional activity of androgen receptor with short polyglutamine tract. *PLoS One* 6, e25040 (2011).
47. Lee, S.H. et al. Identification of a novel role of ZMIZ2 protein in regulating the activity of the Wnt/beta-catenin signaling pathway. *J Biol Chem* 288, 35913-24 (2013).
48. Sharrocks, A.D. PIAS proteins and transcriptional regulation--more than just SUMO E3 ligases? *Genes Dev* 20, 754-8 (2006).
49. Dobreva, G., Dambacher, J. & Grosschedl, R. SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin mu gene expression. *Genes Dev* 17, 3048-61 (2003).
50. Lee, H. et al. PIAS1 confers DNA-binding specificity on the Msx1 homeoprotein. *Genes Dev* 20, 784-94 (2006).
51. Sachdev, S. et al. PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. *Genes Dev* 15, 3088-103 (2001).

52. Leach, D.A. & Buchanan, G. Stromal Androgen Receptor in Prostate Cancer Development and Progression. *Cancers (Basel)* 9(2017).
53. Gross, M., Yang, R., Top, I., Gasper, C. & Shuai, K. PIASy-mediated repression of the androgen receptor is independent of sumoylation. *Oncogene* 23, 3059-66 (2004).
54. Toropainen, S. et al. SUMO ligase PIAS1 functions as a target gene selective androgen receptor coregulator on prostate cancer cell chromatin. *Nucleic Acids Res* 43, 848-61 (2015).
55. Junicho, A. et al. Protein inhibitor of activated STAT3 regulates androgen receptor signaling in prostate carcinoma cells. *Biochem Biophys Res Commun* 278, 9-13 (2000).
56. Rabellino, A., Andreani, C. & Scaglioni, P.P. The Role of PIAS SUMO E3-Ligases in Cancer. *Cancer Res* 77, 1542-1547 (2017).
57. Kotaja, N., Aittomaki, S., Silvennoinen, O., Palvimo, J.J. & Janne, O.A. ARIP3 (androgen receptor-interacting protein 3) and other PIAS (protein inhibitor of activated STAT) proteins differ in their ability to modulate steroid receptor-dependent transcriptional activation. *Mol Endocrinol* 14, 1986-2000 (2000).
58. Yamamoto, T. et al. Molecular interactions between STAT3 and protein inhibitor of activated STAT3, and androgen receptor. *Biochem Biophys Res Commun* 306, 610-5 (2003).
59. Peng, Y., Lee, J., Zhu, C. & Sun, Z. A novel role for protein inhibitor of activated STAT (PIAS) proteins in modulating the activity of Zimp7, a novel PIAS-like protein, in androgen receptor-mediated transcription. *J Biol Chem* 285, 11465-75 (2010).
60. Colella, S., Nardo, T., Botta, E., Lehmann, A.R. & Stefanini, M. Identical mutations in the CSB gene associated with either Cockayne syndrome or the DeSanctis-cacchione variant of xeroderma pigmentosum. *Hum Mol Genet* 9, 1171-5 (2000).
61. DiGiovanna, J.J. & Kraemer, K.H. Shining a light on xeroderma pigmentosum. *J Invest Dermatol* 132, 785-96 (2012).
62. Marteijn, J.A., Lans, H., Vermeulen, W. & Hoeijmakers, J.H. Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat Rev Mol Cell Biol* 15, 465-81 (2014).
63. Laugel, V. et al. Mutation update for the CSB/ERCC6 and CSA/ERCC8 genes involved in Cockayne syndrome. *Hum Mutat* 31, 113-26 (2010).
64. Laugel, V. Cockayne syndrome: the expanding clinical and mutational spectrum. *Mech Ageing Dev* 134, 161-70 (2013).
65. Bradford, P.T. et al. Cancer and neurologic degeneration in xeroderma pigmentosum: long term follow-up characterises the role of DNA repair. *J Med Genet* 48, 168-76 (2011).
66. Barnes, D.E. & Lindahl, T. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu Rev Genet* 38, 445-76 (2004).
67. Rahbar, Z. & Naraghi, M. De Sanctis-Cacchione syndrome: A case report and literature review. *Int J Womens Dermatol* 1, 136-139 (2015).
68. Ljungman, M. & Zhang, F. Blockage of RNA polymerase as a possible trigger for u.v. light-induced apoptosis. *Oncogene* 13, 823-31 (1996).
69. Vrouwe, M.G., Pines, A., Overmeer, R.M., Hanada, K. & Mullenders, L.H. UV-induced photolesions elicit ATR-kinase-dependent signaling in non-cycling cells through nucleotide excision repair-dependent and -independent pathways. *J Cell Sci* 124, 435-46 (2011).
70. Ljungman, M. & Lane, D.P. Transcription - guarding the genome by sensing DNA damage. *Nat Rev Cancer* 4, 727-37 (2004).
71. Brooks, P.J. 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 (Amst)* 12, 656-71 (2013).
72. Kraemer, K.H. et al. Xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome: a complex genotype-phenotype relationship. *Neuroscience* 145, 1388-96 (2007).
73. Balajee, A.S., May, A., Dianov, G.L., Friedberg, E.C. & Bohr, V.A. Reduced RNA polymerase II transcription in intact and permeabilized Cockayne syndrome group B cells. *Proc Natl Acad Sci U S A* 94, 4306-11 (1997).
74. Selby, C.P. & Sancar, A. Cockayne syndrome group B protein enhances elongation by RNA polymerase II. *Proc Natl Acad Sci U S A* 94, 11205-9 (1997).

75. van Gool, A.J. et al. The Cockayne syndrome B protein, involved in transcription-coupled DNA repair, resides in an RNA polymerase II-containing complex. *EMBO J* 16, 5955-65 (1997).
76. Menoni, H., Hoeijmakers, J.H. & Vermeulen, W. Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions in vivo. *J Cell Biol* 199, 1037-46 (2012).
77. Aamann, M.D. et al. Cockayne syndrome group B protein promotes mitochondrial DNA stability by supporting the DNA repair association with the mitochondrial membrane. *FASEB J* 24, 2334-46 (2010).
78. Scheibye-Knudsen, M., Croteau, D.L. & Bohr, V.A. Mitochondrial deficiency in Cockayne syndrome. *Mech Ageing Dev* 134, 275-83 (2013).
79. Menoni, H. et al. The transcription-coupled DNA repair-initiating protein CSB promotes XRCC1 recruitment to oxidative DNA damage. *Nucleic Acids Res* 46, 7747-7756 (2018).
80. Canugovi, C., Misiak, M., Ferrarelli, L.K., Croteau, D.L. & Bohr, V.A. The role of DNA repair in brain related disease pathology. *DNA Repair (Amst)* 12, 578-87 (2013).
81. Narciso, L. et al. The Response to Oxidative DNA Damage in Neurons: Mechanisms and Disease. *Neural Plast* 2016, 3619274 (2016).
82. Horibata, K. et al. Complete absence of Cockayne syndrome group B gene product gives rise to UV-sensitive syndrome but not Cockayne syndrome. *Proc Natl Acad Sci U S A* 101, 15410-5 (2004).
83. Giang, J. et al. Complement Activation in Inflammatory Skin Diseases. *Front Immunol* 9, 639 (2018).
84. Davis, A.E., III, Lu, F. & Mejia, P. C1 inhibitor, a multi-functional serine protease inhibitor. *Thromb. Haemost* 104, 886-893 (2010).
85. Gettins, P.G. & Olson, S.T. Inhibitory serpins. New insights into their folding, polymerization, regulation and clearance. *Biochem J* 473, 2273-93 (2016).
86. Huntington, J.A. Serpin structure, function and dysfunction. *J. Thromb. Haemost* 9 Suppl 1, 26-34 (2011).
87. Huntington, J.A., Read, R.J. & Carrell, R.W. Structure of a serpin-protease complex shows inhibition by deformation. *Nature* 407, 923-926 (2000).
88. Cugno, M., Zanichelli, A., Foieni, F., Caccia, S. & Cicardi, M. C1-inhibitor deficiency and angioedema: molecular mechanisms and clinical progress. *Trends Mol. Med* 15, 69-78 (2009).
89. Carugati, A., Pappalardo, E., Zingale, L.C. & Cicardi, M. C1-inhibitor deficiency and angioedema. *Mol. Immunol* 38, 161-173 (2001).
90. Prematta, M.J., Prematta, T. & Craig, T.J. Treatment of hereditary angioedema with plasma-derived C1 inhibitor. *Ther Clin Risk Manag* 4, 975-82 (2008).
91. Henry Li, H., Riedl, M. & Kashkin, J. Update on the Use of C1-Esterase Inhibitor Replacement Therapy in the Acute and Prophylactic Treatment of Hereditary Angioedema. *Clin Rev Allergy Immunol* 56, 207-218 (2019).
92. van Veen, H.A. et al. Characterization of recombinant human C1 inhibitor secreted in milk of transgenic rabbits. *J. Biotechnol* 162, 319-326 (2012).
93. Caldwell, E.E. et al. Heparin binding and augmentation of C1 inhibitor activity. *Arch. Biochem. Biophys* 361, 215-222 (1999).
94. Wuillemin, W.A. et al. Potentiation of C1 inhibitor by glycosaminoglycans: dextran sulfate species are effective inhibitors of in vitro complement activation in plasma. *J. Immunol* 159, 1953-1960 (1997).
95. Zhou, Z.H., Rajabi, M., Chen, T., Karnaukhova, E. & Kozlowski, S. Oversulfated chondroitin sulfate inhibits the complement classical pathway by potentiating C1 inhibitor. *PLoS. One* 7, e47296 (2012).
96. Dijk, M. et al. How Dextran Sulfate Affects C1-inhibitor Activity: A Model for Polysaccharide Potentiation. *Structure* 24, 2182-2189 (2016).
97. Bos, I.G. et al. The potentiation of human C1-inhibitor by dextran sulphate is transient in vivo: studies in a rat model. *Int. Immunopharmacol* 1, 1583-1595 (2001).