Mini-review

Regulation of UV-induced DNA damage response by ubiquitylation

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Abstract

Like many other cellular processes, regulation of the DNA damage response (DDR) is regulated at different levels, ranging from transcriptional control to an array of distinct post-translational modifications. Involvement of ubiquitylation and the ubiquitin proteasome system in adjusting DDR are such protein modifications that were receiving increasing attention in the field. In this review we summarize and discuss a few recent key publications addressing the issue of DDR factor ubiquitylation, focusing on UV-induced DDR. We discuss the implications of these modifications to allow swift adaptation and regulation of genome surveillance factors.

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ubiquitin modifications have sprouted—one of these being the DNA damage response (DDR) factors where many proteins need to be activated or altered in a highly orchestrated manner.

Each cell is confronted with a rich spectrum of DNA damages during its normal live cycle. To circumvent the severe consequences on cellular performance of these genomic insults a complex array of DDR mechanisms has evolved. These include different DNA repair systems, damage avoidance processes to prevent replication blockage and damage signalling systems [2]. Which of the DDR processes is activated after genomic stress depends on the type of lesions as well as their genomic location and timing within the cell cycle [3]. DNA lesions particularly interfere with replication and transcription, however each of these processes apply entirely different strategies to cope with obstructing lesions. Stalled replication forks at DNA lesions are resolved by either template switching or recombination, or by temporarily invoking the action of alternative polymerases to perform translesion synthesis (TLS). To avoid the severe cytotoxic effect of lesion-blocked transcription a highly specialized transcription-coupled repair (TCR) mechanism is activated. Obviously, delicate adjustments of repair and coordination between repair, transcription, replication and cell cycle checkpoints are required in DDR. Cells utilize multiple regulation mechanisms in (adaptive) response mechanisms, such as transcriptional regulation and post-translational protein modifications (PTM) to fine-tune cellular homeostasis. In this perspective/review we will mainly focus on ubiquitin-related mechanisms in DDR to UV-induced DNA lesions, as discussed in a few recent key publications addressing this item.

Ubiquitin conjugation to substrates is catalysed by a three-step enzymatic mechanism. First the ‘E1 ubiquitin activating’ enzyme transfers ubiquitin in an ATP-dependent manner to a subsequent ‘E2 ubiquitin conjugating’ enzyme that catalyses the covalent attachment of ubiquitin, either with the help of the ‘E3 ligase’ (RING domain, subclass) or by transferring the ubiquitin to the E3 (HECT domain, subclass) after which the E3 conjugates the ubiquitin to the substrate [4]. Ubiquitin conjugation may elicit widely different actions on the protein substrate, such as refolding, or remodelling, altering enzymatic action or intracellular sorting as well as breakdown by the ubiquitin proteasome system (UPS) [4].

1. DDR to UV-induced DNA lesions

UV-light is a highly mutagenic and cytotoxic agent for all species. Multiple defence mechanisms have emerged during evolution [5]. Within placental mammals NER is the dominant response to UV-lesions in addition to checkpoint activation via ATR, Chk1 and p53 and application of alternative translesion DNA polymerases [6–8].

Dealing with UV-lesions is a prime example of different strategies that cells apply to respond to this genomic insult. This response likely reflects general rules within DNA damage response, the beauty in studying UV-induced DDR is that the applied genotoxic agents can be administered within the second timescale to virtually all model organisms and laboratory cell cultures and allows direct monitoring of the consequences. The response depends on the genomic position, type of the lesion (cyclobutane pyrimidine dimer (CPD) or 6-4 photo products (6-4PP)) and when the lesion is encountered. Two distinct damage recognition pathways are operational within NER, global genome repair (GGR) and TCR. GGR is targeted to lesions located anywhere in the genome, following a ‘just-in-case’ type of scenario to deal with genomic insults. GGR efficiently removes 6-4PPs, however, the most abundant UV-lesion; CPDs, appeared a poor substrate for this pathway. As a consequence a large number of CPDs remain unrepaird and form a roadblock for elongating DNA and RNA polymerases. Replication problems are solved by damage avoidance processes and TLS (see below). Since there are no alternative RNA polymerases nor the possibility to switch template in transcription a complete different strategy has emerged to neutralize the highly cytotoxic transcription blocking lesions, that is by TCR, which efficiently removes lesions (including CPDs) from the transcribed strand (only) of actively transcribed genes [9]. These ‘just-in-time’ strategies to cope with genomic injuries can be considered as emergency mechanisms. As with most crisis management the applied procedures to counteract the catastrophe are usually less specific and may harm other cellular activities. For this reason it is obvious that particularly these emergency scenarios should be tightly controlled. In the DNA damage tolerance process for example, TLS low fidelity alternative DNA polymerases are recruited to stalled replication forks (see below). Exaggerated contribution of these polymerases to normal replication would lead to enhanced mutagenesis, thus their action should be restricted to replication forks where lesions are encountered. In addition, DNA damage recognition proteins have affinity for undamaged DNA as well, creating a potential hazardous situation. Post-translational modifications such as, phosphorylation, ubiquitylation and SUMOylation have turned out to play a key role in this regulation, both to control and to achieve swift adaptation. Several strategies are applied to minimize fortuitous or illegitimate actions, ranging from re-localization, to altered activity and even proteolytic degradation. The evolutionary benefit appears obvious, given the large number of critical steps in genome maintenance that are regulated in this manner (for examples in the UV-induced DNA damage response see Fig. 1).

2. Stalled replication forks and ubiquitylation

Perhaps the best-explored DNA damage-dependent ubiquitylation is the differential modification of the proliferating cell nuclear antigen (PCNA). PCNA forms a clamp on the parental DNA to support the action of DNA polymerases. Most knowledge on the different modifications of PCNA have been acquired from studies in yeast the implications of these have been extensively reviewed by others [10,11] and will therefore not be discussed here. In short: lysine 164 of PCNA can be SUMOylated, monoubiquitylated or polyubiquitylated [12,13]. Polyubiquitylated PCNA in yeast is functioning in error-free, damage-avoiding replication, whereas monoubiquitylated PCNA is implicated in error-prone translesion synthesis [12]. In mammalian cells monoubiquitylation [12,14] and recently
Fig. 1 – UV-induced DNA damage-dependent ubiquitylations. Schematic representation of the different DNA damage response pathways that are differentially modified by ubiquitylation, which are activated after UV-irradiation, depending on genomic location (TCR at the transcribed strand, GGR at other genomic loci) and time within the cell cycle (damage-induced replication stress). For simplicity, DNA is presented as a ladder and the lesion as a small triangle. Within global genome nucleotide excision repair (GGR) UV-induced DNA lesions located in the non-transcribed part of the genome or on the non-transcribed strand are recognized by the UV–DDB and XPC/HR23B/Cen2 complexes (left panel). UV–DDB resides in a complex containing DDB1, DDB2 (XPE), Cul4A and Roc1 that harbors E3 ligase activity and the Cop9 Signalosome (CSN) that displays de-ubiquitylation activity. After binding to lesions the CSN dissociates allowing the intrinsic E3 ligase to (poly)ubiquitylate DDB2 and subsequently also XPC. After subsequent recruitment of the TFIIH complex and other NER factors, XPC and DDB dissociate from the DNA. Free polyubiquitylated DDB is degraded whereas polyubiquitylated XPC is not. Lesions induced in the transcribed strand cause stalling of the elongating RNA polymerase (RNApol II) complex, this triggers transcription-coupled NER (TCR) (middle panel). The CSB protein plays a crucial role in the subsequent recruitment of the core NER factors leading to removal of elongating blocking lesion. In addition, CSB is also important for binding of the CSA-containing DDB1/Cul4A/Roc1/CSN to the stalled complex. After lesion removal CSB is degraded after polyubiquitylation in a CSA-dependent fashion. This CSB break down is thought to be required for subsequent transcription resumption. Lesions refractory to repair cause prolonged staling and the subsequent polyubiquitylation and degradation of the largest subunit (Rpb1) of RNApol II. Lesion obstructed DNA polymerase (during DNA replication in S-phase) induces monoubiquitylation of PCNA. This modified form PCNA is a substrate for alternative or translesion (TLS) polymerases. Most of these TLS polymerases, including pol eta, contain a ubiquitin-interacting domain that facilitates the interaction of monoubiquitylated PCNA and the polymerase.

also polyubiquitylation [15,16] have been reported. Whereas PCNA SUMOylation has recently been shown to occur in avian cells [17]. In yeast Rad6 (E2) and Rad18 (E3) were identified to establish the monoubiquitylation of PCNA whereupon Mms2/Ubc13 (E2) together with Rad5 (E3) further polyubiquitylate via a lys 63 chain PCNA [12]. Monoubiquitylation of PCNA is essential for the recruitment of polymerase η-mediated translesion synthesis (polymerase η (polη) is one of the TLS polymerases, able to efficiently bypass UV-induced lesions) to lesion-stalled replication forks. Polη, but not DNA polymerase 6, specifically binds monoubiquitylated PCNA [14,18]. Most, if not all, DNA polymerases have a PCNA-interacting domain. In addition, the Y-family polymerases harbor different variations of ubiquitin-interacting domains [19]. It was suggested that PCNA ubiquitylation forms a molecular switch between normal synthesis polymerases and lesion bypass polymerases.
yeast SUMOylation of PCNA on lys164 is likely involved in suppressing recombination during S-phase by the recruitment of the Srs2 helicase [20,21].

PCNA has yet another essential function in the damage response. At the end of normal S-phase the S-phase licensing factor Cdc510-dependent transcript1 (Cdt1) is degraded to suppress further rounds of replication. Cdt1 is polyubiquitylated on the chromatin by the Cul4–DDB1–Roc1 complex. Although Cdt1 binds DDB1 directly, polyubiquitylation is initiated by the binding of Cdt to PCNA [22,23]. DNA damage induces proteolysis of Cdt1, possibly to inhibit cells containing genomic insults to entry S-phase [24–26]. For DNA damage-induced Cdt1 degradation the PCNA interaction is essential as well [23,25]. All these observations underline the essential roles that PCNA and the post-translational modifications of PCNA (and those mediated by them) play in the cellular defence response towards genotoxic insults.

3. DNA damage-dependent ubiquitylation of RNA polymerase II

Lesion-stalled RNA polymerase II (RNAPII), when not properly handled can even provoke apoptosis [27]. As mentioned above TCR is an immediate response to relieve this cellular hazard. However, in yeast it has been shown that prolonged stalling either by DNA damage [28,29] or by other means of elongation pausing [30], induces polyubiquitylation and subsequent degradation by the 26S proteasome of the largest subunit of RNAPII (Rpb1) [31]. Polyubiquitylation of Rpb1/RNAPII is dependent on the yeast gene Def1. Although Def1 is in complex with the TCR protein Rad26 (yeast counterpart of CSB), the latter is not essential for Rpb1/RNAPII polyubiquitylation [31,32]. In contrast, the DNA damage-induced ubiquitylation of RNAPII is dependent on both Cockayne syndrome A (CSA) and CSB in humans [33]. A true mammalian ortholog for Def1 was not identified [31], possibly reflecting that during evolution different strategies have emerged to solve this problem [34]. Both the E2 (Ubcs5/Ubc4) and E3 (Rsp5) were identified as the enzymes that catalyse RNAPII ubiquitylation [28,30]. Elegant yeast work has shown that polyubiquitylation of Rpb1 was more efficient on the immobilized ternary elongation complex consisting of DNA template, RNAPII and nascent RNA than on free RNAPII or promoter-bound polymerase [30]. This difference is determined by the phosphorylation status of RNAPII’s C-terminal repeat domain (CTD). The ubiquitylation of RNAPII is inhibited by phosphorylation of the serine 5 residue CTD, a specific phosphorylation associated with early (promoter-bound) transcription. Ser2 phosphorylation of the CTD, associated with elongating RNAPII, is however competent/adequate for ubiquitylation. This cooperative action of distinct post-translational modifications illustrates that a high selectivity can be reached, specifically paused elongating RNAPII is vulnerable to proteolytic degradation rather than the more frequently occurring temporal entrapment of RNAPII (abortive initiation) in the transition from initiation to early elongation. In contrast, it was recently shown that Ser5-phosphorylated RNAPII ubiquitylation occurred after cisplatin-induced DNA damage, possibly explained by an alternative (non lys48) ubiquitylation [35].

The degradation of the large subunit of RNAPII is most likely a last “emergency break” to avoid irreversible stalling of the RNAPII. In addition, the TCR option (dependent on the action of the CSA and CSB proteins) is also subject to ubiquitin-dependent modulation. The CSB protein is a SWI/SNF-like DNA-dependent ATPase, that possesses nucleosome remodeling activity [36] and is likely involved in transcription elongation [37,38]. CSB accumulates at repair sites [39] and plays a crucial role in the further assembly of a functional TCR-complex [40]. CSA resides in an E3 ligase complex, containing DDB1, Roc1, Cull4A and the COP9 signalosome, i.e. the same E3 ligase complex implicated in global genome repair, except that CSA is substituted for DDB2 (see below) [41]. This complex is recruited to the nuclear matrix in a CSB-dependent fashion after UV-light, likely to modify stalled transcription complexes [42]. Recently CSB was identified as (one of the) substrate(s) for the CSA associated E3 ligase complex. UV-irradiation induced the degradation of CSB via the ubiquitin proteasome system in a CSA-dependent manner [43]. Interestingly CSB degradation occurs in a relatively late step of TCR, several hours post UV-irradiation and this degradation appeared to play an important role in the recovery of RNA synthesis and subsequent survival of the damaged cell [44]. In this model first loading of CSB is essential to initiate TCR, whereas its subsequent ubiquitin-dependent degradation is required to restart transcription, again showing that modification by ubiquitin plays an important regulatory role in DDR.

4. Ubiquitylation of the DNA damage binding (DDB) protein

Initiation of global genome NER (GGR) is performed by two protein complexes, i.e. DDB and XPC, each with an intrinsic high affinity towards DNA-helix distortions [45–48]. DDB was identified as a UV-induced DNA damage binding protein deficient in xeroderma pigmentosum group E (XP-E) cells [49]. Initially this heterodimeric protein complex, consisting of the DDB1 and DDB2 polypeptides (respectively p127 and p48), was thought to be specifically required for CPD removal in GGR [50], however recent evidence also suggest a stimulatory effect on 6-4PP repair [51,52]. A multifaceted – and seemingly contradicting – mechanism of regulating the activity of this complex was found by controlling the level of DDB2 both at the transcriptional and the post-translational level with entirely different kinetics. A delayed UV-induced and p53-dependent transcriptional up-regulation of DDB2 expression was observed with maximal induction 48 h post UV [53]. Paradoxically, within a few hours post UV a surprising strong reduction in the total cellular amount of this p48 polypeptide was reported [54]. In earlier studies Shiyanov and co-workers found that the DDB complex interacts with Cull4A (one of the members of the cullin family subunit of ubiquitin-protein (E3) ligases) [55]. Cull4A stimulates the degradation of the p48 subunit of DDB2 through the ubiquitin-proteasome pathway [56]. More recently, DDB1 and DDB2 were found to be part of a larger ubiquitin ligase complex, almost identical to the CSA-containing complex apart from the absence of CSA [41]. The associated COP9 signalosome (CSN) complex suppresses (poly)ubiquitylation, displayed by the Cull4A E3 ligase [57],
to repair most of the UV-induced 6-4PP, the main target for UV-irradiation [63], which matches with the time required for polyubiquitylation of DDB2 that is subsequently degraded by the proteasome [41,54,58].

The damage-induced degradation suggests that the activity induced by DDB2 should be restricted to the very early response, i.e. removal of 6-4PPs. This early degradation is contrary to the expectation in view of the notion that DDB was thought to be specifically required for CPD removal [59]. CPD’s are slowly repaired and the bulk of these lesions are still not removed when most DDB2 is degraded. The exact biological function of this early degradation remains enigmatic. Transcriptional regulation by p53 likely functions to restore DDB2 levels and the elevated levels (as compared to the non-challenged situation) might function as an adaptation mechanism. Since DDB2 bridges the Cul4A complex to the damaged site (within the context of highly organised chromatin) it is tempting to speculate that other proteins are targeted by the E3 ligase of this complex. Regulation by swift degradation might be necessary to avoid massive chromatin ubiquitylation.

5. DDB-dependent ubiquitylation of XPC

DDB bound to lesions stimulates the recruitment of the heterotrimeric XPC complex (i.e. XPC, HR23B and cen2 [60,61]) to repair sites [51,52]. Recently, two independent studies described the UV-dependent ubiquitylation of the DNA damage sensor XPC [62,63]. This ubiquitylation is also mediated by the DDB–Cul4A complex, as it fails in cells from XP-E patients (mutated in DDB2) but not in any of the other NER-deficient XP fibroblasts [63]. Although both recognition complexes are polyubiquitylated when bound to lesions, the consequences of this event are remarkably different for each. Polyubiquitylated DDB2 dissociates from the damage and is rapidly degraded, but polyubiquitylated XPC is not degraded [63]. Instead, the latter displays an increased affinity for both damaged and undamaged DNA as measured by an in vitro binding assay. Modified XPC persisted for several hours post UV and was found in both DNA-bound and DNA-free cellular fractions, suggesting that also after release from the repair site XPC remains ubiquitylated. The higher affinity for damaged DNA might boost the efficiency of the entire NER reaction. Identification of the targeted lysine(s) and subsequent experiments using ubiquitylation-negative XPC mutants will be helpful to further establish the effect of the XPC ubiquitylation properly.

UV-induced PTM of XPC is further complicated by the observed additional SUMOylation [62]. Contrary to ubiquitylation this SUMOylation is dependent on the further progression of the NER reaction, since in fully NER-defective XP-A cells it does not occur. Polyubiquitylated XPC in XP-A cells is rapidly degraded, suggesting that SUMO-1 modification protects XPC from proteasomal processing. How SUMOylation protects XPC from the proteasomal degradation invoked by polyubiquitylation remains to be elucidated. The amount of polyubiquitylated XPC species gradually reduces from 4 h after UV-irradiation [63], which matches with the time required to repair most of the UV-induced 6-4PP, the main target for early GGR. Thus, it appeared that XPC ubiquitylation is most abundant when a maximal GGR activity is required. Within a speculative model, it might be suggested that polyubiquitylated XPC occurs early after UV-irradiation, has increased affinity for DNA lesions, and is protected from breakdown at later stages, thereby providing an acute adaptation strategy to optimise NER competence until the majority of the severe cytotoxic lesions (6-4PP) are removed.

Previously, it was shown that the subunit HR23B (or HR23A) of the heterotrimeric complex XPC/HR23B/Cen2 prevents XPC’s proteasomal degradation [64] and enhances its repair capacity in vitro [65] as well as in vivo (in yeast [66] our own unpublished observations in mammalian cells). This HR23B-dependent XPC stabilization is contrary to the expectation as the main function of this protein (as it is in far excess over the XPC protein and implicated in many other cellular processes) [67,68] is being a shuttling vehicle from ubiquitylated substrates to the proteasome. The function of Rad23 in NER is however better studied in yeast, although its involvement in this process has been a matter of dispute among different research groups. Since it is not the scope of this synopsis to provide any preferences for one model over the other we will simply summarise the postulated roles of Rad23 in regulating NER. These different Rad23 functions range from a protective role in proteolytic degradation of Rad4 (yeast ortholog of XPC) [69] to a non-proteolytic function of Rad23 [70]. Different studies (reviewed by Reed and Gilette [71]) have shown that at least one additional function of Rad23 is regulating the recruitment of the 19S regulatory complex to the NER machinery in which it plays a role in modulating NER-activity in a non-proteolytic manner. Even evidence for a transcription-dependent regulation of Rad4 via Rad23 was recently reported [72] in this latter report a two-branched model for regulating yeast NER via UPS was postulated. The yeast XPC ortholog Rad4 is ubiquitylated as well [72], but in contrast to mammalian XPC, Rad4 is degraded after UV-irradiation. Genetic analysis further revealed that a novel cullin-based E3 ligase complex containing Rad7, Rad16, Elc1 and Cul3 performed UV-induced ubiquitylation. Ubiquitylation was shown to adjust repair activity whereas degradation did not affect NER potential. Within a tempting model the authors describe the functional, but in this case not structural evolutionary conservation of this type of repair adjustment of an early NER factor (Rad4/XPC): i.e. the GGR-specific yeast Rad7 and Rad16 factors (of which no structural orthologs were identified in mammals) mimic the function of the mammalian UV–DDB complex, also functioning in GGR. Unfortunately, the explicit difference between both species in initiating NER was not addressed: the yeast Rad4 is essential for both GGR and TCR, whereas its mammalian ortholog (XPC) only functions in GGR. This functional difference between both orthologs might also explain the surprising difference in the fate of ubiquitylation of Rad4 when compared to XPC, at least in mammals no such damage-dependent degradation of XPC was observed. This also indicates that the subtle regulation of NER efficiency is not as conserved as the overall reaction.

Apparently, a complex interplay between SUMOylation, ubiquitylation and the binding partners of XPC is required to determine the stability, affinity and action of XPC on DNA lesions. One could speculate, that damage-dependent...
post-translational modifications are necessary to suppress illegitimate binding to different types of DNA-structure aberrations, to which this protein has intrinsic affinity [73–75], in order to avoid futile initiation of the NER reaction. The delicate regulation of these NER initiators extends beyond post-translational modifications, as both DDB2 and XPC expression are controlled by a complex set of different regulatory mechanisms at the transcriptional level in a p53-dependent fashion [53,76,77]. Clearly, cells need to keep critical control on the level of XPC and DDB2; further studies on the behaviour of these proteins (and defined mutants) in living cells are expected to provide more insight in how all these mechanisms are regulated and coordinated in space and in time.

6. The Ssl1/p44 subunit of TFIIH is a ubiquitin ligase

Among the recent publications that integrate the DNA damage response with the UPS, the identification of a ubiquitin ligase activity (E3 ligase) within the transcription/repair factor TFIIH by Takagi et al. [78] is an intriguing and as yet enigmatic finding. The TFIIH complex is composed of 10 subunits [79,80]. It is essential for RNA polymerase II (RNAPII) initiation and for NER [81], it plays a role in RNA polymerase I driven transcription [82,83] is involved in nuclear receptor-mediated transcriptional regulation [84], and likely functions in cell cycle regulation [85,86]. The spectrum of intrinsic enzymatic activities comprising ATPases, helicases and kinase functions required to achieve TFIIH’s multifunctionality has now been broadened to ubiquitin ligase, carried out by the core TFIIH subunit Ssl1 (the yeast ortholog of mammalian GTF2H2 hereafter called p44).

Within TFIIH both Ssl1/p44 and the CAK component Tfb3/MAT1 contain the consensus amino acid sequence indicative for E3 RING ligases [87]. Takagi et al. showed that the ‘core’ complex (TFIIH without the trimeric CAK component) harbors indeed E3 ligase activity. In addition, isolated recombinant Ssl1 possesses E3 ligase activity, although reduced as compared to the intact TFIIH complex. Apparently, presence of the other TFIIH core components especially the Tfb4 (p34) subunit stimulate this function. Mutation studies showed that E3 activity depends on an intact RING finger domain (RNF) of Ssl1.

This E3 ligase activity appeared not to be essential for transcription, since cells carrying mutated Ssl1 can still grow, although at a lower rate and in a temperature-sensitive fashion. However, these mutant cells showed increased sensitivities towards the DNA damaging agents UV and MMS, suggesting a role of the Ssl1 ligase in the cellular response to DNA damage. Despite the pivotal role of TFIIH in NER, the authors claimed that the Ssl1-E3 ligase was not required for NER in vitro, suggesting that the increased DNA damage sensitivity is derived from an altered TFIIH function outside this process. Alternatively, the absence of any detectable influence on in vitro NER capacity may reflect the limitations of the applied assay. A reduction of the NER efficiency by less than 50% (as observed by Takagi and co-workers in the survival assays) can still cause a severe carcinogenic condition, as presented by mice and humans lacking the GGR-specific DDB2(XPD) gene [88–90]. Note that purified UV–DDB (DDB1 and DDB2/XPE complex) does not significantly enhance mammalian NER capacity in vitro [91]. On the other hand, Takagi et al. found evidence for an indirect action of the TFIIH-associated E3 on UV-induced DDR by transcriptional profiling of E3-deficient Ssl1 mutants. These mutants have a reduced DNA damage-dependent (MMS) transcriptional regulation of certain DNA repair genes. The mechanism for this change in the transcriptional response induced by interference with this enzyme was not uncovered nor the target substrate for the newly identified E3.

It is not yet clear how this novel E3 ligase activity fits in other links between TFIIH and the ubiquitin proteasome system. For instance, the TFIIH subunit XPB interacts with the AAA-ATPase rpt6 (Sug1) a subunit of the proteasomal 19S complex found by a yeast two-hybrid screen [92] and a large-scale proteomics approach (a tandem-affinity purification followed by mass spectrometry) [93]. Initially, the 19S particle of the proteasome was thought to function only in the ubiquitin-dependent 26S-mediated proteasomal degradation. Polyubiquitylated proteins are recruited to be deubiquitylated, unfolded and translocated into the 20S catalytic particle by the 19S particle after which actual proteolysis is performed [4]. However, both in transcription and NER this subcomplex also has a non-proteolytic function in regulating these processes [70,94]. In a recent review the connection between transcription and NER regulation via 19S is nicely outlined and will therefore not be addressed here [71]. A role in transcription for rpt6 (Sug1) and rpt4 (Sug2), as subunits of the 19S particle, was first established in yeast [95,96]. Moreover, Rpt6 and Rpt4 facilitate the connection between H2B ubiquitylation and H3 K4 and K79 di- and tri-methylation, necessary in discriminating active and inactive genes. Mutated rpt6 yeast cells fail to methylate K4 and K79, although H2B ubiquitylation stays intact [97]. A likely mechanism for the different functions of the 19S particle might be established by interacting with distinct binding partners, on top of that different PTM’s might even further fine-tune 19S action. Currently, it is not clear whether the E3 ligase activity of TFIIH is mechanistically or functionally linked to the 19S particle via Rpt6. Since Rpt6 is involved in regulating transcription by influencing PTM’s on histones and expression of NER genes in particular [72] it would be interesting to investigate whether the change in gene expression pattern upon DNA damage, as found by Takagi et al., is associated or correlated with a change in the histone makeup.

7. UV-induced histone ubiquitylation

Next to a role in transcriptional regulation, histone modifications have recently also been implicated in DDR. Yeast cells carrying the histone H2B ubiquitin mutant (htb1-K123R) lack the UV-induced phosphorylation of Rad53, a checkpoint kinase involved in establishing checkpoint response to DNA damage. This links H2B ubiquitylation to checkpoint signalling after UV-induced DNA damage [98]. It is striking to note that H2B ubiquitylation is performed via the E2 activity of the Rad6/Bre1 complex [99]. The same protein, Rad6 (the first identified link between DDR and ubiquitylation [100]), is also the E2
responsible for ubiquitylating the polymerase clamp PCNA in the control of TLS [12]. Thus a dual role in DDR for the canonical H2B E2 enzyme Rad6 [99], in addition to its role in PCNA ubiquitylation, can be envisaged. Evidence for this possible dual action of Rad6 in DDR is further provided by the fact that in yeast cells Rad6 deletion mutants are far more sensitive to various types of DNA damage than Rad18 mutant cells (the E3 for PCNA ubiquitylation [12]). Whereas, double mutants of both Rad18 and Bre1 were indeed as sensitive to DNA damage (X-rays) as the Rad6 knock out [101], thus implying that H2B both Rad18 and Bre1 were indeed as sensitive to DNA damage E3 for PCNA ubiquitylation [12]). Whereas, double mutants of both Rad18 and Bre1 were indeed as sensitive to DNA damage (X-rays) as the Rad6 knock out [101], thus implying that H2B ubiquitylation causes part of the Rad6 sensitivity.

Very recently, also differential histone ubiquitylations in mammals as a response to UV-induced DNA damage was reported [102–104]. These three studies each report on three distinct (in terms of kinetics, histone targets and underlying ubiquitylation mechanisms) ubiquitylation events. However, these superficially conflicting data are not necessary incompatible with each other. First Kapetanaki et al. reported about a Cul4A-dependent de-ubiquitylation of H2A directly (detectable at 0 min) after UV-irradiation [103]. At longer time periods post UV, starting at 30 min, ubiquitylated H2A recovers to normal levels in normal lymphoblastoids, however cells mutated in DDB2 (XP group E) do not exhibit this recovery. The authors suggest that both Cul4A and DDB2 are implicated in this H2A ubiquitylation, by co-localization studies. This observation conflicts with previous reports that the canonical ligase for H2A-ubiquitylation is Ring2 [105,106]. Shortly after this we reported on UV-induced H2A ubiquitylation, increased ubiquitylation rather than decreased, but however in a different timescale [104]. This process appeared to depend on active NER, since in a series of defined NER mutants this reaction was not observed [104]. Based on these findings we suggest that this event at least requires NER-induced incision and thus occurs late in the NER reaction. Moreover, this process appeared to depend on the canonical Ring2. Surprisingly, H2A-ubiquitylation was severely attenuated in ATR-mutated cells [104]. ATR is a DNA damage signalling kinase, playing an important role in particularly UV-induced DDR [7,107] and thus suggests a role in damage signalling rather than directly affecting the repair process [104]. Simultaneously, Wang et al. showed UV-induced ubiquitylation of H3 and H4, which is dependent on the E3 ligase activity of the Cul4A complex [102], known to co-localize at DNA damaged sites via its DDB2 interaction [41,108]. Typical differences exist between the two types of histone ubiquitylation events. Firstly, the amounts of histone modifications are distinct. The very low base levels of H3 and H4 ubiquitylation (0.3% and 0.1% respectively [102]) are significantly boosted by UV, but remain low in absolute terms. In contrast, the modest relative increase in ubiquitylated H2A after UV, though less conspicuous, must involve large amounts of H2A in view of its constantly high background (5–15% of all H2A [109]). A second difference concerns the timing of the H3/H4 and H2A ubiquitylation responses. The first is probably involved in early steps of NER, where the delivery of ubiquitin moieties to H3 and H4 might even come from the UV-induced short term de-ubiquitylation of H2A [103], while the latter is dependent on both NER and ATR, and most likely functions in later step of the DDR. These quantitative and kinetic differences strongly suggest distinct functions. H3/H4 ubiquitylation probably triggers local effects that may improve NER efficiency. Indeed, in Cul4A-knocked-down cells XPC fails to accumulate at locally damaged sites [102]. Remarkably, sheer absence of the Cul4A complex at these sites (as observed in XP-E cells) does not prevent XPC from accumulation, it only reduces the rate of XPC recruitment [52]. Regarding UV-induced H2A ubiquitylation, its increase surrounding repaired lesions is not likely to evoke a very specific response in view of the high basal levels. Perhaps its effect may relate to the known transcriptional repression function of ubiquitylated H2A [105,106]. Whether or how H2A ubiquitylation can contribute to UV-induced inhibition of transcription remains a subject of further study. One other hypothetical option is that the relatively strong depletion of the available free ubiquitin pool in the nucleoplasm by binding it to H2A has an enormous impact on the entire cellular UPS and as a consequence on cellular homeostasis, in a similar (but opposite direction) fashion as was previously reported when proteotoxic stress was elicited causing accumulation of ubiquitylated substrates in the cytosol with a concomitant depletion of ubiquitylated H2A [110].

8. DNA damage-induced signalling responses and ubiquitin

Ubiquitylation as a response to genotoxic insult influences not only repair activity but also has an important role in damage signalling as discussed above by the damage-induced ubiquitylation and subsequent degradation of Cdt1. Other examples of proteins implicated in DNA damage-induced cell cycle control that are regulated via this ubiquitylation-dependent destructive response to damage are p21 and Chk1. At relatively low doses of UV p21 is degraded in an ATR-dependent fashion. Failure to degrade p21 results in repair inhibition [111], although some reports state that p21 degradation does not occur after genotoxic stress [112]. Several studies reported on a direct involvement of p21 in NER via its interaction with PCNA [113]. It has been hypothesised that degradation of p21 is required to allow efficient DNA repair synthesis [111,113]. However, this model has been challenged by the fact that deletion of p21 hardly affects NER [114]. The exact role of this protein in NER remains enigmatic [115], although recent evidence suggested that downregulation of p21 is required for efficient PCNA ubiquitylation [116]. Despite the lack of a generally accepted function of p21 in DNA damage repair it further illustrates the central role that PCNA plays in DDR. More recently, DNA damage-dependent degradation of activated Chk1 has been reported. The checkpoint regulator Chk1 can be activated by the protein kinases ATM (ataxia-telangiectasia mutated) and ATR (ATM-Rad3-related). Activated Chk1 is thought to quickly spread the stress signal by a swift redistribution throughout the nucleoplasm, thus affecting replication controllers to temporarily halt S-phase progression not only locally near the site of the lesion but throughout the entire nucleus [117,118]. In time, the alarm bell (activated Chk1) is degraded, possibly to allow resumption of S-phase, when lesions are removed [119]. The complex ubiquitylation events in response to DNA damage suggest that malfunctioning of the ubiquitylation machinery would reduce survival after DNA damage. Indeed cells that fail to ubiquitylate either by the
usage of temperature sensitive E1 or chemical inhibitors fail to repair UV-induced lesions efficiently [120].

9. Different outcomes of ubiquitylation

The different DDR pathways collectively counteract the biological consequences of genotoxic insults. However, intrinsic properties of some of these DDR enzymes, i.e. probing and binding to DNA lesions which directly act on the DNA template may perturb genetic-homeostasis by uncontrolled actions. Obviously, orchestration of processes that directly interfere with the genome require a tight control, particularly under non-challenging conditions these activities need to be restricted in their action. This regulation (or restriction) of DDR pathways takes place at multiple levels e.g. transcriptional changes [76], alterations in the composition of regulatory-protein complexes or by post-translational modification of the proteins involved. Many ubiquitylation events and other post-translational modifications within the DDR as well as their functional implication remain to be discovered. The regulatory nature of ubiquitylation and the relative fast way to induce this process make it very likely that more DNA damage-induced ubiquitylation events will be reported in the near future. Different consequences of ubiquitylation on DDR targets have been reported, such as: (i) the enhancement/alteration of the activity or affinity for substrate of a target protein (e.g. XPC); (ii) the switch between cellular pathways (e.g. PCNA); (iii) the termination of a process achieved either by reversing the modification of the protein or, more fundamentally, by degrading it (e.g. DDB2, RNAPII, Chk1).

Subcellular redistribution, transient interactions and expression levels are known handles to control the action of complex pathways. In the recent years a wealth of information has become available on cellular networks ranging from transcriptome, interactome, proteome and additional ‘ome’ analyses, providing a more global view on cellular homeostasis. Clearly, post-translational modifications provide the cell an additional tool to control pathway flow in an even more dynamic manner. This appears to be particularly relevant to sudden emergency situations such as occasional high exposure to genotoxic agents. The few examples of ubiquitylation that is used to establish pathway control and can only be restricted in their action. This regulation (or restriction) of DDR pathways takes place at multiple levels e.g. by differential post-translational modifications. Undoubtedly, ubiquitylation is only one aspect of the complicated spectrum of post-translational modifications that is used to establish pathway control and can only be properly interpreted on the background of phosphorylation, SUMOylation and other post-translational modifications that remain to be discovered.

REFERENCES


