Chapter 5

# The Roles of Cullin RING Ligases and the Anaphase Promoting Complex/Cyclosome in the Regulation of DNA Double Strand Break Repair

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**Abstract**

Historically, genome maintenance has been viewed as the largely independent activities of (1) ubiquitin ligases driving unidirectional cell cycle progression and, (2) the activity of cellular checkpoints that monitor DNA integrity and DNA replication. It is well established that the DNA damage response (DDR) checkpoint machinery promotes the activation of repair mechanisms in addition to opening a window for repair. Emerging evidence demonstrates an integrated network of the central cell cycle driving E3 ubiquitin ligases and the checkpoint machinery, as well as deubiquitinating enzymes, which intermittently cooperate and antagonize one another to define windows of checkpoint and repair activities to optimize genome stability and cellular health. A growing number of components of the ubiquitin machinery are involved in the DDR. Herein, we focus on the regulation of cell cycle checkpoints and the DNA repair mechanisms for double strand breaks (DSBs) by the coordinated activities of Cullin RING ligases (CRLs) and the anaphase promoting complex/cyclosome (APC/C).

**Keywords:** DNA repair, deubiquitinating enzymes, E3 ubiquitin ligase, APC/C, Cullin-RING ligase, SCF, homologous repair, non-homologous end-joining

## 1. Introduction

Our cells face a multitude of DNA damaging insults, both internally and externally derived, on a daily basis. The majority of our cells is not cycling and must simply respond by rapidly repairing the damaged DNA to maintain homeostasis. For those cells that are cycling, however, the precise maintenance of the genome is of even more critical importance to ensure the faithful transmission of identical copies of an undamaged genome to the next generation of

cells. Of critical concern for cycling cells is the precise duplication of one exact copy of the genome followed by the accurate segregation of the two copies. To ensure that these events happen once, only once, and in the proper order, cells utilize the periodic synthesis and ubiquitin-mediated degradation of a host of proteins to control the timely activity of multiple enzymatic activities, such as kinases and polymerases, to drive the unidirectional transit through the cell cycle.

Upon the occurrence of DNA damage, cycling cells must not only sense and respond to the insult, but must also coordinate cell cycle progression with repair. Moreover, as damage can occur during any of the many processes taking place during the cell cycle, proliferating cells have evolved a number of mechanisms for repairing and overcoming damage to maintain the genome. However, the proper selection of the repair mechanism to use is nearly as important as sensing the damage as some mechanism could be highly mutagenic if utilized at the wrong point in the cell cycle or if used without proper control. Such errors would clearly result in the generation of mutations that could lead to a number of human pathologies, most notably cancer. Moreover, the induction of DNA damage is a central therapeutic strategy for treatment of the majority of cancer types. And, while clinically useful, such treatments lack specificity and are often limited by toxicities. The dissection of genome maintenance pathways thus holds the potential to define new therapeutic targets that may ultimately lead to more effective therapeutic strategies. This review will focus on recent advances in our understanding of how the interplay of the major cell cycle-associated ubiquitin ligases, the DNA-monitoring checkpoint machinery, and deubiquitinating enzymes coordinate cell cycle progression with the response to the proper repair of DNA damage. In particular, the focus will be on the use of mechanisms of repairing DNA double strand breaks and stalled replication forks.

## 2. Modular E3 ubiquitin ligases in the cell cycle and genome repair

### 2.1. Cullin RING ligases (CRLs)

The cullin family proteins (Cul1, 2, 4A, 4B, 5, and 7) function as the central scaffolds for the assembly of multi-subunit ubiquitin ligases [1]. The cullin C-terminus adopts a globular conformation that provides a docking site for the RING finger proteins Rbx1 or Rbx2. The RING fingers recruit the ubiquitin E2 enzymes and catalyze the transfer of ubiquitin to substrates. At the cullin N-terminus is a helical domain that is the site of interaction with an adapter protein (s), which recruits substrate receptors. In general, each cullin associates with a distinct set of substrate receptors. For example, the CRL1 ligases utilize the adaptor protein Skp1 to interact the F-box family of proteins defined for a Skp1-interacting motif defined in the archetypical F-box, Cyclin F. The CRLs are denoted by the identity of the cullin family member and the associate receptor. For example, $CRL1^{Cyclin\ F}$ denotes a cullin1-based ligase complexed with the substrate adapter Cyclin F. The human genome contains nearly 200 cullin-associated substrate receptors thus allowing CRLs to regulate myriad cellular processes [1]. The extent of this functional diversity is exemplified by the fact that a single CRL can have either oncogenic or tumor suppressive activity depending on the substrate adaptor, for example $CRL1^{Skp2}$ and $CRL1^{Fbw7}$, respectively [2–5].

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With nearly 200 E3 ligases regulating an estimated 20% of the human proteome and a growing number of cellular process, it is not surprising that the function of CRLs is highly regulated at multiple levels, including; regulation of substrate receptor availability (e.g., regulated expression and degradation of receptors), activation/inactivation by the reversible neddylation of the cullin subunit, CAND1-mediated exchange of substrate receptors, regulation of substrate-receptor interactions (e.g., post-translational modification of substrates such as phosphorylation and glycosylation) and the activity of deubiquitinating enzymes [1, 6].

### *2.1.1. CRL1 (a.k.a. SCF) complexes*

The Cullin1-based CRL1 ligases are more commonly known as the Skp1-Cullin1-F-box (SCF) ligases. There are nearly 70 F-box proteins in the human genome, although only a subset has been studied in great detail. For the purposes of this review we will utilize the SCF rather than CRL1, nomenclature. Multiple SCF ligases are involved in cell cycle control and the response to and repair of DNA damage. In consideration of space constraints, we will give overviews of two key SCF, rather than CRL1, ligases as more specific examples of the function of this group of enzymes.

#### *2.1.1.1. $SCF^{Skp2}$*

$SCF^{Skp2}$ functions as a driver of S-phase and exhibits oncogenic activity in multiple settings. Skp2 activity is regulated by its controlled expression and degradation. In addition, even when the Skp2 protein is present and complexed with Cullin1 and Skp1, its ability to recruit substrates for ubiquitination requires site-specific phosphorylation of its target proteins to create a phosphodegron that is recognized by Skp2. Many Skp2 substrates are phosphorylated in a cell cycle-specific fashion, adding an additional layer of control. Skp2 is predominantly known for its role in driving S-phase entry by degrading the Cdk inhibitors p21 and p27 to drive S-phase entry. It is frequently overexpressed in tumours of varying origins and exhibits oncogenic activity [7].

#### *2.1.1.2. $SCF^{\beta TrCP}$*

$SCF^{\beta TrCP}$ is a collective term for two SCF complexes defined by the F-box proteins βTrcp1 and βTrCp2, which are largely, but not exclusively interchangeable. In contrast to the fluctuating levels of Skp2, βTrcp levels are relatively constant throughout the cell cycle, and a major determinant $SCF^{\beta TrCP}$ activity is the creation of a consensus DpSGxxpS phosphodegron upon substrates. Multiple kinases are involved in the generation of phospho-DSGxxS in substrates, including GSK3β, CK2, Polo-like kinases (e.g., Plk1) and Chk1. Thus, some substrates, for example those directed to $SCF^{\beta TrCP}$ by Plk1, are degraded in a cell cycle specific manner owing to the regulated expression of Plk1 itself [7].

### *2.1.2. CRL4 complexes*

The cullin 4-based ligases, encompassing cullin 4A or 4B, display almost complete functional redundancy and are generally referred to collectively as CRL4. These ligase complexes incorporate the adapter protein damage DNA-binding 1 (DDB1) and associate with ~25 substrate receptors known as the DDB1 and Cul4 associated factors (DCAFs) [8, 9]. As with the SCF

ligases, the majority of CRL4 complexes have not been studied in detail, yet it is clear that the CRL4 ligases are involved in a multitude of processes, including embryogenesis and haematopoiesis and impact both tumorigenesis and tumour suppression depending on context. CRL4 ligases are best characterized for their roles in cell cycle progression (predominantly controlling replication) and DNA repair. In regard to the latter, $CRL4^{CSA}$ and $CRL4^{DDB2}$ are well characterized for their roles in nucleotide excision repair (NER) in response to UV irradiation [10].

#### *2.1.2.1. $CRL4^{Cdt2}$*

$CRL4^{Cdt2}$ is a central component of the S-phase machinery, which acts to ensure that genome replication is limited to a single round per cell cycle. $CRL4^{Cdt2}$ couples destruction of these targets to replication through a partnership with PCNA, which interacts with a host of proteins to maintain genomic integrity, including licensing factors, helicases, methyltransferase, repair enzymes, and the translesion (TLS) polymerases [11]. The regulated recruitment of these proteins is critical for preparing the genome for faithful transmission to the next generation as spurious engagement of several PCNA-binding proteins has been shown to have deleterious effects [8, 12–17]. Importantly, the majority of these factors engage the same interaction surface on PCNA via a PCNA-interacting protein (PIP)-box motif. Interestingly, the PIP-box of a subset of PCNA-interacting proteins, such as the Cdk inhibitor p21 and the replication licensing factor Cdt1, when bound to PCNA, acts to recruit the $CRL4^{Cdt2}$ leading to the ubiquitination and destruction of these proteins [13, 18]. Notably, these $CRL4^{Cdt2}$-PCNA-substrate interactions only occur when PCNA is bound to DNA to allow recruitment of additional factors [11]. A number of mechanisms regulate these interactions with PCNA, but a critical determinant is the strength of the PCNA-PIP-box interface.20 The PIP-box of the tumour suppressor p21 has the highest known affinity for PCNA, allowing it to prevent PCNA interactions with other PIP-box proteins [19]. In this way, p21 acts to prevent spurious replication and prevent the inappropriate engagement of the error-prone polymerases, which are able to continue DNA replication despite damaged DNA. However, upon replication blocks such as UV-induced damage, p21 is degraded by $CRL4^{Cdt2}$ to allow TLS. Subsequently, the bypassed sites of damage can be repaired by NER.

### 2.2. Anaphase promoting complex/cyclosome (APC/C)

The APC/C is a large, multi-subunit E3 ubiquitin ligase conserved from yeast to humans. By targeting a multitude of proteins for destruction by the 26S proteasome, the APC is a major driver of cell cycle progression, as well as regulating many diverse processes including meiosis, TFGβ signalling, synaptic maturation and differentiation [20–31]. Although not itself a cullin, the central APC2 subunit bares significant homology to the cullins and like these proteins provides a scaffold for the assembly of the multi-subunit APC/C E3 ligase. APC2 contains a binding site for APC11, the RING finger and catalytic component of the APC/C. The APC/C, like CRLs, is involved in numerous cellular processes. However, in contrast to the CRLs, substrate recognition by the APC/C is mediated by a bipartite receptor made up of the APC/C core component APC10 and one of only two substrate receptor/activator proteins, Cdc20 and Cdh1.

Recognition of substrates is mediated by several cis-acting sequence motifs (degrons). It is generally thought that D-boxes and KEN-boxes are responsible for the destruction of all APC substrates [32, 33]. Indeed, most substrates contain one (often multiple) of these two degrons;

however, there are a growing number of motifs identified as critical for APC/C-mediated ubiquitination in the ever-increasing number of APC/C substrates. Recent structural analyses have identified the molecular basis for the interaction of substrates with Cdc20 and Cdh1, which suggests that non-canonical APC/C degrons interact with the activators in manners analogous to the canonical degrons.

$APC^{Cdc20}$, essential for cell division and viability, is indirectly inhibited by clinically relevant agents (*e.g.* paclitaxel, an anti-cancer drug Taxol), and has received substantial evaluation for pharmacological manipulation. In contrast, $APC^{Cdh1}$ activity is not required for viability, although increasing data demonstrate a role for $APC^{Cdh1}$ in genomic stability and tumor suppression [34, 35]. Indeed, many $APC^{Cdh1}$ substrates (*e.g*. Cyclin A, Skp2, Aurora A, Plk1, and Id2) are associated with oncogenesis, and the regulation of the stability of these substrates has been extensively linked to highly malignant cancers [36]. However, increased Cdh1 activity is also deleterious to cells.

APC/C activity must be tightly controlled and this is accomplished by several mechanisms. First, the activators are regulated at the level of expression with both Cdc20 and Cdh1 accumulating during S and G2 phases. At the end of mitosis, Cdc20 is then degraded by $APC/C^{Cdh1}$. $APC/C^{Cdh1}$ activity remains high in G1 and its inactivation is critical for commitment to S-phase. Down regulation of $APC/C^{Cdh1}$ activity involves APC/C-mediated degradation of its primary E2 enzyme, UbcH10, Cdk-mediated phosphorylation of Cdh1 which antagonizes its binding to the APC/C holoenzyme, degradation of Cdh1, and the interaction of APC/C with Emi1. Binding of Emi1 prevents substrate engagement and ubiquitination activities and is critical for inhibition of $APC/C^{Cdh1}$.

### 2.3. Crosstalk between CRL and APC/C ligases

There is increasing understanding that crosstalk between the CRL ligases and APC/C ligases is required for efficient cell cycle. For example, Skp2 is a substrate of $APC/C^{Cdh1}$ and as cells near the G1/S transition, Cyclin E-Cdk2 complexes initiate the inactivation of $APC/C^{Cdh1}$, which promotes early accumulation of APC substrates such as Cyclin A and Skp2 (and the activation of $SCF^{Skp2}$) which promotes further Cdk activity as well as the expression of Emi1, leading to rapid abrogation of $APC/C^{Cdh1}$ activity [37–47]. Then, as cells transit S and G2 the accumulation of the $APC/C^{Cdh1}$ Plk1 leads to the $SCF^{\beta TrCP}$-mediated degradation of Emi1 at the G2/M transition to allow APC/C to become active in mitosis [48–51]. Recently, it was discovered that in addition to APC/C-mediated degradation of Cdh1 in late G1, Plk1 also directs the $SCF^{\beta TrCP}$-mediated degradation of Cdh1 as cells enter S-phase [52]. $SCF^{Fbw7}$ via its ability to target and regulate the levels of Cyclin E and Plk1 adds another input to this regulatory circuit [53].

## 3. DNA damage responses

### 3.1. The double strand break response

The generation of double strand breaks (DSBs) is of potentially grave consequence to cells at any stage of the cell cycle and must be dealt with immediately. In response to DSBs the MRE11-RAD50-NBS1 (MRN) complex and the inactive dimers of the ATM kinase localize to

the damaged site, resulting in the autophosphorylation and activation of ATM monomers (**Figure 1**). The MRN-dependent activation of ATM is facilitated by the non-degradative, K63 linked ubiquitination of NBS1 by $SCF^{Skp2}$ [54]. Phosphorylation of histone H2AX by ATM leads to the recruitment of the checkpoint mediator MDC1, which recruits additional MRN-ATM complexes, to amplify the checkpoint signal, and promotes ubiquitination of histone H2AK15, by the concerted actions of the RNF8 and RNF168 ubiquitin ligases [55–61]. The PR-Set7 and MMSET methyltransferases are also recruited to sites of DNA damage where they catalyse methylation of histone H4K20 [62–66]. Together the ubiquitination of H2A and the methylation of H4 provide a high-affinity binding sight for the checkpoint mediator 53BP1 at sites of damage [67, 68]. 53BP1 further stimulates ATM activity by interacting with MRN complexes and sets the stage for repair. While ATM provides local regulation of the DDR, global regulation is carried out by the effector kinase Chk2, which is activated by ATM. Chk2 phosphorylates numerous proteins, including Cdc25 family phosphatases (to promote/maintain inhibitory phosphorylation of Cdks and cell cycle arrest), p53, and the repair protein BRCA1.

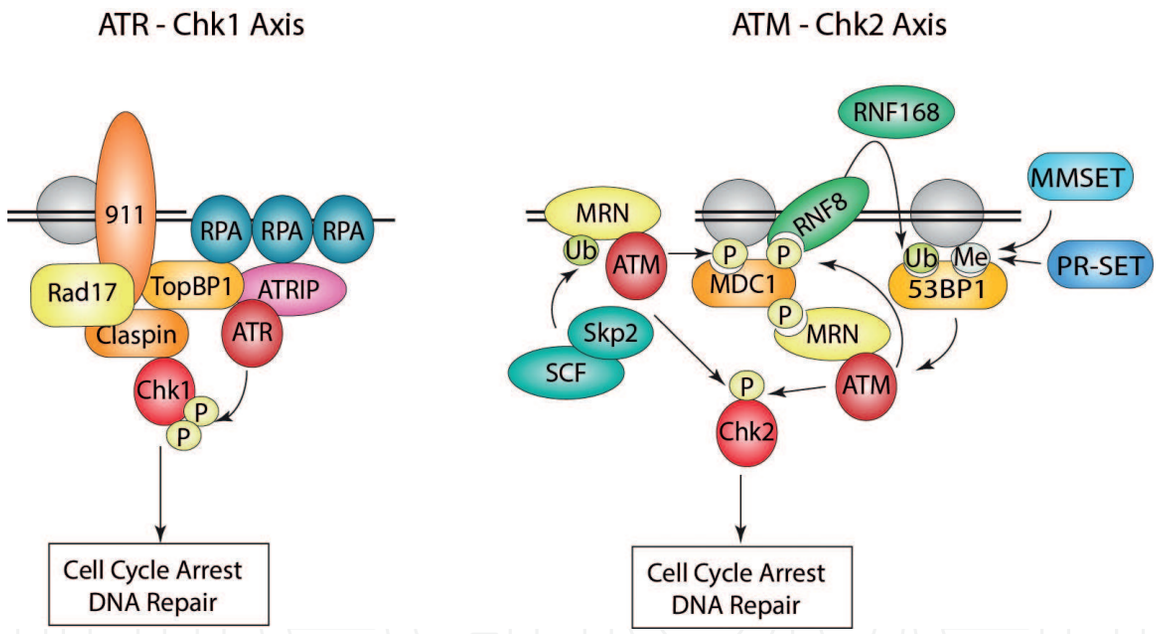


**Figure 1.** Activation of the ATR and ATM kinase cascades upon DNA damage. Left—Single stranded DNA, generated by blocks to DNA replication or the resection of double strand breaks (DSBs) is coated by RPA, which acts to recruit the ATR-ATRIP heterodimer. The RAD9-RAD1-HUS1 [1] complex is loaded by RAD17. 9-1-1 recruits the ATR activator TopBP1. The mediator protein, Claspin then recruits Chk1 to the site of damage where it is activated by ATR to effect the checkpoint. Right—The induction of a DSB leads to the direct binding of the MRE11-RAD50-NBS1 (MRN)—ATM complex, which phosphorylates histone H2AX (grey spheres represent the histone octamer). The checkpoint mediator MDC1 binds to the phosphorylated histone and is then bound by another MRN-ATM complex. Phosphorylation of MDC1 by ATM recruits the E3 ubiquitin ligase RNF8, which in conjunction with RNF168, ubiquitinates histone H2K15. The ubiquitin ligase $SCF^{Skp2}$ also promotes the MRN-ATM complex formation. The methyltransferases MMSET and PR-SET catalyse methylation of histone H4K20. The H2K15-Ub and H4K20me marks are recognized by 53BP1 leading to further stimulation of ATM activity and the ultimate induction of cell cycle arrest and DNA repair by the effector kinase Chk2.

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### 3.2. Mechanisms of DSB repair

Cells with DNA damage in the form of double strand breaks (DSBs) predominantly use two mechanisms to repair these lesions (**Figure 2**) [69, 70]. The least error-prone of these mechanisms, homologous recombination (HR), utilizes the non-damaged sister chromatid as a template to inform repair of the damaged DNA and is thus limited to S and G2 phases of the cell cycle, where the sister template is available [69]. Indeed, damage incurred during S-phase, whether DSB, interstrand cross-links, or collapsed replication forks rely heavily on HR for repair. The alternative repair pathway, non-homologous end-joining (NHEJ), as the name suggests, involves the sequence-independent ligation of broken DNA ends. Although, some NHEJ (alt-NHEJ or microhomology-mediated, mmNHEJ) do utilize very small regions of homology to identify DNA ends for ligation, canonical NHEJ has no requirement for any

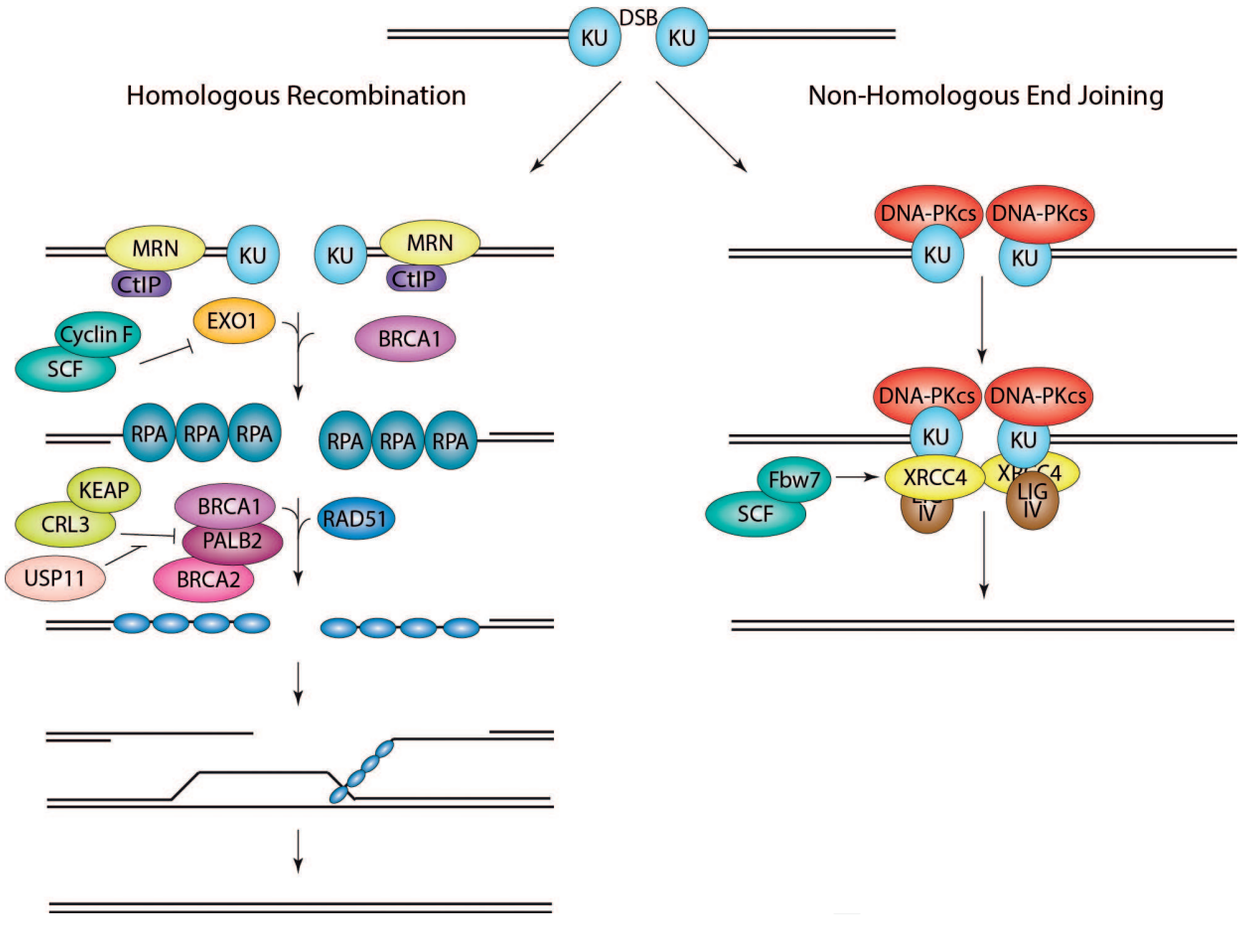


**Figure 2.** Mechanisms of DSB repair. Upon induction of DSBs, KU proteins are recruited to the broken DNA, protecting the ends. MRN complexes are then recruited. In the presence of BRCA1 and CtIP, the DNA ends are resected, recruiting additional nucleases, including EXO1. Resection leads to the removal of KU proteins. $SCF^{Cyclin\ F}$ prevents excessive resection by targeting EXO1 for degradation. ssDNA generated by resection is coated by RPA. The BRCA1, BRCA2, PALB2 complex then stimulates the replacement of RPA with RAD51, which promotes strand invasion of the sister chromatid template, leading to homology directed repair of the break. PALB2 function is negatively regulated by the $CRL3^{KEAP}$ ligase and promoted by USP11. In the absence of resection, DNA-PKcs is recruited by the KU proteins, which leads to the recruitment of additional factors, including XRCC4, which is stimulated by $SCF^{Fbw7}$ ligase activity, and DNA ligase IV, which ultimately joins the DNA fragments together.

sequence homology in the selection of ends to be ligated and NHEJ is thus potentially error prone and mutagenic [71]. A key step in NHEJ is the rapid recruitment of Ku70/80 proteins to the severed DNA ends, which function to hold the broken fragments together, limiting the mutagenic potential of this mechanism (**Figure 2**) [72, 73]. Ku70/80 recruits the DNA-PKcs to form the functional DNA-dependent protein kinase, which directs NHEJ. Small gaps in the broken DNA are filled by polymerase μ in to generate blunt ends, which are then ligated by DNA ligase IV in conjunction with XRCC4 [70, 74]. NHEJ is further stimulated by the K63-linked ubiquitination of XRCC4 by $SCF^{Fbw7}$ [74]. The end-joining process is rapid and likely of relatively little genetic consequence [75]. Small deletions could readily occur [71]. If, however, the damage is extensive, processing of DNA ends in an ATM and MRN dependent manner is required, which may lead to larger deletions and in the case of multiple damage sites can produce mutagenic evens on the scale of chromosomal rearrangements [71].

In contrast to blunt-ended ligation of NHEJ, the use of the sister chromatid as a template for HR requires the formation of a synapse between the damaged DNA and the undamaged sister (**Figure 2**). Synapse formation requires resection of the DNA from at the site of the break to generate ssDNA. Resection is driven by stimulation of the nuclease activity of the MRN complex by CtIP [76–79]. The ability of CtIP to drive resection is controlled by the balance of BRCA1 and 53BP1 on the chromatin [79–86]. The presence of 53BP1 forms a barrier that limits the accessibility of chromatin to HR-driving nucleases (**Figure 3**). A major role of BRCA1 in HR is to antagonize the binding of 53BP1 to chromatin to al-low resection and repair. Indeed, loss of 53BP1 function in BRCA1 mutant cells improves resection and overall genomic stability (**Figure 2**) [81, 85]. BRCA1 recruitment to damaged chromatin is multifactorial and it is thought that BRCA1-Ctip-MRN complex accesses chromatin directly while BRCA1-PALB2-BRCA2 complexes promote the loading of Rad51 on the resected DNA [78, 79, 87]. Rad51 functions to coat the ssDNA and facilitates synapse formation with the template DNA. In contrast the BRCA1-A (BRCA1-MERIT40-BRCC36-BRCC45-ABRAXAS) complex is recruited

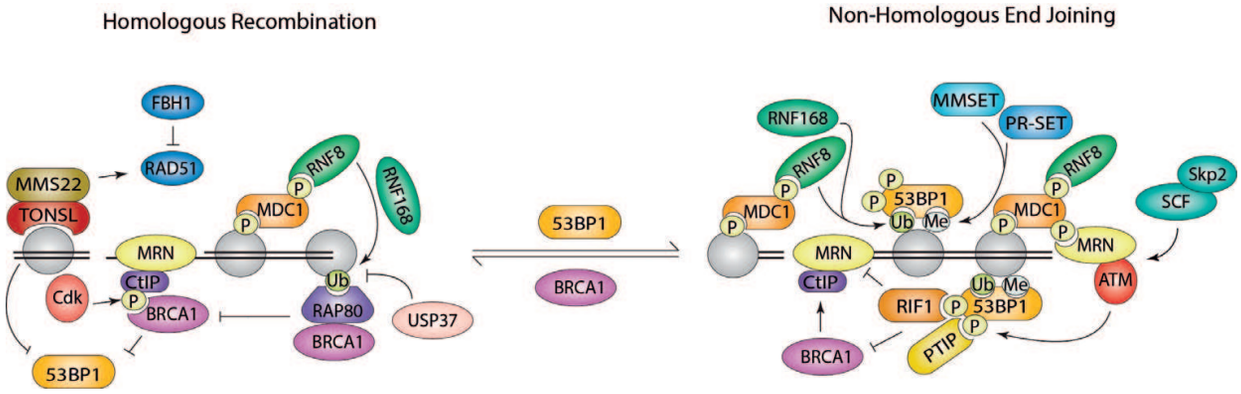


**Figure 3.** 53BP1 and BRCA1 determine the choice between DNA repair mechanisms. Homologous recombination, takes place in S- and G2 phases after DNA replication. Histones (gray circles) of newly replicated DNA lack methylation at H4K20, which weakens the interaction with 53BP1. Moreover, MMS22-TONSL binds the non-methylated H4, which may further antagonize 53BP1, and promotes RAD51 function, which is antagonized by FBH1. BRCA1 is then able to complex with CDK phosphorylated CtIP to drive end resection, preventing NHEJ and setting the stage for HR. BRCA1 further blocks 53BP1 binding, in part by competing for the histone H2K15-Ub marks when complexed with RAP80. This complex also limits resection and is antagonized by the deubiquitinase USP37. In G1 and G2 upon *de novo* H4K20 methylation, 53BP1 is recruited to chromatin to form a barrier to resection. ATM phosphorylation of 53BP1 recruits RIF1 and PTIP, which antagonize BRCA1 and promote recruitment of additional factors to promote repair by NHEJ.

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to chromatin by the RNF168-mediated ubiquitination of histones, via the ubiquitin-binding RAP80 protein (**Figure 3**) [88–90]. Interestingly, formation of this complex limits the access of BRCA1 to the damaged DNA, suppressing resection [88].

The critical distinction between these two pathways is the dependency of HR on the resection of the broken DNA end to generate ssDNA to form the synapse with the template DNA. In addition, because NHEJ relies on the ligation of blunt DNA ends and once resection is initiated NHEJ cannot be used. Thus, regulation of resection is central to the choice between mechanisms of repair. Moreover, the inappropriate induction of resection can also give rise to the use of alt-NHEJ. Given that cells that are HR-deficient, (e.g., BRCA1 mutant cells) exhibit sensitivity to DSBs suggests that NHEJ is either too mutagenic or simply does not function efficiently during S-phase, when HR normally predominates. These two possibilities are not mutually exclusive. In addition to DSB repair, HR is also critical for the stabilization and restart of replication forks after prolonged replication stress. As discussed below, multiple layers do, in fact, limit the use of NHEJ during S-phase.

### 3.3. The replication stress checkpoint

Cells encounter a multitude of intrinsic and extrinsic barriers in attempting to achieve accurate DNA replication. To ensure that replication is error free, eukaryotes possess a conserved checkpoint that monitors replication progress. Upon replication stress (e.g., stalled replication fork, nucleotide deficiency, DNA damage), extensive regions of ssDNA are formed, which are coated by Replication Protein A (RPA) which mediates the recruitment of the apical kinase ATR to the DNA [91]. The Rad17 protein then promotes the loading of a protein complex including the ATR activator, TopBP1, and the checkpoint mediator, Claspin, that then recruits the effector kinase Chk1, which is ultimately phosphorylated by ATR at S317 and S345 that allow Chk1 to adopt an open, active conformation. In turn, Chk1 phosphorylates many proteins, including the Cdk-activating Cdc25 phosphatases, the CDK inhibitory kinase WEE1, and the key HR protein Rad51 [92]. Notably, phosphorylation of Cdc25A by Chk1 leads to $SCF^{\beta TrCP}$-mediated degradation. Chk1 ultimately controls origin firing and entry into mitosis as well as promoting replication fork restart and repair, which is predominantly dependent upon the HR machinery.

In the absence of Chk1 recruitment and activation, cells undergoing replication stress maintain high levels of Cdk activity and, continue to fire origins. Under these conditions, replication forks may be prone to stalling and will likely collapse to form DSBs leading to chromosomal abnormalities. These cells are thus highly sensitive to additional replication stress. Importantly, high levels of replication stress are associated with high rates of proliferation during early development and expression of multiple oncogenes (e.g., Cyclin E, c-Myc) [93–98]. Chk1 activity is essential for embryonic development and it follows that surviving the process of transformation requires Chk1 function to survive with abnormal levels of replication stress [99]. As a result, transformed cells are highly dependent on the ATR-Claspin-Chk1 pathway for survival and are sensitive to agents that either induce additional stress or inhibit this critical checkpoint [94, 97, 98]. Indeed, mice possessing an extra copy of Chk1 are more susceptible to oncogenic stimuli. Intriguingly, premature Chk1 activation may drive S-phase entry and failure to down-regulate Chk1 activation is also detrimental.

### 3.4. Crosstalk between the ATM-Chk2 and ATR-Chk1 axes

As described above, it would seem that the ATM-Chk2 and ATR-Chk1 pathways function in isolation, depending on cell cycle stage and type of insult. However, there is clear cross-talk between the two and, at least in some cell types, the G2 DDR is dramatically weakened, if not abrogated, in the absence of Chk1 function. Resection of damaged DNA ends upon initiation of the HR pro-cess yields ssDNA similar to replication stress, which is also coated by RPA and serves as a scaffold upon which to activate the ATR-Chk1 cascade.

## 4. CRLs and APC/C in DNA damage checkpoint responses

### 4.1. The G2 DNA damage checkpoint

Initial evidence that Cdh1 possesses a function in the DDR was obtained from chicken DT40 cells in which Cdh1 gene had been deleted [100]. Surprisingly, these Cdh1 knock-out cells were unable to maintain a G2 arrest in the presence of DNA damage. This result was unexpected as $APC/C^{Cdh1}$ is largely thought to be inactive in S and G2 cells due to Cyclin A- and Cyclin E-Cdk-mediated phosphorylation of Cdh1, which both prevents its binding to the APC/C holo-enzyme and, at least at the G1/S transition promotes the creation of a phosphodegron that is recognized by $SCF^{\beta TrCP}$ leading to Cdh1 degradation [40, 41, 52, 53, 101]. In addition, Emi1, which binds to the APC/C with high affinity and prevents ubiquitination of substrates, is maximally expressed from late G1 through early mitosis [44, 46, 47, 49–51, 102]. Moreover, key APC/C substrates, including Cyclin A, Cyclin B, and Skp2 remain stable during a G2 arrest [103]. Indeed, nearly all APC/C targets tested do remain stable upon DNA damage in G2, with the exception of Plk1 [103]. This is an important distinction as APC/C substrates can have dramatically different impacts on the checkpoint. Plk1 dampens the checkpoint by phosphorylating 53BP1 and Chk2 to inhibit ATM signalling (**Figure 4**) [104]. In addition, Plk1 catalyzes the $SCF^{\beta TrCP}$-mediated inhibition of ATR-Chk1 signalling (**Figure 4**) [105–109]. Down-regulation of Plk1 protein levels upon DNA damage was demonstrated to be the result of $APC/C^{Cdh1}$ activation (**Figure 4**) [103]. A critical question stemming from these studies is how $APC/C^{Cdh1}$ targets only Plk1 under these conditions.

The studies in both chicken and human cells indicate that active $APC/C^{Cdh1}$ complexes form upon DNA damage in G2 [100, 103]. Previous analyses had suggested the existence of an Emi1-free pool of the APC/C during interphase [44]. Consistent with this idea, an increased APC/C-Cdh1 association was detected upon damage whereas changes in the abundance of either Emi1 protein or in amount of Emi1-bound APC/C were not observed upon DNA damage [103]. Given that Cdk activity is diminished upon DNA damage (**Figure** 4), these data suggest that a pool of APC/C exists that is independent of Emi1 and regulated largely by inhibitory phosphorylation of Cdh1. The failure to phosphorylate Cdh1 may result in the dephosphorylation and activation of this pool of Cdh1 due to a shift in the balance of kinase phosphatase activities brought about by the inactivation of Cdks by the DDR. In addition, it has been shown that specific release of the Cdc14B phosphatase from the nucleolus upon DNA damage contributes to the dephosphorylation of Cdh1, promoting $APC/C^{Cdh1}$ formation [103].

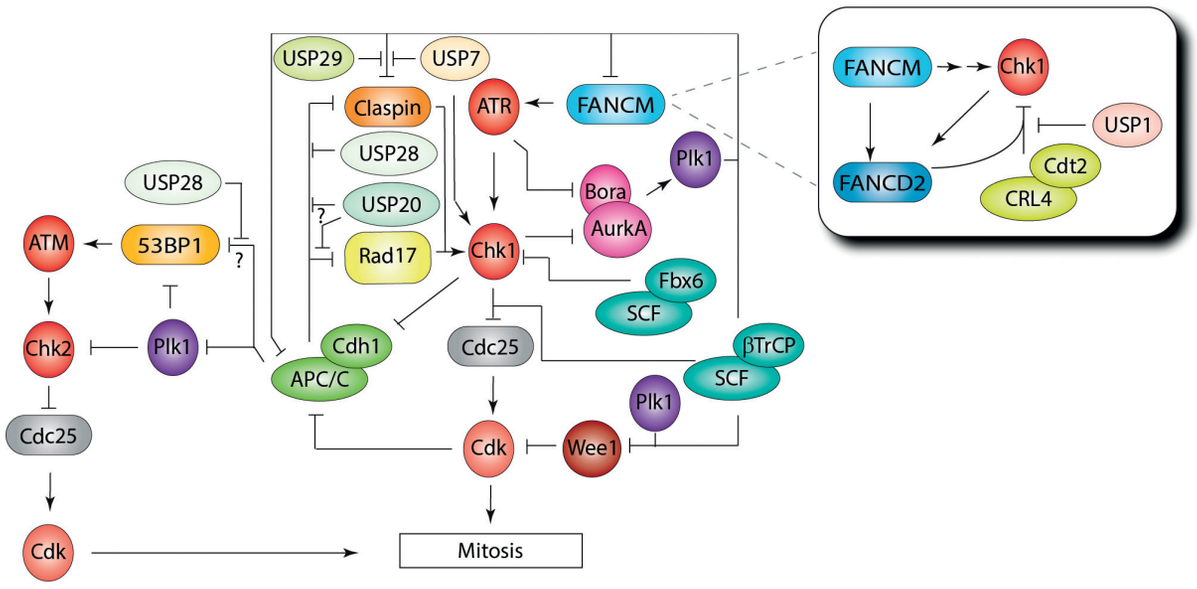


**Figure 4.** Interplay between Plk1 kinase, ubiquitin machinery and DNA damage checkpoint activity. The circuitry in the main figure depicts the crosstalk between APC/C, SCF ligases and the checkpoint. Plk1 is a critical factor in checkpoint recovery, silencing both the ATM and ATR cascades. Plk1 phosphorylates and inhibit both 53BP1 and Plk1 to allow Cdk1 activity. Similarly, Plk1 triggers the $SCF^{\beta TrCP}$-dependent destruction of Claspin and FANCM to silence the ATR-Chk1 axis. Plk1 activity may also contribute to the silencing of $APC/C^{Cdh1}$ activity upon replication stress and during recovery from $APC/C^{Cdh1}$ activation during the G2 DDR. In G2 $APC/C^{Cdh1}$ targets Plk1 for degradation, while USP28 prevents it from targeting Claspin. USP28 also stabilizes 53BP1 after DNA damage as well, possibly from $APC/C^{Cdh1}$ (represented by, "?"). Chk1 activation requires Claspin function, which is protected from $SCF^{\beta TrCP}$-mediated degradation by USP29 and USP7. USP20 stabilizes both Claspin and Rad17 to promote Chk1 activity, possibly from $APC/C^{Cdh1}$ ("?") as they are both substrates of the ligases. ATR and Chk1 prevent checkpoint recovery by inhibiting the Plk1 activators Aurora and Bora. Irreversible checkpoint activation is prevented by the degradation of active Chk1 by $SCF^{Fbx6}$. USP7 prevents the complete destabilization of Chk1. The inset shows a potential feedback loop between ATR-Chk1 and the Fanconi pathway. FANCM promotes Chk1 activation (indirectly via ATR). Chk1 promotes FANCM-promoted FANCD2 monoubiquitination. In turn, FANCD2-Ub promotes the $CRL4^{Cdt2}$-mediated degradation of Chk1. USP1 deubiquitinates FANCD2, stabilizing Chk1. The negative feedback loop favors silencing of Chk1 due to the inactivation of USP1 upon DNA damage.

However, whether this Cdc14B contributes to the G2 checkpoint activity or DNA repair functions of $APC/C^{Cdh1}$ is unclear and could be influenced by cell type [110–112].

Activation of this pool of $APC/C^{Cdh1}$ may not be sufficient to target the majority of APC/C substrates. As dephosphorylated Cdh1 localizes to the nucleus, substrates such as Cyclin B, which are localized in the cytoplasm in G2 would be likely to remain safe from this pool of $APC/C^{Cdh1}$ [113]. How other nuclear APC/C substrates (e.g., Cyclin A) remain stable is an open question. One potential mechanism by which APC/C substrates may evade degradation is via the antagonistic activity of DUBs. Indeed, there is evidence that USP28 activity prevents $APC/C^{Cdh1}$-mediated degradation of Claspin after DNA damage (**Figure** 4) [103, 114]. Given the apparently small size of the APC/C pool activated by DNA damage, it is likely that selective targeting of APC/C substrates may also be achieved by specific localization of DNA damage-activated $APC/C^{Cdh1}$, for example to sites of DNA damage where proteins such as Claspin and Plk1 are expected be found. This idea remains to be tested, but it is worth noting that $APC/C^{Cdh1}$ is found on chromatin in S-phase and $APC/C^{Cdh1}$- mediated regulation of Cdc7-Dbf4 activity appears to be via specific targeting of the chromatin-bound fraction of the

kinase [115]. Thus it is possible that the apparent substrate-specificity may be due to limited access to substrates in conjunction with antagonism by DUBs. In keeping with this idea, it is worth noting that Plk1 levels are diminished, but not abolished by damage-activated APC/$C^{Cdh1}$, perhaps reflecting degradation of a pool of Plk1 in the vicinity of the sites of damage, where phosphorylation of key substrates such as Claspin and 53BP1 will eventually be phosphorylated to promote checkpoint recovery [103, 104]. In addition, 53BP1 has recently been identified as an APC/C substrate and, intriguingly, was also identified as an USP28 substrate raising the possibility that it too may be protected from APC/C at sites of DNA damage, but idea has not been tested (**Figure** 4) [114, 116].

### 4.2. The replication stress checkpoint

Whereas it is clearly established that APC/$C^{Cdh1}$controls entry into S-phase, multiple recent lines of evidence suggest that the E3 is also a key regulator of the replication stress response as well. However, in contrast to its role as a positive regulator of the G2 DNA damage checkpoint, APC/$C^{Cdh1}$ appears to be a negative regulator of the replication stress checkpoint, as it targets two critical regulators of the checkpoint, Rad17 and Claspin, which are central to the activation of Chk1 (**Figure 4**) [103, 117, 118]. Indeed, in the absence of Cdh1, failure to degrade Claspin leads to unscheduled Chk1 activation, which is associated with premature S-phase entry [117]. Given the importance of these proteins for the stress response, both UV-irradiation and induction of replication stress by treatment with hydroxyurea lead to the degradation of Cdh1 and, at least in the case of UV, to the stabilization of Rad17 [115, 118]. Activation of Chk1 then feeds back to further enhance its own activation by triggering Cdh1 destruction [115]. Claspin stability is also dependent on Chk1 activity, suggesting that down-regulation of Cdh1 contributes to this arm of a Chk1 auto-amplification loop as well [119]. Notably, Claspin stability is also dependent on context dependent DUB activity as well. ATR activation leads to the stabilization of USP20, which promotes Claspin stability during S-phase [120, 121]. USP20 has also been demonstrated to stabilize Rad17, suggesting perhaps that this DUB may antagonize APC/$C^{Cdh1}$-mediated destruction of these proteins to promote ATR-Chk1 function (**Figure 4**) [122, 123]. USP9x has also been identified as a DUB for Claspin during replication stress and USP7 has been found to counteract the degradation of Claspin by $SCF^{\beta TrCP}$, but not APC/$C^{Cdh1}$, during replication stress as well [123, 124]. A similar role has been demonstrated for USP29 (**Figure 4**).

The mechanism for Chk1-mediated degradation of Cdh1 is not well-defined, but in the case of HU-induced stress, APC/C-mediated destruction has been implicated [115]. However, degradation induced by UV exposure, which would presumably be augmented by Chk1 as well, involves a region of Cdh1, which is not known to mediate interactions with the APC/C, but does lie between two regions of the Cdh1 N-terminal domain (NTD) that make critical contacts with the APC/C and are negatively regulated by phosphorylation [125, 126]. Thus, Chk1 may directly or indirectly alter the association of Cdh1 with the APC/C to promote its degradation. In addition, the region containing the UV-responsive degron in Cdh1 also encompasses the $SCF^{\beta TrCP}$ phosphodegron [53, 115, 125]. Notably, while phosphorylation by Plk1 has been identified as critical for creating this phosphodegron there are additional phosphorylation events, mediated by unknown kinases, which contribute to recognition by

$SCF^{\beta TrCP}$ [53]. It is tempting to speculate that Chk1 directly or indirectly promotes the $SCF^{\beta TrCP}$-mediated destruction of Cdh1 as well.

Chk1 itself is also targeted for destruction. Upon activation, Chk1 adopts an open conformation, which exposes degrons that are recognized by $SCF^{Fbw6}$ and $CRL4^{Cdt2}$ ubiquitin ligases (**Figure 4**) [127–129]. DUB activity also plays a role in the maintenance of Chk1 levels. Surprisingly, there are few examples of Chk1 stabilization by DUBs in comparison to their involvement stabilizing Claspin to promote Chk1 activation. To date, only USP7 and USP1 have been implicated in the maintenance of active Chk1 levels. USP7 directly deubiquitinates Chk1 and this activity is enhanced by ATM activation [124, 130, 131]. Whether ATR may also promote USP7-mediated Chk1 activity is not clear. Active Chk1 levels are indirectly protected by USP1 via its ability to antagonize the ubiquitination FANCD2, which induces CRL4-mediated degradation of Chk1 (**Figure 4**) [132]. USP1 is also an $APC/C^{Cdh1}$ substrate adding another level of complexity to the Chk1-Cdh1 feedback loop [133].

The relationship between Chk1 and USP1 also begins to lend some insight into how the feedback loop is faulted to allow checkpoint recovery (**Figure 4**). First, the ATR-Chk1 axis promotes FANCD2 ubiquitination, which would begin to induce down-regulation of active Chk1 [132, 134–136]. Second, USP1 activity is inhibited by multiple mechanisms after UV-damage or the induction of replication stress [137–140]. Thus as the damage or stress-inducing events are resolved and ATR signalling is diminished, active Chk1 becomes susceptible to degradation, which would allow the accumulation of Cdh1 protein. Stabilization of Cdh1 leads to degradation of Rad17 to further inhibit activation of additional Chk1 [118]. Diminished activity of ATR and Chk1 promotes the stabilization of Bora and allows Aurora A activity, respectively, which are critical for Plk1 activation **(Figure 4**) [108, 134, 141]. Plk1, in turn, phosphorylates FANCM and Claspin to promote their $SCF^{\beta TrCP}$-mediated degradation to further silence ATR and Chk1 activity, respectively, and further promote loss of Chk1 activity (**Figure 4**) [109, 142]. A key remaining question is how APC/C activity is then restrained to allow normal cell cycle progression. The increase in Plk1 activity also triggers $SCF^{\beta TrCP}$-mediated degradation of Wee1, preventing the inhibitory phosphorylation of Cdks [143, 144]. A straightforward mechanistic model is that increased Cdk activity following stabilization of Cdc25A levels and loss of Wee1 promote increased Cdk-mediated inhibitory phosphorylation of Cdh1 to return to normal levels of $APC/C^{Cdh1}$ activity. It is currently unclear, however, why Cdh1 is able reaccumulate during checkpoint recovery despite rising activity of the $SCF^{\beta TrCP}$-targeting kinase, Plk1.

## 5. CRLs and APC/C influence the selection of DSB repair mechanism

Given their many roles in the regulation and execution of checkpoints that monitor the integrity of DNA, it is not surprising that the CRL and APC/C ligases also have roles in regulating DNA repair pathways. $SCF^{Fbw7}$ has been demonstrated to promote NHEJ by catalyzing K63-linked ubiquitination of XRCC4 to enhance its interaction with Ku70/80 complex [74]. However, for the most part the concerted efforts of these ligases do not appear to exert a predominant effect on the decision between NHEJ and HR repair pathways at the moment of damage. Rather their activities appear to promote proper and efficient use of NHEJ and HR.

A critical distinction between these two pathways is the dependency of HR on the resection of the broken DNA end to generate ssDNA that forms a synapse with the template DNA.

APC/C$^{Cdh1}$ activity is required for faithful repair, possibly independent of its checkpoint role. Indeed, APC/C$^{Cdh1}$ regulates multiple components of these pathways. Recently, it was shown that CtIP levels are regulated by APC/C$^{Cdh1}$ upon mitotic exit and after DNA damage, thus limiting the potential for attempting HR in G1, which would likely be mutagenic, and limiting the potential frequency of HR upon damage in G2 (**Figure 5**) [145]. Interestingly, failure to

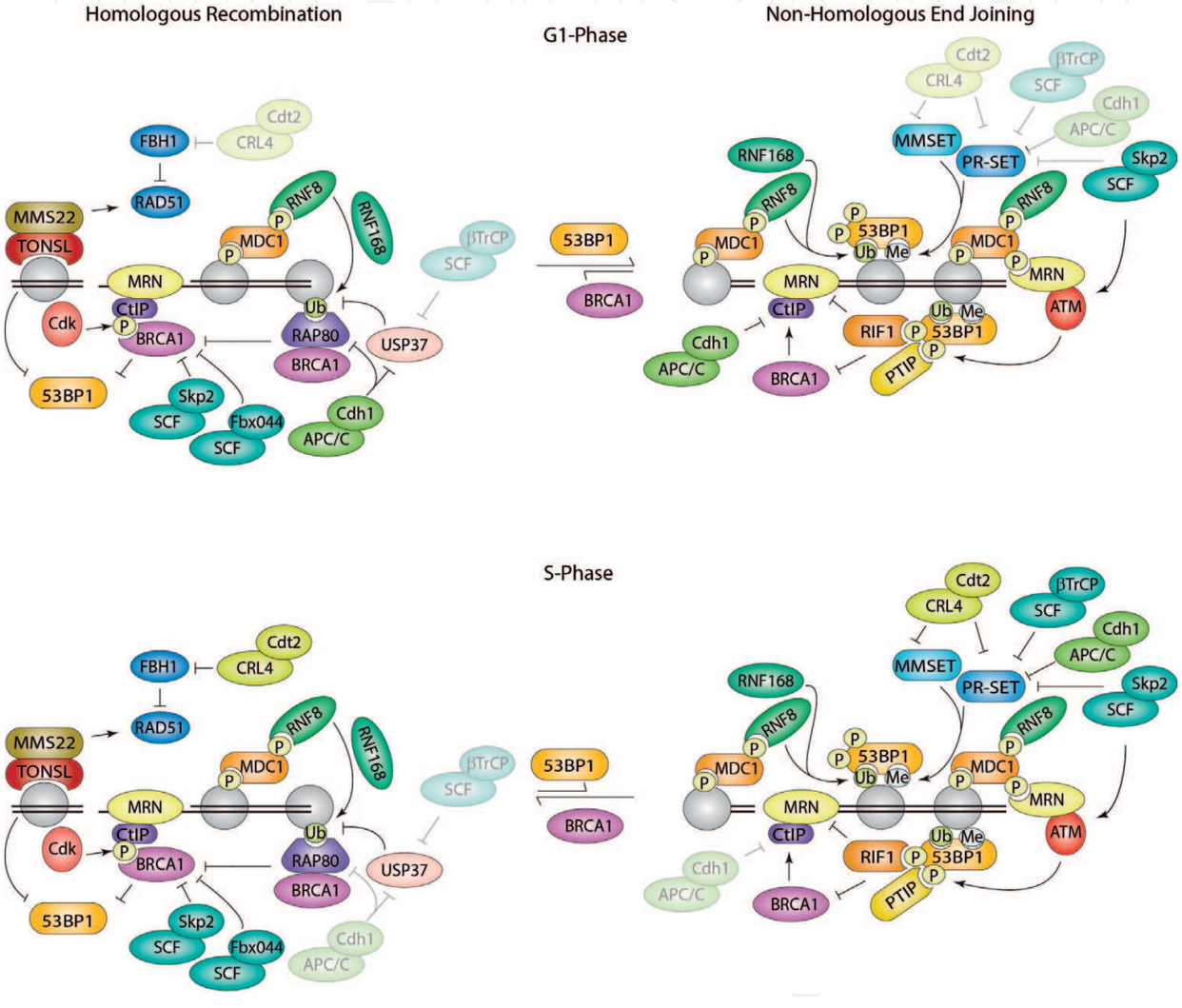


**Figure 5.** Cell cycle ligases set the stage for the selection of NHEJ and HR in G1 and S phases, respectively. During G1 (upper panel) APC/C$^{Cdh1}$ mediates the degradation of pro-HR factors, CtIP, USP37 and RAP80, whereas pro-NHEJ factors MMSET and PR-SET remain stable. Together these factors promote the recruitment of 53BP1 to chromatin favoring NHEJ. The anti-HR factor FBH1 also remains stable. During S-phase (lower panel) replication coupled, CRL4$^{Cdt2}$-mediated degradation of FBH1 favors HR. Similarly, CRL4$^{Cdt2}$ targets the methyltransferase MMSET2 and PR-SET7 for destruction. PR-SET7 is also targeted for destruction by SCF$^{Skp2}$ and SCF$^{\beta TrCP}$. This destruction hinders the recruitment of 53BP1 to chromatin favoring HR. Finally, Cdk activity, positively regulated by SCF$^{Skp2}$ and the downregulation of APC/C$^{Cdh1}$activity in S-phase further promotes the use of HR. The activities of SCF$^{Skp2}$ and SCF$^{Fbxo44}$ limit BRCA1-CtIP–mediated resection are depicted in both phases, although the cell cycle-dependence of these events is not clear. Similarly, the potential for APC/C$^{Cdh1}$–mediated regulation of PR-SET7 is depicted, but remains unclear.

down-regulate CtIP levels by APC/$C^{Cdh1}$ leads to increased resection and inefficient repair, potentially due to interference with the use of NHEJ as well [145]. Although it remains to be tested, it would stand to reason that degradation of Cdh1 upon replication stress may also lead to enhanced stabilization of CtIP to promote HR. In contrast to limiting resection by targeting CtIP, APC/$C^{Cdh1}$ also targets the HR-limiting factor RAP80, which localizes BRCA1 to regions flanking DSB in an ubiquitin-dependent manner, but represses BRCA1-mediated HR [88, 146]. APC/C-mediated destruction limits RAP80 expression during G1, presumably to diminish competition for H2AK15-Ub binding with 53BP1 at DSBs to promote the use of NHEJ in the absence of a homologous template (**Figure 5**). During S and G2, BRCA1-dependent HR is thought to involve the degradation of RAP80 and, although the activation of APC/$C^{Cdh1}$ by DSBs in G2 suggests that it may be, it remains to be determined whether the APC/$C^{Cdh1}$ is involved in this destruction event. In addition, APC/$C^{Cdh1}$ and $SCF^{\beta TrCP}$ cooperate to limit the expression of USP37 to S-phase and early G2 (**Figure 5**). USP37, along with the related USP26, has been shown to antagonize RAP80 to promote BRCA1-dependent HR [147–149]. $SCF^{Skp2}$ is also required for efficient HR, in part via promotion of checkpoint signaling [54]. In addition, $SCF^{Skp2}$ and $SCF^{Fbxo44}$ ubiquitinate BRCA1 to control the extent of resection (**Figure 5**) [150]. The balance of $CRL3^{Keap}$ and USP11 activities also regulates HR by targeting PALB2 (**Figure 2**) [151]. $CRL4^{Cdt2}$ catalyzes the degradation of FBH1, which negatively regulates Rad51 function to limit HR prior to replication-dependent generation of the template. Interestingly, the interaction of FBH1 with PCNA may promote the use of TLS [152–156]. The APC/C may also contribute to the use of HR by antagonizing the expression of the NHEJ-promoting protein 53BP1 [116]. However, it is not clear whether APC/C impacts NHEJ activation via regulation 53BP1. Interestingly, a proteomic screen identified several additional pro-NHEJ factors in association with APC/$C^{Cdh1}$ [145]. Yet, it remains to be determined whether these are substrates of the ligase.

In addition to restricting the use of HR to S-phase and G2 by regulating the levels of key HR factors to these phases, the coordinated efforts of APC/C and CRL ligases also limit the use of NHEJ during S-phase. The methyltransferases PR-SET7 and MMSET promote NHEJ by directing recruitment methylating H4K20 to recruit 53BP1 (**Figure 5**) [62–65]. Whereas global H4K20 methylation is not significantly altered by the induction of DSBs, de novo methylation of H4K20 at sites of damage has been demonstrated to mediate recruit 53BP1 and promote NHEJ. Importantly, histones deposited during replication lack H4K20 methylation. Multiple ligases, APC/$C^{Cdh1}$, $SCF^{\beta TrCP}$, $SCF^{Skp2}$, and $CRL4^{Cdt2}$ restrict expression and activity of the methyltransferase PR-Set7 to G2, mitosis, and early G1 (**Figure 5**) [66, 157–161]. In addition, $CRL4^{Cdt2}$ targets MMSET for replication-coupled degradation (**Figure 5**) [162]. Thus, with little capacity to generate NHEJ promoting methylation marks, DSBs occurring in S-phase, and likely early G2 as well, are not permissive for the recruitment of NHEJ factors allowing relatively uncontested access to the damaged sites by the HR machinery. In addition, the deposition of histones lacking H4K20 methylation marks in newly replicated DNA recruits MMS22L-TONSL complex, which directly promotes HR (**Figure 5**) [163–166]. Finally, the window of kinase activities, (cyclin-Cdk activity in particular) opened to promote the transition into and through S-phase also catalyze the phosphorylation of multiple components of the HR machinery, which promote the activity of this pathway [77, 78, 167, 168].

## 6. Conclusion

For many years, the importance of the CRL and APC/C ligases in cancer and genome stability has been appreciated. It was long thought that these roles were attributed to their ability to control cell cycle transitions, particularly their abilities to regulate one another. As discussed herein, we have more recently begun to elucidate that these ligases possess more direct, highly regulated and interconnected roles in the response to and repair of DNA damage as well.

While alterations in the mechanisms controlling genome stability lead to disease such as cancer, the induction of DNA damage is a tested and potent anti-cancer strategy. Moreover, manipulating these pathways has obvious therapeutic potential. Indeed, recent advances in inhibitors of DNA checkpoint and repair proteins (e.g., Chk1) suggest that manipulating the DDR response offers a therapeutic advantage over DNA damage based therapies alone. However, these strategies have faced challenges in translation. As we move ever closer to the realization of personalized medicine, it is of increasing importance that we understand not only the full cadre of players in a given pathway, but also those regulating it as well. Only with this knowledge can we fully appreciate the impact of altering that pathway, whether in dissecting pathophysiological changes of disease or in the development of potential therapeutic manipulations. We are increasingly successful in targeting components of the ubiquitin proteasome system and there are now small molecules capable of inhibiting specific SCF complexes with potential for substrate specificity. Similar accomplishments have been made in the targeting of the APC/C as well as DUBs, including USP1 and USP7. Finally, while we have focused on the role of these ligases in the major responses to DNA damage and the impact they have on DSB repair, there is mounting evidence that the activities of these enzymes impact multiple damage response and repair pathways. Thus, as we increase our understanding of the how these components of the ubiquitin machinery impact the choice and efficient use of DNA repair mechanisms we also increase our opportunities for improved therapeutic options.

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## References


[1] Lydeard JR, Schulman BA, Harper JW. Building and remodelling Cullin-RING E3 ubiquitin ligases. EMBO Reports. 2013;**14**:1050-1061

[2] Zheng N, Zhou Q, Wang Z, Wei W. Recent advances in SCF ubiquitin ligase complex: Clinical implications. Biochimica et Biophysica Acta. 2016;**1866**:12-22

[3] Kitagawa K, Kitagawa M. The SCF-type E3 ubiquitin ligases as cancer targets. Current Cancer Drug Targets. 2016;**16**:119-129

[4] Kitagawa K, Kotake Y, Kitagawa M. Ubiquitin-mediated control of oncogene and tumor suppressor gene products. Cancer Science. 2009;**100**:1374-1381

[5] Nakayama KI, Nakayama K. Regulation of the cell cycle by SCF-type ubiquitin ligases. Seminars in Cell & Developmental Biology. 2005;**16**:323-333

[6] Soucy TA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009;**458**:732-736

[7] Frescas D, Pagano M. Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: Tipping the scales of cancer. Nature Reviews. Cancer. 2008;**8**:438-449

[8] Jin J, Arias EE, Chen J, Harper JW, Walter JC. A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. Molecular Cell. 2006;**23**:709-721

[9] Angers S, et al. Molecular architecture and assembly of the DDB1-CUL4A ubiquitin ligase machinery. Nature. 2006;**443**:590-593

[10] Hannah J, Zhou P. Regulation of DNA damage response pathways by the cullin-RING ubiquitin ligases. DNA Repair (Amst). 2009;**8**:536-543

[11] Mailand N, Gibbs-Seymour I, Bekker-Jensen S. Regulation of PCNA-protein interactions for genome stability. Nature Reviews. Molecular Cell Biology. 2013;**14**:269-282

[12] Mansilla SF, et al. UV-triggered p21 degradation facilitates damaged-DNA replication and preserves genomic stability. Nucleic Acids Research. 2013;**41**:6942-6951

[13] Havens CG, Walter JC. Docking of a specialized PIP Box onto chromatin-bound PCNA creates a degron for the ubiquitin ligase CRL4Cdt2. Molecular Cell. 2009;**35**:93-104

[14] Soria G, Speroni J, Podhajcer OL, Prives C, Gottifredi V. P21 differentially regulates DNA replication and DNA-repair-associated processes after UV irradiation. Journal of Cell Science. 2008;**121**:3271-3282

[15] Soria G, Podhajcer O, Prives C, Gottifredi V. P21Cip1/WAF1 downregulation is required for efficient PCNA ubiquitination after UV irradiation. Oncogene. 2006;**25**:2829-2838

[16] Gottifredi V, McKinney K, Poyurovsky MV, Prives C. Decreased p21 levels are required for efficient restart of DNA synthesis after S phase block. The Journal of Biological Chemistry. 2004;**279**:5802-5810

[17] Jones MJ, Colnaghi L, Huang TT. Dysregulation of DNA polymerase kappa recruitment to replication forks results in genomic instability. The EMBO Journal. 2012;**31**:908-918

[18] Havens CG, Walter JC. Mechanism of CRL4(Cdt2), a PCNA-dependent E3 ubiquitin ligase. Genes & Development. 2011;**25**:1568-1582

[19] Bruning JB, Shamoo Y. Structural and thermodynamic analysis of human PCNA with peptides derived from DNA polymerase-delta p66 subunit and flap endonuclease-1. Structure 2004;**12**:2209-2219

[20] Pesin JA, Orr-Weaver TL. Regulation of APC/C activators in mitosis and meiosis. Annual Review of Cell and Developmental Biology. 2008;**24**:475-499

[21] Manchado E, Eguren M, Malumbres M. The anaphase-promoting complex/cyclosome (APC/C): Cell-cycle-dependent and -independent functions. Biochemical Society Transactions. 2010;**38**:65-71

[22] Peters JM. The anaphase promoting complex/cyclosome: A machine designed to destroy. Nature Reviews. Molecular Cell Biology. 2006;**7**:644-656

[23] Wasch R, Robbins JA, Cross FR. The emerging role of APC/CCdh1 in controlling differentiation, genomic stability and tumor suppression. Oncogene. 2010;**29**:1-10

[24] Simpson-Lavy KJ, et al. Fifteen years of APC/cyclosome: A short and impressive biography. Biochemical Society Transactions. 2010;**38**:78-82

[25] Bassermann F, Pagano M. Dissecting the role of ubiquitylation in the DNA damage response checkpoint in G2. Cell Death and Differentiation. 2010;**17**:78-85

[26] Skaar JR, Pagano M. Control of cell growth by the SCF and APC/C ubiquitin ligases. Current Opinion in Cell Biology. 2009;**21**:816-824

[27] Li M, Zhang P. The function of APC/CCdh1 in cell cycle and beyond. Cell Division. 2009;**4**:2

[28] van Leuken R, Clijsters L, Wolthuis R. To cell cycle, swing the APC/C. Biochimica et Biophysica Acta. 2008;**1786**:49-59

[29] Lindon C. Control of mitotic exit and cytokinesis by the APC/C. Biochemical Society Transactions. 2008;**36**:405-410

[30] Sullivan M, Morgan DO. Finishing mitosis, one step at a time. Nature Reviews. Molecular Cell Biology. 2007;**8**:894-903

[31] Kim AH, Bonni A. Thinking within the D box: Initial identification of Cdh1-APC substrates in the nervous system. Molecular and Cellular Neurosciences. 2007;**34**:281-287

[32] Pfleger CM, Kirschner MW. The KEN box: An APC recognition signal distinct from the D box targeted by Cdh1. Genes & Development. 2000;**14**:655-665

[33] Glotzer M, Murray AW, Kirschner MW. Cyclin is degraded by the ubiquitin pathway. Nature. 1991;**349**:132-138

[34] Garcia-Higuera T, et al. Genomic stability and tumour suppression by the APC/C cofactor Cdh1. Nature Cell Biology. 2008;**10**:802-811

[35] Engelbert D, Schnerch D, Baumgarten A, Wasch R. The ubiquitin ligase APC(Cdh1) is required to maintain genome integrity in primary human cells. Oncogene. 2008;**27**:907-917

[36] Lehman NL, et al. Oncogenic regulators and substrates of the anaphase promoting complex/cyclosome are frequently overexpressed in malignant tumors. The American Journal of Pathology. 2007;**170**:1793-1805

[37] Wei W, et al. Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. Nature. 2004;**428**:194-198

[38] Bashir T, Dorrello NV, Amador V, Guardavaccaro D, Pagano M. Control of the SCF(Skp2-Cks1) ubiquitin ligase by the APC/C(Cdh1) ubiquitin ligase. Nature. 2004;**428**:190-193

[39] Cappell SD, Chung M, Jaimovich A, Spencer SL, Meyer T. Irreversible APC(Cdh1) inactivation underlies the point of no return for cell-cycle entry. Cell. 2016;**166**:167-180

[40] Sorensen CS, et al. A conserved cyclin-binding domain determines functional interplay between anaphase-promoting complex-Cdh1 and cyclin A-Cdk2 during cell cycle progression. Molecular and Cellular Biology. 2001;**21**:3692-3703

[41] Sorensen CS, et al. Nonperiodic activity of the human anaphase-promoting complex-Cdh1 ubiquitin ligase results in continuous DNA synthesis uncoupled from mitosis. Molecular and Cellular Biology. 2000;**20**:7613-7623

[42] Lukas C, et al. Accumulation of cyclin B1 requires E2F and cyclin-A-dependent rearrangement of the anaphase-promoting complex. Nature. 1999;**401**:815-818

[43] Verschuren EW, Ban KH, Masek MA, Lehman NL, Jackson PK. Loss of emi1-dependent anaphase-promoting complex/cyclosome inhibition deregulates E2F target expression and elicits DNA damage-induced senescence. Molecular and Cellular Biology. 2007;**27**:7955-7965

[44] Miller JJ, et al. Emi1 stably binds and inhibits the anaphase-promoting complex/cyclosome as a pseudosubstrate inhibitor. Genes & Development. 2006;**20**:2410-2420

[45] Eldridge AG, et al. The evi5 oncogene regulates cyclin accumulation by stabilizing the anaphase-promoting complex inhibitor emi1. Cell. 2006;**124**:367-380

[46] Hsu JY, Reimann JD, Sorensen CS, Lukas J, Jackson PK. E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APC(Cdh1). Nature Cell Biology. 2002;**4**:358-366

[47] Reimann JD, et al. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. Cell. 2001;**105**:645-655

[48] Moshe Y, Boulaire J, Pagano M, Hershko A. Role of Polo-like kinase in the degradation of early mitotic inhibitor 1, a regulator of the anaphase promoting complex/cyclosome.

Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:7937-7942

[49] Hansen DV, Loktev AV, Ban KH, Jackson PK. Plk1 regulates activation of the anaphase promoting complex by phosphorylating and triggering SCFbetaTrCP-dependent destruction of the APC inhibitor Emi1. Molecular Biology of the Cell. 2004;**15**:5623-5634

[50] Margottin-Goguet F, et al. Prophase destruction of Emi1 by the SCF(betaTrCP/Slimb) ubiquitin ligase activates the anaphase promoting complex to allow progression beyond prometaphase. Developmental Cell. 2003;**4**:813-826

[51] Guardavaccaro D, et al. Control of meiotic and mitotic progression by the F box protein beta-Trcp1 in vivo. Developmental Cell. 2003;**4**:799-812

[52] Lau AW, et al. Regulation of APC(Cdh1) E3 ligase activity by the Fbw7/cyclin E signaling axis contributes to the tumor suppressor function of Fbw7. Cell Research. 2013;**23**:947-961

[53] Fukushima H, et al. SCF-mediated Cdh1 degradation defines a negative feedback system that coordinates cell-cycle progression. Cell Reports. 2013;**4**:803-816

[54] Wu J, et al. Skp2 E3 ligase integrates ATM activation and homologous recombination repair by ubiquitinating NBS1. Molecular Cell. 2012;**46**:351-361

[55] Mailand N, et al. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell. 2007;**131**:887-900

[56] Kolas NK, et al. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science. 2007;**318**:1637-1640

[57] Huen MS, et al. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell. 2007;**131**:901-914

[58] Mochan TA, Venere M, DiTullio RA Jr, Halazonetis TD, 53BP1 and NFBD1/MDC1-Nbs1 function in parallel interacting pathways activating ataxia-telangiectasia mutated (ATM) in response to DNA damage. Cancer Research. 2003;**63**:8586-8591

[59] Mattiroli F, et al. RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell. 2012;**150**:1182-1195

[60] Stewart GS, et al. The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. Cell. 2009;**136**:420-434

[61] Doil C, et al. RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. Cell. 2009;**136**:435-446

[62] Pei H, et al. The histone methyltransferase MMSET regulates class switch recombination. Journal of Immunology. 2013;**190**:756-763

[63] Pei H, et al. MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. Nature. 2011;**470**:124-128

[64] Tuzon CT, et al. Concerted activities of distinct H4K20 methyltransferases at DNA double-strand breaks regulate 53BP1 nucleation and NHEJ-directed repair. Cell Reports. 2014;**8**:430-438

[65] Dulev S, Tkach J, Lin S, Batada NN. SET8 methyltransferase activity during the DNA double-strand break response is required for recruitment of 53BP1. EMBO Reports. 2014;**15**:1163-1174

[66] Oda H. et al. Regulation of the histone H4 monomethylase PR-Set7 by CRL4(Cdt2)-mediated PCNA-dependent degradation during DNA damage. Molecular Cell. 2010;**40**:364-376

[67] Wilson MD, et al. The structural basis of modified nucleosome recognition by 53BP1. Nature. 2016;**536**:100-103

[68] Fradet-Turcotte A, et al. 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. Nature. 2013;**499**:50-54

[69] Karanam K, Kafri R, Loewer A, Lahav G. Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. Molecular Cell. 2012;**47**:320-329

[70] Deriano L, Roth DB. Modernizing the nonhomologous end-joining repertoire: Alternative and classical NHEJ share the stage. Annual Review of Genetics. 2013;**47**:433-455

[71] Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. Trends in Cell Biology. 2016;**26**:52-64

[72] Pierce AJ, Hu P, Han M, Ellis N, Jasin M. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. Genes & Development. 2001;**15**:3237-3242

[73] Mari PO, et al. Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:18597-18602

[74] Zhang Q, et al. FBXW7 facilitates nonhomologous end-joining via K63-linked polyubiquitylation of XRCC4. Molecular Cell. 2016;**61**:419-433

[75] Betermier M, Bertrand P, Lopez BS. Is non-homologous end-joining really an inherently error-prone process? PLoS Genetics. 2014;**10**:e1004086

[76] Sartori AA, et al. Human CtIP promotes DNA end resection. Nature. 2007;**450**:509-514

[77] Huertas P, Jackson SP. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. The Journal of Biological Chemistry. 2009;**284**:9558-9565

[78] Wang H, et al. The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair. PLoS Genetics. 2013;**9**:e1003277

[79] Escribano-Diaz C, et al. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. Molecular Cell. 2013;**49**:872-883

[80] Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. Science. 2013;**339**:700-704

[81] Li M, et al. 53BP1 ablation rescues genomic instability in mice expressing 'RING-less' BRCA1. EMBO Reports. 2016;**17**:1532-1541

[82] Feng L, et al. Cell cycle-dependent inhibition of 53BP1 signaling by BRCA1. Cell Discovery. 2015;**1**:15019

[83] Feng L, Fong KW, Wang J, Wang W, Chen J. RIF1 counteracts BRCA1-mediated end resection during DNA repair. The Journal of Biological Chemistry. 2013;**288**:11135-11143

[84] Chapman JR, et al. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. Molecular Cell. 2013;**49**:858-871

[85] Bunting SF, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell. 2010;**141**:243-254

[86] Bouwman P, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nature Structural & Molecular Biology. 2010;**17**:688-695

[87] Park JY, Zhang F, Andreassen PR. PALB2: The hub of a network of tumor suppressors involved in DNA damage responses. Biochimica et Biophysica Acta. 2014;**1846**:263-275

[88] Coleman KA, Greenberg RA. The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. The Journal of Biological Chemistry. 2011;**286**:13669-13680

[89] Shao G, et al. The Rap80-BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8-Ubc13-dependent ubiquitination events at DNA double strand breaks. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**: 3166-3171

[90] Sobhian B, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science. 2007;**316**:1198-1202

[91] Nam EA, Cortez D. ATR signalling: More than meeting at the fork. The Biochemical Journal. 2011;**436**:527-536

[92] Zhang Y, Hunter T. Roles of Chk1 in cell biology and cancer therapy. International Journal of Cancer. 2014;**134**:1013-1023

[93] Bester AC, et al., Nucleotide deficiency promotes genomic instability in early stages of cancer development. Cell. 2011;**145**:435-446

[94] Hoglund A, et al., Therapeutic implications for the induced levels of Chk1 in Myc-expressing cancer cells. Clin Cancer Res. 2011;**17**:7067-7079

[95] Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science. 2008;**319**:1352-1355

[96] Bartkova J. et al., Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature. 2006;**444**:633-637

[97] Murga M. et al., Exploiting oncogene-induced replicative stress for the selective killing of Myc-driven tumors. Nat Struct Mol Biol. 2011;**18**:1331-1335

[98] Schoppy DW. et al., Oncogenic stress sensitizes murine cancers to hypomorphic suppression of ATR. J Clin Invest. 2012;**122**:241-252

[99] Zachos G, Rainey MD, Gillespie DA, Chk1-dependent S-M checkpoint delay in vertebrate cells is linked to maintenance of viable replication structures. Mol Cell Biol. 2005;**25**:563-574

[100] Sudo T, et al. Activation of Cdh1-dependent APC is required for G1 cell cycle arrest and DNA damage-induced G2 checkpoint in vertebrate cells. The EMBO Journal. 2001;**20**: 6499-6508

[101] Keck JM, et al. Cyclin E overexpression impairs progression through mitosis by inhibiting APC(Cdh1). The Journal of Cell Biology. 2007;**178**:371-385

[102] Machida YJ, Dutta A. The APC/C inhibitor, Emi1, is essential for prevention of rereplication. Genes & Development. 2007;**21**;184-194

[103] Bassermann F, et al. The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. Cell. 2008;**134**:256-267

[104] van Vugt MA, et al. A mitotic phosphorylation feedback network connects Cdk1, Plk1, 53BP1, and Chk2 to inactivate the G(2)/M DNA damage checkpoint. PLoS Biology. 2010;**8**:e1000287

[105] Peschiaroli A, et al. SCFbetaTrCP-mediated degradation of Claspin regulates recovery from the DNA replication checkpoint response. Molecular Cell. 2006;**23**:319-329

[106] Mamely I, et al. Polo-like kinase-1 controls proteasome-dependent degradation of Claspin during checkpoint recovery. Current Biology. 2006;**16**:1950-1955

[107] Mailand N, Bekker-Jensen S, Bartek J, Lukas J. Destruction of Claspin by SCFbetaTrCP restrains Chk1 activation and facilitates recovery from genotoxic stress. Molecular Cell. 2006;**23**:307-318

[108] Qin B, Gao B, Yu J, Yuan J, Lou Z. Ataxia telangiectasia-mutated- and Rad3-related protein regulates the DNA damage-induced G2/M checkpoint through the Aurora A cofactor Bora protein. The Journal of Biological Chemistry. 2013;**288**:16139-16144

[109] Schwab RA, Blackford AN, Niedzwiedz W. ATR activation and replication fork restart are defective in FANCM-deficient cells. The EMBO Journal. 2010;**29**:806-818

[110] Lin H, et al. Cdc14A and Cdc14B redundantly regulate DNA double-strand break repair. Molecular and Cellular Biology. 2015;**35**:3657-3668

[111] Wei Z, et al. Early-onset aging and defective DNA damage response in Cdc14b-deficient mice. Molecular and Cellular Biology. 2011;**31**:1470-1477

[112] Mocciaro A, et al. Vertebrate cells genetically deficient for Cdc14A or Cdc14B retain DNA damage checkpoint proficiency but are impaired in DNA repair. The Journal of Cell Biology. 2010;**189**:631-639

[113] Zhou Y, Ching YP, Chun AC, Jin DY. Nuclear localization of the cell cycle regulator CDH1 and its regulation by phosphorylation. The Journal of Biological Chemistry. 2003; **278**:12530-12536

[114] Zhang D, Zaugg K, Mak TW, Elledge SJ. A role for the deubiquitinating enzyme USP28 in control of the DNA-damage response. Cell. 2006;**126**:529-542

[115] Yamada M, et al. ATR-Chk1-APC/CCdh1-dependent stabilization of Cdc7-ASK (Dbf4) kinase is required for DNA lesion bypass under replication stress. Genes & Development. 2013;**27**:2459-2472

[116] Kucharski TJ, Minshall PE, Moustafa-Kamal M, Turnell AS, Teodoro JG. Reciprocal regulation between 53BP1 and the anaphase-promoting complex/cyclosome is required for genomic stability during mitotic stress. Cell Reports. 2017;**18**:1982-1995

[117] Gao D, et al. Cdh1 regulates cell cycle through modulating the claspin/Chk1 and the Rb/E2F1 pathways. Molecular Biology of the Cell. 2009;**20**:3305-3316

[118] Zhang L. et al. Proteolysis of Rad17 by Cdh1/APC regulates checkpoint termination and recovery from genotoxic stress. The EMBO Journal. 2010;**29**:1726-1737

[119] Chini CC, Wood J, Chen J. Chk1 is required to maintain claspin stability. Oncogene. 2006;**25**:4165-4171

[120] Zhu M, Zhao H, Liao J, Xu X. HERC2/USP20 coordinates CHK1 activation by modulating CLASPIN stability. Nucleic Acids Research. 2014;**42**:13074-13081

[121] Yuan J. et al. HERC2-USP20 axis regulates DNA damage checkpoint through Claspin. Nucleic Acids Research. 2014;**42**:13110-13121

[122] Shanmugam I, et al. Ubiquitin-specific peptidase 20 regulates Rad17 stability, checkpoint kinase 1 phosphorylation and DNA repair by homologous recombination. The Journal of Biological Chemistry. 2014;**289**:22739-22748

[123] McGarry E, et al. The deubiquitinase USP9X maintains DNA replication fork stability and DNA damage checkpoint responses by regulating CLASPIN during S-Phase. Cancer Research. 2016;**76**:2384-2393

[124] Faustrup H, Bekker-Jensen S, Bartek J, Lukas J, Mailand N. USP7 counteracts SCFbetaTrCP- but not APCCdh1-mediated proteolysis of Claspin. The Journal of Cell Biology. 2009;**184**:13-19

[125] Liu W, Li W, Fujita T, Yang Q, Wan Y. Proteolysis of CDH1 enhances susceptibility to UV radiation-induced apoptosis. Carcinogenesis. 2008;**29**:263-272

[126] Chang L, Zhang Z, Yang Y, McLaughlin SH, Barford D. Atomic structure of the APC/C and its mechanism of protein ubiquitination. Nature. 2015;**522**:450-454

[127] Zhang YW, et al. The F box protein Fbx6 regulates Chk1 stability and cellular sensitivity to replication stress. Molecular Cell. 2009;**35**:442-453

[128] Leung-Pineda V, Huh J, Piwnica-Worms H. DDB1 targets Chk1 to the Cul4 E3 ligase complex in normal cycling cells and in cells experiencing replication stress. Cancer Research. 2009;**69**:2630-2637

[129] Huh J, Piwnica-Worms H. CRL4(CDT2) targets CHK1 for PCNA-independent destruction. Molecular and Cellular Biology. 2013;**33**:213-226

[130] Zhang P, et al. ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance through CHK1. Nature Cell Biology. 2014;**16**:864-875

[131] Alonso-de Vega I, Martin Y, Smits VA. USP7 controls Chk1 protein stability by direct deubiquitination. Cell Cycle. 2014;**13**:3921-3926

[132] Guervilly JH, Renaud E, Takata M, Rosselli F. USP1 deubiquitinase maintains phosphorylated CHK1 by limiting its DDB1-dependent degradation. Human Molecular Genetics. 2011;**20**:2171-2181

[133] Cotto-Rios XM, Jones MJ, Busino L, Pagano M, Huang TT. APC/CCdh1-dependent proteolysis of USP1 regulates the response to UV-mediated DNA damage. The Journal of Cell Biology. 2011;**194**:177-186

[134] Zhi G, et al. Fanconi anemia complementation group FANCD2 protein serine 331 phosphorylation is important for fanconi anemia pathway function and BRCA2 interaction. Cancer Research. 2009;**69**:8775-8783

[135] Guervilly JH, Mace-Aime G, Rosselli F. Loss of CHK1 function impedes DNA damage-induced FANCD2 monoubiquitination but normalizes the abnormal G2 arrest in Fanconi anemia. Human Molecular Genetics. 2008;**17**:679-689

[136] Wang X, et al. Chk1-mediated phosphorylation of FANCE is required for the Fanconi anemia/BRCA pathway. Molecular and Cellular Biology. 2007;**27**:3098-3108

[137] Kim JM, et al. Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. Developmental Cell. 2009;**16**:314-320

[138] Cohn MA, et al. A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. Molecular Cell. 2007;**28**:786-797

[139] Huang TT, et al. Regulation of monoubiquitinated PCNA by DUB autocleavage. Nature Cell Biology. 2006;**8**:339-347

[140] Nijman SM, et al. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Molecular Cell. 2005;**17**:331-339

[141] Macurek L, et al. Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. Nature. 2008;**455**:119-123

[142] Kee Y, Kim JM, D'Andrea AD. Regulated degradation of FANCM in the Fanconi anemia pathway during mitosis. Genes & Development. 2009;**23**:555-560

[143] Watanabe N, et al. Cyclin-dependent kinase (CDK) phosphorylation destabilizes somatic Wee1 via multiple pathways. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:11663-11668

[144] Watanabe N, et al. M-phase kinases induce phospho-dependent ubiquitination of somatic Wee1 by SCFbeta-TrCP. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:4419-4424

[145] Lafranchi L, et al. APC/C(Cdh1) controls CtIP stability during the cell cycle and in response to DNA damage. The EMBO Journal. 2014;**33**:2860-2879

[146] Cho HJ, et al. Degradation of human RAP80 is cell cycle regulated by Cdc20 and Cdh1 ubiquitin ligases. Molecular Cancer Research. 2012;**10**:615-625

[147] Burrows AC, Prokop J, Summers MK. Skp1-Cul1-F-box ubiquitin ligase (SCF (betaTrCP))-mediated destruction of the ubiquitin-specific protease USP37 during G2-phase promotes mitotic entry. The Journal of Biological Chemistry. 2012;**287**:39021-39029

[148] Huang X, et al. Deubiquitinase USP37 is activated by CDK2 to antagonize APC(CDH1) and promote S phase entry. Molecular Cell. 2011;**42**:511-523

[149] Typas D, et al. The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. Nucleic Acids Research. 2015;**43**:6919-6933

[150] Parameswaran B, et al. Damage-induced BRCA1 phosphorylation by Chk2 contributes to the timing of end resection. Cell Cycle. 2015;**14**:437-448

[151] Orthwein A, et al. A mechanism for the suppression of homologous recombination in G1 cells. Nature. 2015;**528**:422-426

[152] Chu WK, et al. FBH1 influences DNA replication fork stability and homologous recombination through ubiquitylation of RAD51. Nature Communications. 2015;**6**:5931

[153] Simandlova J, et al. FBH1 helicase disrupts RAD51 filaments in vitro and modulates homologous recombination in mammalian cells. The Journal of Biological Chemistry. 2013;**288**:34168-34180

[154] Bacquin A, et al. The helicase FBH1 is tightly regulated by PCNA via CRL4(Cdt2)-mediated proteolysis in human cells. Nucleic Acids Research. 2013;**41**:6501-6513

[155] Laulier C, Cheng A, Huang N, Stark JM. Mammalian Fbh1 is important to restore normal mitotic progression following decatenation stress. DNA Repair (Amst). 2010;**9**: 708-717

[156] Lorenz A, Osman F, Folkyte V, Sofueva S, Whitby MC. Fbh1 limits Rad51-dependent recombination at blocked replication forks. Molecular and Cellular Biology. 2009;**29**: 4742-4756

[157] Wang Z, et al. SCF(beta-TRCP) promotes cell growth by targeting PR-Set7/Set8 for degradation. Nature Communications. 2015;**6**:10185

[158] Jorgensen S, et al. SET8 is degraded via PCNA-coupled CRL4(CDT2) ubiquitylation in S phase and after UV irradiation. The Journal of Cell Biology. 2011;**192**:43-54

[159] Wu S, et al. Dynamic regulation of the PR-Set7 histone methyltransferase is required for normal cell cycle progression. Genes & Development. 2010;**24**:2531-2542

[160] Centore RC, et al. CRL4(Cdt2)-mediated destruction of the histone methyltransferase Set8 prevents premature chromatin compaction in S phase. Molecular Cell. 2010;**40**:22-33

[161] Abbas T, et al. CRL4(Cdt2) regulates cell proliferation and histone gene expression by targeting PR-Set7/Set8 for degradation. Molecular Cell. 2010;**40**:9-21

[162] Evans DL, et al. MMSET is dynamically regulated during cell-cycle progression and promotes normal DNA replication. Cell Cycle. 2016;**15**:95-105

[163] Saredi G, et al. H4K20me0 marks post-replicative chromatin and recruits the TONSL-MMS22L DNA repair complex. Nature. 2016;**534**:714-718

[164] Piwko W, et al. The MMS22L-TONSL heterodimer directly promotes RAD51-dependent recombination upon replication stress. The EMBO Journal. 2016;**35**:2584-2601

[165] O'Donnell L, et al. The MMS22L-TONSL complex mediates recovery from replication stress and homologous recombination. Molecular Cell. 2010;**40**:619-631

[166] Duro E, et al. Identification of the MMS22L-TONSL complex that promotes homologous recombination. Molecular Cell. 2010;**40**:632-644

[167] Tomimatsu N, et al. Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice. Nature Communications. 2014;**5**:3561

[168] Hustedt N, Durocher D. The control of DNA repair by the cell cycle. Nature Cell Biology. 2016;**19**:1-9

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