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# [The role of the APC/C-Cdh1 in the response to DNA damage]

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University of Groningen

Marloes Roorda
S2296632

Supervisor: M. van Vugt

## Abstract

The anaphase-promoting complex (APC/C) is a ubiquitin ligase that has a crucial function in the regulation of the cell cycle. The APC/C is active during mitosis and G1 of the cell cycle and ubiquitylates proteins to mark them for degradation, but only when activating proteins Cdc20 or Cdh1 interact with the APC/C. Targets of the APC/C are regulators of the cell cycle, such as Cyclins, Securin and kinases (Aurora A, Plk1). Degradation of these proteins controls processes such as entry of mitosis, the metaphase to anaphase transition and exit from mitosis and is thereby responsible for initiating the transition to the next phase of the cell cycle. Appropriate regulation of the APC/C contributes to the correct sequence of events through the cell cycle.

In response to DNA damage, DNA repair mechanisms will cooperate with cell cycle regulators to halt the cell cycle. This will create more time for reparation of DNA and prevent the cell from initiating premature transition to the next phase while damaged. Cellular responses to DNA damage are regulated by two kinase signalling cascades, the ATR-Chk1 and ATM-Chk2 pathways. This induces a fast-acting but transient response, mediated by kinases, and a slow-acting response that is mediated by a transcription-dependent (p53/p21) pathway. Both pathways result in the inhibition of Cyclin-Cdk complexes and thereby induce cell cycle arrest.

In response to DNA damage in G2, transition to mitosis must be prevented. Remarkably, APC/$C^{Cdh1}$ is activated in this process, although the APC/C only gets activated during mitosis and G2 of the unperturbed cell cycle. Cdc14b activity and p21-dependent inhibition of Emi1 induce this APC/$C^{Cdh1}$ activity. The activation of APC/$C^{Cdh1}$ may have interesting functions in the DNA damage response, such as maintaining a stable G2 arrest, permanent cell cycle exit, promoting DNA repair or induce mitotic catastrophe.


## Content

# 1. Introduction

One of the most important things in living organisms is to maintain genomic integrity, in order to proliferate and produce daughter cells with the same genomic information. A cell reproduces by performing a few successive events in which the DNA is replicated, followed by cell division. This cycle of events is known as the cell cycle. To prevent the formation of aberrant cells, it is important that these events follow each other in the correct order. The principal behind this mechanism is a series of biochemical switches, that can initiate the next event in the cell cycle. The main components in the cell cycle control system are cyclin-dependent kinases (Cdks). Their function is to phosphorylate proteins involved in the regulation of several events in the cell cycle, such as DNA replication, the condensation of chromosomes, breakdown of the nuclear envelope and assembly of the spindles. The activity of Cdks changes over time, and is controlled by proteins called Cyclins. Without the interaction with Cyclins, Cdks have no phosphorylation activity and cannot trigger the initiation of the next cell cycle events. The availability of Cyclins changes over time, in a cycle of synthesis and degradation.[1] Degradation of Cyclins is executed by the proteasome, however this process can only occur when Cyclins are marked with ubiquitin residues for recognition by the proteasome. The process of ubiquitylation is regulated by a protein complex called the anaphase promoting complex (APC/C).[2] The APC/C is activated in late G2 when activator protein Cdc20 is bound to the APC/C. In this phase, APC/$C^{Cdc20}$ is responsible for the metaphase to anaphase transition. After this, activator protein Cdh1 replaces Cdc20 and continues Cyclin degradation. Cdh1 remains active up until G1, where it has a function in the formation of pre-replicative forks.[2]

Despite the accurate regulation of the cell cycle, the cell can obtain errors. The DNA is constantly exposed to various sources of DNA damage. These sources can be exogenous, such as radiation of chemicals. DNA damage can also be induced by endogenous factors, such as replication errors or oxidative stress due to normal metabolism. Cells have an extensive mechanism to react to DNA damage. In response to genomic stress, cells will go in cell cycle arrest to generate more time to repair the damaged DNA. The condition of the DNA is constantly monitored, by a pathway called the DNA damage response (DDR), together with signals from the environment. When DNA damage occurs, this control system can halt the cell cycle in so-called cell cycle checkpoints and start DNA repair. [3]

When DNA damage occurs in G2 phase of the cell cycle, the G2/M checkpoint will prevent the cell from transitioning to mitosis through several signalling cascades. Besides this, the APC/$C^{Cdh1}$ gets activated.[4] This is remarkable, since APC/$C^{Cdh1}$ is suppressed by Cdk activity during this phase of the cell cycle, and is normally only activated during mitosis and early G1 phase. This has lead to the question how it is possible that the APC/$C^{Cdh1}$ can be activated during G2.

In this thesis I will describe the importance of the APC/C ubiquitin ligase in the unperturbed cell cycle and the different repair mechanisms that cells can use in response to DNA damage. Furthermore, I will describe the mechanisms through which activation of the APC/$C^{Cdh1}$ can be realized specifically after DNA damage in G2. Finally, theories about the function of APC/$C^{Cdh1}$ in G2 will be provided to understand how this could contribute to the maintenance of genomic integrity.

## 2. The role of the APC/C-Cdh1 in the unperturbed cell cycle

To maintain a well-functioning organism it is essential for cells to replicate, in order to contribute to organismal development, or to replace damaged cells. The duplication of cells is an important process that needs proper regulation, which is done at various transitions of the cell cycle. Progression through the cell cycle should be monitored to maintain the integrity of the cell and the genome. Therefore, different events in the cell cycle (cell growth, DNA replication and cell division) need to happen in the correct sequence. The key players that drive the cell cycle engine are Cdks (cyclin-dependent kinases) and cyclins, regulatory proteins that interact with Cdks. Each cyclin-Cdk complex targets different substrates and influences different stages of the cell cycle. These cyclin-Cdk complexes can be divided in three major classes: (1) G1 cyclin-Cdk complexes that regulate progression through G1 and initiation of S-phase, (2) S cyclin-Cdk complexes that trigger DNA replication and (3) M cyclin-Cdk complexes that are responsible for mitosis. The activity of each of these complexes is not regulated by the presence of Cdks, as their expression levels are stable during the cell cycle. Rather, the activity of the Cdk-cyclin compexes are controlled by the availability of cyclins. Their expression levels fluctuate extensively because of changes in the extent of transcription or by degradation by proteolysis.[5]

The ubiquitin-proteosome system (UPS) has a major role in the regulation of proteolysis. An important function of the UPS is to degrade proteins of which the abundance needs to change (quickly) over time.[6] The ligation of ubiquitin (Ub) to a target protein is a 'marker' for its degradation by the proteosome. Three subsequent acting enzymes are needed for this process. An activating enzyme (E1) activates the last amino acid of ubiquitin (Gly76) in an ATP-dependent way. The activated ubiquitin is transferred to a Cys-residue of the Ub-conjugating enzyme (E2) and subsequently linked to a Lys-residue of the substrate protein. This last step is catalyzed by a ubiquitin-protein ligase enzyme (E3). When ubiquitinated, the target protein can be recognised by the 26S proteasome and is degraded.[7] The anaphase promoting complex (APC/C) is an E3 ubiquitin ligase that is able to target Cyclins for degradation by proteolysis. Since Cyclins can be seen as the engine driving the cell cycle, the APC/C has an important function in the control of cell cycle progression. Without the APC/C, cells cannot successfully divide in two daughter cells during mitosis, exit from mitosis or initiate a new round of replication in a new cycle.

### 2.1 The molecular organization of the APC/C

The APC/C is a large complex protein, consisting of twelve subunits, that can be divided in four categories: (a) catalytic and substrate recognition domains, (b) scaffolding domains, (c) tetrico-peptide repeat (TPRs) domains and (d) activating domains. Together these subunits have a mass of 1,4-1,5 MDa.[8,9]

The APC/C is listed as a RING-Cullin E3 ligase, because its catalytic and substrate-recognition domain contains an APC11 protein, which has a $Zn^{2+}$-binding RING ('Really Interesting New Gene') domain, as well as a Cullin-like protein APC2.[10,11] The catalytic subunits are essential for ubiquitylation. APC11 is the subunit that has E3 ligase activity and interacts with E2 enzymes, transferring the ubiquitin residues from E2 to a lysine residue of the substrate.[12] APC2 is bound to small subunit called Doc1/APC10, which is important for the interaction with substrates.[13] (Figure 1)

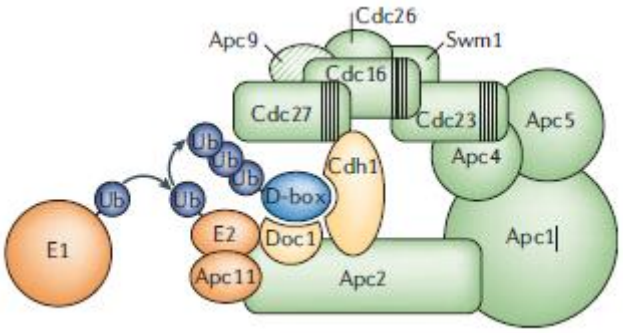


**Figure 1.** Ubiquitin is activated by E1 and transferred to the E2 enzyme. This enzyme interacts with the APC/C subunit Apc11, which has E3 ligase activity. Apc11 is able to ubiquitylate the substrate protein on a lysine residue. Substrates possessing a D-box or KEN-box can be recognized by activator proteins Cdh1 (or Cdc20; not shown). The activator protein interacts with the APC/C by binding to Cdc27 (a TPR protein) and Apc2. Other TPR proteins are Cdc16 and Cdc23. (Image from ref. 22)

The scaffolding domain is the largest part of the APC/C and is composed of Apc1, Apc4 and Apc5. The scaffolding domain connects the catalytic subunits to the TPR-domain proteins (Cdc27, Cdc16 and Cdc23).[14,15] (Figure 1)

Finally, the APC/C can only function when it is bound by activating proteins, which promote the strong binding of substrates and ubiquitylation. Two activating proteins are known: Cdc20 and Cdh1, however, only one activator can be bound to the APC/C at the same time. Both activating proteins are members of the Cdc20 family of tryptophan-aspartate (WD) repeat proteins.[16] These repeating domains can recognize so-called D-boxes and KEN-boxes of the substrates.[17,18] Furthermore, these proteins are characterized by sequence elements as the C-box[19] and the IR-tail.[14] Through these structures the activating proteins can interact with the APC/C complex by binding to the TPR subunit Cdc27 (via the IR-tail) and APC2 (via the C-box).[20, 15]

Cryo-EM provided insight in the structural configuration of the APC/C. The APC/C complex resembles an asymmetric triangular structure with an internal pocket. The activator and cullin-like domains are located at the outside of the complex.[20] Since these proteins are involved in substrate recognition and ubiquitylation, it is thought that ubiquitylation takes place at the outside of the APC/C complex. The complex can be divided in two large domains, called the 'platform' and 'arc lamp', but their positions are flexible to each other. Also when activator Cdh1 links to the APC/C, their relative positions can change, indicating that the binding of Cdh1 could induce conformational changes in the structure of APC/C.[21] (Figure 2)

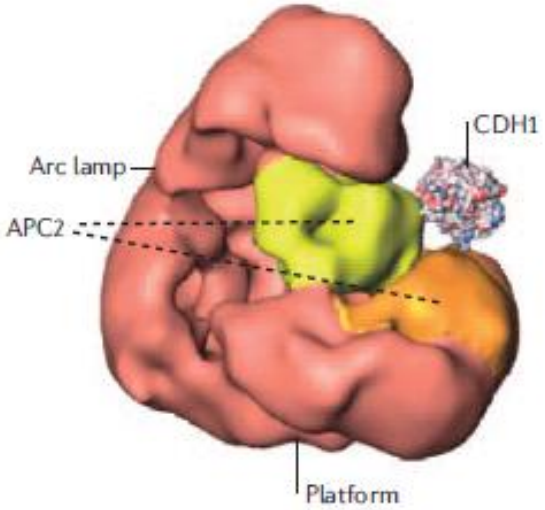


**Figure 2.** A 3D-model of the APC/C reconstructed by using cryo-EM. The protein is divided in two parts, the 'arc lamp' and 'platform' domains that are flexible to each other, so the protein can have different conformations from a more open state to a closed state. Activator protein Cdh1 is located on the outside of the protein. (Image from ref.22)

## 2.2 Targets of activators Cdc20 and Cdh1 and the regulation of degradation by the APC/C$^{Cdh1}$

The function of the APC/C and its substrate specificity is dependent on which activator protein interacts with the APC/C core complex, because they determine which substrates are targeted for degradation. Since these activator proteins have a big influence on cell cycle progression, they have to be regulated carefully.

In short, Cdc20 is active during the first phase of mitosis and is responsible for the degradation of Securin and Cyclins, leading to the metaphase to anaphase transition, whereas Cdh1 degrades Cyclins and many other substrates, resulting in mitotic exit and the initiation of G1 phase.[22]

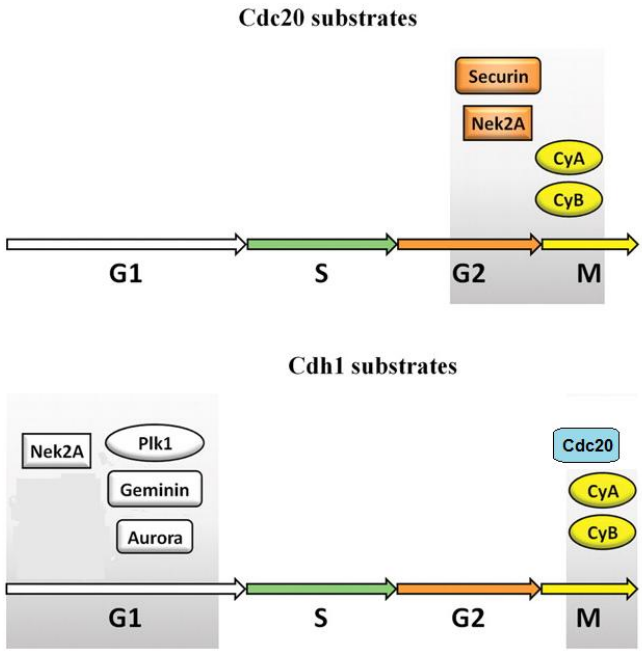


**Figure 3.** Cdc20 is activated during G2 and mitosis and is responsible for the metaphase to anaphase transition by ubiquitylating Nek2A, Securin and Cyclins A and B. The degradation of Cyclins inhibits kinase activity of Cdks, resulting in the activation of Cdh1. Cdh1 activity degrades Cdc20, thereby inactivating it, and continues Cyclin degradation during mitosis. Cdh1 promotes mitotic exit but stays active during G1 to keep kinase activity low and to promote the formation of the pre-replicative complexes. *(Image adapted from http://dx.doi.org/10.1093/cvr/cvp244)*

Transcription and translation of Cdc20 takes place in S and G2 phase, but it can only interact efficiently with the APC/C when subunits of the APC/C are phosphorylated by mitotic kinases such as Cdk1 during mitosis. In contrast, Cdh1 cannot interact with the APC/C when it is phosphorylated and therefore it is not active during this phase.[21,22]

Cdc20 activity starts with the degradation of Cyclin A and Nek2A in prometaphase.[23,24] Cdc20 is also responsible for the metaphase to anaphase transition by promoting the ubiquitylation of Securin, which is an inhibitor of Separase. During this process, Separase is activated and cleaves Cohesin complexes that hold the chromatids together, inducing separation of sister chromatids.[25,26] (Figure 3) However, the metaphase to anaphase transition may only take place when all chromatids are aligned properly and kinetochores are attached to the microtubuli to proceed to anaphase. Therefore, the ubiquitylation of Securin and Cyclin B by APC/C$^{Cdc20}$ needs to be inhibited until this is the case. The

spindle assembly checkpoint (SAC) regulates this process, by inhibiting Cdc20 from its interaction with the APC/C complex. The SAC has substrate specificity, because it inhibits degradation of Securin and Cyclin B, while the degradation of Cyclin A and Nek2A is not affected.[2,27] A few proteins involved in the SAC are present on unattached kinetochores.[27] Mad2 and BubR1 have demonstrated to inhibit ubiquitylation, however the exact mechanism behind this is unknown.[28] BubR1 has shown to interact with Bub3 and Mad2 and forms the mitotic checkpoint complex (MCC). By binding to Cdc20 this complex can inhibit Cdc20 activity.[29]

The inhibition of APC/$C^{Cdc20}$ by the SAC stops not until all sister chromatids are attached properly to the spindle microtubules during metaphase. APC/$C^{Cdc20}$ then starts ubiquitylating Securin and Cyclin B as well as Cyclin A and Nek2A. Cyclin B is an activator of Cdk1 and when absent, Cdk1 will be inactivated through a conformational change that prevents fitting of substrates into the active site.[30,31] Meanwhile, phosphatase activity will dephosphorylate Cdk1 substrates, which initiates mitotic exit. The low Cdk1 activity induced by degradation of Cyclin B also leads to dephosphorylation of Cdh1, which results in its activation in anaphase and telophase up until G1 phase. APC/$C^{Cdh1}$ contributes to the degradation of Cyclins and Securin initiated by Cdc20 but also has other targets, such as kinases Polo-like kinase 1 (Plk1), Aurora A and B and Cdc20[32], which keeps kinase activity low, stimulates mitotic exit and stops APC/$C^{Cdc20}$ activity.

Besides these functions in mitosis, APC/$C^{Cdh1}$ also regulates the duration of G1 and the G1 to S-phase transition. APC/$C^{Cdh1}$ remains active during G1, degrading Cyclins and thereby inactivating Cdks. This low activity of Cdks is essential for the formation of the pre-replicative complexes (pre-RCs). Cdks inhibit the formation of these complexes at the origins of replication. The pre-RCs are required for binding of DNA polymerases in order to replicate DNA in S-phase.[33] DNA replication is also favoured by the ubiquitylation of Geminin by APC/$C^{Cdh1}$. This protein inhibits replication factor CDT1, preventing the assembly of the pre-RC.[34] Thus, only when Geminin and Cdks are (either directly or indirectly) inactivated through ubiquitylation by APC/$C^{Cdh1}$, pre-RCs can form, and a new round of replication can be started. Hence, the APC/$C^{Cdh1}$ regulates mitotic exit and but also has an important function during G1 of the cell cycle, assuring that no new round of replication is possible before the previous cycle has ended correctly.

However, when approaching S-phase, Cyclin levels need to increase, since Cyclin A and E are required for the G1 to S-phase transition. The transcription of these Cyclins is regulated by transcription factor E2F. During early G1, the retinoblastoma (Rb) protein inhibits E2F. Only when Cyclin D-dependent kinases phosphorylate Rb, the brake on transcription of Cyclins A and E is released.[50] Also during S-phase high levels of Cyclins are needed for chromosome duplication and later on to initiate mitosis. Thus, in order to increase Cyclin levels, it is necessary to stop the activity of APC/$C^{Cdh1}$. It was thought the accumulation of Cyclin A is crucial to form Cyclin A-Cdk2 complexes that can inactivate APC/$C^{Cdh1}$.[35] Yet, Cyclin A itself is a target for APC/$C^{Cdh1}$ and therefore it could never accumulate to inhibit APC/$C^{Cdh1}$. Two mechanisms have been suggested that could explain the accumulation of Cyclin A.

The first explanation states that Cyclin A degradation is dependent on the presence of an E2 enzyme called UBCH10. In G1 phase, UBCH10 itself is a target for APC/$C^{Cdh1}$, so degradation of this enzyme would lead to the stabilization and accumulation of Cyclin A. This allows the formation of Cyclin A-Cdk2 complexes, which can inhibit the function of APC/$C^{Cdh1}$.[36] In contrast to this auto-inhibition mediated by UBCH10, another explanation describes EMI1 (early mitotic inhibitor 1) as an inhibitor

of APC/$C^{Cdh1}$. During the G1-S transition the transcription factor E2F promotes the expression of EMI1 and thus supports the accumulation of APC/$C^{Cdh1}$ substrates such as Cyclin A.[37] Yet, the exact mechanism of this process is still quite unknown. In vitro, EMI1 shows to compete with APC/C substrates by binding to N-terminal fragments of the APC/C activators.[38] Nevertheless, this explanation does not fit with our understanding of the interaction between APC/C substrates and APC/C activators, because it is known that substrates bind to the C-terminal WD-repeat domains of Cdh1.[18]

The importance of the APC/C and its regulators Cdc20 and Cdh1 is supported by experiments in which Cdc20 and Cdh1 were depleted using siRNA or knockout cells and mice. Loss of the APC/C causes lethality in all species.[39] The stabilization of many mitotic proteins due to inactivation of the APC/C inhibits cell proliferation or shows cellular aberrations.

The loss of Cdc20 in $Cdc20^{-/-}$ mice has shown to be lethal, because cells remained in metaphase arrest already in the two-cell stage of the embryo.[40] Further experiments in a conditional Cdc20 knockout mouse showed metaphase arrest in proliferative tissues.[41] The importance of Cdc20 in cell proliferation was further shown when these mice were given skin tumors. Compared with control mice, the Cdc20 depletion resulted in the complete inhibition of tumor growth.[41] Resembling knockout Cdc20 mice, Cdh1 knockout mouse models also lead to embryonic lethality.[42] Multiple experiments with Cdh1-depleted cells showed an increase in genomic instability, confirming the importance of a proper regulation of Cdh1. During mitosis and G1 the downregulation of Cdh1 results in the accumulation of many APC/C target proteins such as Cyclins, Aurora A and Plk1. This results in increased genomic instability, marked by abnormal chromosome separation, the formation of micronuclei, centrosome aberrations and anaphase bridges.[43-46] Stabilization of mitotic kinases such as Plk1 and Aurora A due to Cdh1 inactivation showed defects in the formation of anaphase spindles and cytokinesis, which can result in polyploid cells and multipolar mitosis.[45-47] During mitotic exit Cdh1-depleted cells show accumulation of Cyclins A and B and Geminin. This can hinder the formation of pre-replicative complexes during G1.[45,48,49] These cells showed premature replication starting with less origins of replication. This resulted in slower DNA replication and may causes more errors.[42] These experiments illustrate that life without proper function of the APC/C and its activating proteins is unimaginable.

Concluding, the APC/C is of big importance in the regulation of the cell cycle. Activating protein Cdc20 interacts with the APC/C during late G2 and mitosis, and is responsible for the metaphase to anaphase transition by ubiquitylating Cyclins A and B and proteins such as Securin and Nek2A. The Spindle-Assembly Checkpoint (SAC) mediates this process. The other activating protein, Cdh1, is activated during late mitosis and continues the ubiquitylation of Cyclins, resulting in mitotic exit. Another important feature of APC/$C^{Cdh1}$ is mediating the duration of G1. The APC/$C^{Cdh1}$ activity results in a low Cdk availability, which is needed for the formation of pre-replicative complexes. In this way, the APC/$C^{Cdh1}$ assures that no new round of replication is possible before the previous cycle has ended correctly.

# 3. The response of cells to DNA damage

Our genome is prone to various sources of DNA damage, from endogenous sources (such as replication errors or oxidative stress) as well as exogenous sources (chemicals, irradiation). These sources can cause different kinds of DNA damage. For example, ionizing radiation generates mostly double-strand breaks, which means two phoshodiester bonds in the backbone of the DNA helix break close to each other, in both DNA strands.[3]

The cell uses a complex network to repair DNA damage, often referred as the DNA damage response (DDR). This network includes sensors to recognize DNA damage, transducers to amplify the signal and effectors to execute various functions to restore the cell to a healthy condition. The DDR and cell cycle regulators are closely interconnected to influence the cell cycle. In response to DNA damage, the cell will go in (temporary) arrest to create more time for repair mechanisms and to reduce the chance of mutations in daughter cells.[3]

**Signal initiation**

Several signalling pathways are known to halt the cell cycle by inactivating Cdks.[50] ATM and ATR are two key players in this process. Both proteins are large kinases that start phosphorylating various targets in response to DNA damage, that are related to DNA repair, cell cycle arrest or apoptosis.[51] ATM will get activated after breaks in the DNA, whereas ATR responds to practically all kinds of stress (e.g., hypoxia and alkylating agents) that affect the progression of the replication-fork.[3]

ATM (Ataxia telangiectasia mutated) is present in healthy cells as an inactive homodimer. The kinase domain is blocked because it is bound to an internal domain, surrounding the phosphorylation site (serine 1981).[52] The mediator complex MRN (MRE11/RAD50/NBS1) has a function in sensing DSBs and recruits ATM to the damaged DNA.[53] The sensing of a DSB results in a conformational change of the protein, triggering the kinase domain to phosphorylate serine 1981. The homodimer dissociates and is activated and can phosphorylate various substrates.[52]

ATR co-exists with a binding partner ATRIP (ATR-interacting protein) and plays an important role in the response to DNA replication forks arrest. In case of an arrested replication fork, RPA (replication protein A, a single-stranded DNA-binding protein) is recruited to the ssDNA. RPA enhances the binding of ATRIP and its binding partner ATR to the ssDNA.[54] For this process, other proteins such as claspin and a clamp-loading complex RSR must also be recruited to the ssDNA.[55-57] Only when all these proteins are associated with ATR, it is able to phosphorylate substrates such as Chk1 and RAD17. ATR and Chk1 knock-out cells and animals have shown not to be viable[58], which indicates these proteins have an important role in the normal cell cycle, independent of cellular stress and DNA damage.

### Transducing the signal

Once the DNA damage is detected, the signal is amplified and transduced throughout the cell to start a fast response by activating effector kinases. ATM and ATR collaborate with checkpoint mediators and transducer kinases Chk1 and Chk2. Although all these kinases can cross-talk, Chk2 is mostly phosphorylated by ATM and Chk1 by ATR.[59] Chk1 and Chk2 phosphorylate downstream effector proteins that regulate DNA-repair and cell cycle arrest. Yet, the proximal kinases ATM and ATR are also capable of directly activating effector proteins. Depending on the stage of the cell cycle, different effector proteins can be targeted to halt the cell cycle.

### Arresting the cell cycle

Cell cycle checkpoints monitor constantly whether the cell is ready to proceed to the next stage of the cycle. For example, when DNA replication has been unsuccessful or contains errors, inhibitory signals are sent to prevent the cell from going to M-phase.[60] Eukaryotic cells contain three checkpoints that observe the condition of the cell and that can halt the cell cycle in response to DNA damage: the G1/S checkpoint, the intra-S checkpoint and the G2/M-checkpoint. For each transition from one phase of the cell cycle to another, different proteins such as Cyclins are needed. Those proteins are relevant targets for the ATM/ATR mediated Chk1/Chk2 kinases to prevent transition to the next phase of the cell cycle, in order to slow down the cell cycle or even to cause a prolonged cell cycle arrest. All three checkpoint and their relevant targets for ATM/ATR and Chk1/Chk2 kinases will be explained.

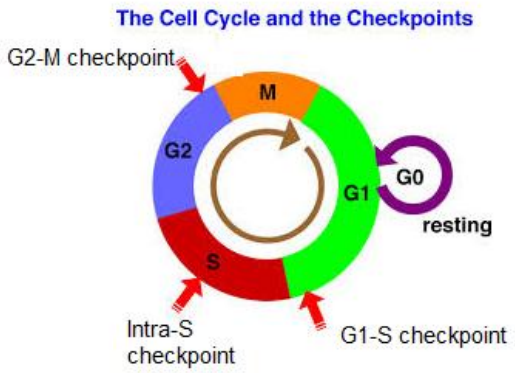


**Figure 4.** The different phases of the cell cycle, and the different checkpoints that monitor the condition of the cell and the DNA. In case of DNA damage, the DNA damage response (DDR) will initiate cell cycle arrest. (Image adapted from http://eishinoguchi.com/checkpoint.htm )

### G1/S checkpoint

The G1/S checkpoint or restriction point monitors whether the environment is supportive for the cell to proliferate, e.g., whether the cell receives enough growth factors and signals to commit to a new cell cycle. After the restriction point, the cell is committed to complete the cell cycle.[61,62] The retinoblastoma protein (Rb) is involved in this process by inactivating transcription factor E2F, that regulates the expression of Cyclins A en E. Since these Cyclins are responsible for the G1 to S phase transition, this pathway can be seen as a brake on the cell cycle. When the environment is

favourable, CyclinD-dependent kinases will inhibit Rb, which will trigger transcription of Cyclins. The CyclinE-Cdk2 complex can in its turn phosphorylate Rb to inactivate it, creating a positive feedback loop.[60,62] (Figure 5 )

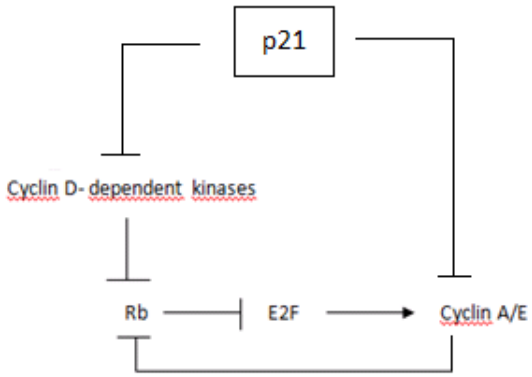


**Figure 5** The transcription of Cyclins A and E is under the control of transcriptionfactor E2F. In early G2, Rb operates as a break on transcription of Cyclins. Accumulation of Cyclin D-dependent kinases phosphorylates Rb, releasing E2F to start transcription of Cyclins A and E, that are crucial for the G1/S-transition. In response to DNA damage, p21 activation will inhibit these processes and thereby induce a cell cycle arrest.

When DNA damage occurs, the cell needs to arrest to prevent the cell from replicating its DNA and subsequent mitosis. Therefore, activated Cyclin-Cdk complexes are relevant targets in this process. To activate these complexes, phosphatase Cdc25a is required to remove a phosphate group from the complex. In response to DNA damage, the ATR-Chk1 pathway will phosphorylate Cdc25a, leading to its degradation. This results in the inhibition of the CyclinE-Cdk2 complex.[63]

Next to this, the cell has another mechanism to respond to DNA damage, which is the ATM/ATR – Chk2/Chk1–MDM2/p53–p21 pathway. This has a long-term inhibitory effect on the cell cycle and can even cause permanent cell arrest.[51,59] The ubiquitin ligase MDM2 is a target of ATM/ATR, thereby inactivating it, although Chk2 and Chk1 are also capable of doing this.[64] Since this ligase is responsible for the degradation of p53, phosphorylation of MDM2 will ensure accumulation of the p53 protein. Stabilization of p53 is also accomplished via another pathway, as ATM and ATR can phosphorylate p53 directly or indirectly via Chk1 and Chk2, only phosphorylating different amino acids of p53.[59,65,66] P53 is a transcription factor that targets genes involved in DNA repair, but can also influence the cell cycle by targeting p21CIP1/WAF1.[51] This is an inhibitor of all Cdks (CKI; cyclin-dependent kinase inhibitor), however in G1 it targets specifically the CyclinE-Cdk2 kinase complex, resulting in cell cycle arrest in this phase of the cell cycle. (Figure 5)

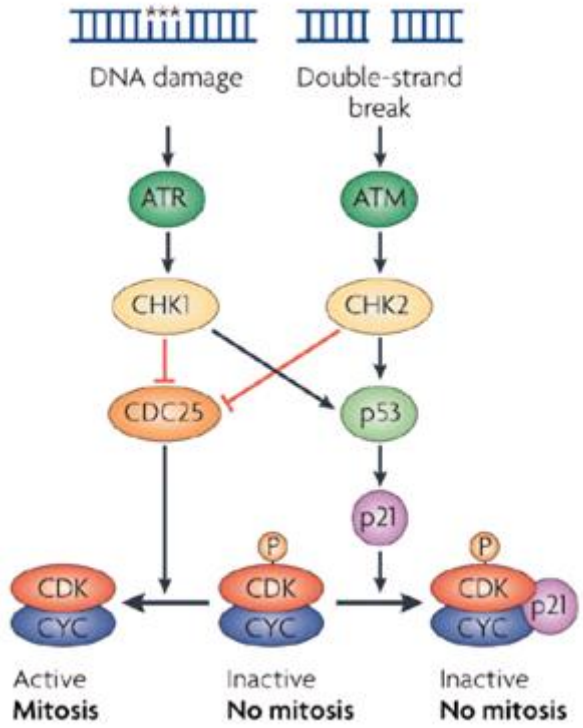


**Figure 5.** DNA damage or double-strand breaks activate kinases ATR and ATM, that can in their turn activate transducer kinases Chk1 and Chk2. One of their functions is to inhibit phosphatase Cdc25. This contributes to cell cycle arrest since the Cyclin-Cdk complex stays in its inactive state. This kinase-driven part of the DDR is acting fast but transiently. To maintain a cell cycle arrest, the p53 pathway is activated. P53 induces activity of Cyclin-dependent kinase inhibitor (CKI) p21. This p53-p21 pathway takes longer to initiate due to transcription, but is stable for a longer period. (Image adapted from De Veylder *et al*., (2007) The ins and outs of the plant cell cycle, Nature Reviews Molecular Cell Biology 8, 655-665)

The process of Cdc25a degradation is a fast-acting mechanism, only taking minutes to respond, acting through post-translational modifications such as phosphorylations. The downside is that this process can only provide the cell to slow down transiently, due to phosphatase activity that counteracts kinase activity. In order to maintain a long-lasting cell cycle arrest, the p53 pathway needs to be activated to suppress Cyclin-Cdk complexes for a longer time. The activation and accumulation of p53 requires transcription and translation: processes that take several hours.[3]

**Intra-S checkpoint**

In S-phase the DNA must be duplicated accurately. The formation of pre-replicative complexes are essential to start this process. In case of DNA damage, the cell cycle must be arrested and further DNA replication must be inhibited. Several mechanisms have been demonstrated to achieve these goals.

The ATM/ATR-Chk1/Chk2-Cdc25a pathway, resulting in the inhibition of Cdks, is a feature of the S-checkpoint, resembling the mechanism in G1 arrest. An additional function of inhibiting Cdk2 is to prevent the loading of Cdc45 onto the chromatin. This protein is needed to mobilize DNA polymerase to the pre-replication complexes and thus for the initiation of DNA replication.[63,67]

A crucial function of the DDR is to inhibit DNA replication and new origin firing during repair, to prevent more genomic instability. Cdt1 plays an important role in this process and is involved in the assembly of the pre-replication complex (pre-RC) as well, by loading helicase MCM2-7 on the origins of replication.[68] In response to DNA damage, Cdt1 is targeted for degradation by an SCF-like ubiquitin ligase, independent on the activation of ATM/ATR.

**G2/M checkpoint**

The G2/M checkpoint prevents the cell from initiating mitosis in case of DNA damage in G2. The main target of this checkpoint is the CyclinB-Cdk1 complex, because this complex is essential for the initiation of mitosis. This complex is also known as M-phase promoting factor (MPF).

When the DNA is damaged or contains errors, the MPF complex is phosphorylated by Wee1 and is thereby inactivated. In this state, the complex is called pre-MPF.[1] To initiate progression into mitosis, phosphatase Cdc25c is needed to remove the inhibiting phosphor group. Cdc25c is therefore a relevant target for Chk1 and Chk2 (mediated by ATM/ATR) after DNA damage. This will result in either degradation, catalytic inhibition or sequestration of Cdc25c through binding to 14-3-3, and its subsequent relocation to the cytosol. Combined, these mechanisms assure that the pre-MPF remains inactive, since it is located in the nucleus.[70-72] The initiating response involving Cdc25c is maintained by regulation trough the p53-p21 pathway, resembling the process during the G1/S checkpoint as described before.

In summary, the cell cycle possesses three checkpoints that monitor the integrity of the DNA and assess whether the cell is prepared to go to the next phase of the cell cycle. In response to DNA damage, the cell uses different repair mechanisms defined as the DNA damage response (DDR). The DDR closely cooperates with cell cycle regulators to arrest the cell cycle in order to create more time for DNA repair. DNA damage is sensed by two large kinases ATM and ATR that initiate the signal. Transducer kinases Chk1 and Chk2 phosphorylate effector proteins. The most important pathways in the DDR are the kinase-driven pathway and the p53/p21-dependent pathway. Kinase activity inactivates phosphatase Cdc25, which keeps Cyclin-Cdk complexes inactivated. Kinase activity is initiated rapidly, but its effect is only temporary. To maintain a proper cell cycle arrest, subsequent activation of the p53 pathway is required. This process takes hours to start up, since p53 and p21 proteins must be synthesized.

# 4. The role of the APC/C$^{Cdh1}$ in the response to DNA damage

## 4.1 The activation of APC/C$^{Cdh1}$ after DNA damage in G2 of the cell cycle

The APC/C is known to be activated during mitosis and early G1 phase. However, experiments have shown that, in response to DNA damage, the APC/C$^{Cdh1}$ is activated in G2 as well. This seems impossible, since Cdk activity during G2 inhibits Cdh1. This indicates another mechanism exists that can activate the APC/C$^{Cdh1}$ specifically in response to DNA damage in G2.

DNA damage checkpoints preserve genomic integrity. As a response to damage, ATR can phosphorylate effector kinase Chk1, a process mediated by Claspin.[73] Under normal conditions, Claspin is degraded via the SCFβ$^{Trcp}$ ubiquitin ligase after phosphorylation by Plk1.[74-76] However, when DNA damage occurs in G2, the ubiquitylation of Claspin by SCF$^{βTrcp}$ must be inhibited in order to activate Chk1. Claspin deubiquitylation by Usp28 remains active, but Claspin protein levels are rather stable, suggesting this process is counteracted by ubiquitylation of Claspin by another ligase, targeting it for degradation after DNA damage. The APC/C$^{Cdh1}$ complex has been identified as the ligase that targets Claspin in G2 specifically after DNA damage.[4] The reactivation of APC/C$^{Cdh1}$ specifically in G2 has also been observed earlier in vertebrate cells.[48] Nevertheless, in normally functioning cells APC/C$^{Cdh1}$ is inactivated in G2 due to Cdk activity during S and G2 phase.

The observed reactivation of APC/C$^{Cdh1}$ happened very rapidly after DNA damage (starting at 90 minutes), indicating this process could not be regulated by a slow-acting response such as p53 activity. To activate APC/C$^{Cdh1}$ as happens in late mitosis in the normal cell cycle, Cdh1 must be dephosphorylated.[35,77] Thus, a phosphatase has to be involved in the process of reactivation of APC/C$^{Cdh1}$ after DNA damage in G2. Basserman *et al.* identified Cdc14B as the phosphatase that translocates from the nucleolus to the nucleoplasm in response to DNA damage and interacts with Cdh1 to activate APC/C$^{Cdh1}$, leading to the degradation of Claspin and other APC/C targets in G2.[4]

However, another mechanism has shown to contribute to the activation of APC/C$^{Cdh1}$ in G2 after DNA damage. As in a normal response to DNA damage, the cell will arrest transiently by inhibition of Cdc25 through the ATM/ATR-Chk1/Chk2 pathway. However, to maintain this arrest p53 and p21 need to be activated to suppress Cdk activity.[63] Next to the suppression of Cdks, p21 has showed to have another function to delay entry into mitosis: it downregulates Emi1, an inhibitor of APC/C$^{Cdh1}$.[78,79] This process activates APC/C$^{Cdh1}$, which will ubiquitylate essential mitotic proteins, making entry into mitosis impossible. In contrast to the fast-acting Cdc14B activity reported by Basserman *et al.*, Wiebusch and Hagemeier support this slower acting response, mediated by p53 and p21. (Figure 6)

### 4.2 The function of APC/C$^{Cdh1}$ in the DNA damage response

Several experiments have shown DNA damage during G2 can induce the activation of APC/C$^{Cdh1}$ by dephosphorylation of Cdh1 by Cdc14B and via the p53/p21-mediated pathway. What is the exact function of the APC/C$^{Cdh1}$ and why is it needed to be active in this phase, when a well-functioning G2/M cell cycle checkpoint exists? A few possible theories have been proposed to answer this question.

**Maintaining a stable G2 arrest**

The study of Basserman *et al.* showed that once activated by Cdc14b, APC/C$^{Cdh1}$ does not target its normal substrates. Of the 15 different observed substrates of Cdh1, only Plk1 was targeted for degradation by APC/C$^{Cdh1}$ after DNA damage.[4] The degradation of Plk1 by APC/C$^{Cdh1}$ is favourable for sustaining Claspin levels, since Plk1 promotes Claspin degradation. ATR needs interaction with Claspin to activate Chk1, resulting in the inactivation of Cdk1. Additionally, Plk1 has an inhibitory effect on Wee1, which is in its turn an Cdk1 inhibitor. Therefore, degradation of Plk1 has a dual function in the inactivation of Cdk1 and is essential to maintain the G2 checkpoint and prevents the cell from initiating mitosis.[4] (Figure 6)

According to this study no other targets of APC/C$^{Cdh1}$ such as Geminin and Cyclins are degraded in cells with DNA damage except for Plk1. It is suggested two pools of APC/C$^{Cdh1}$ are present in G2. The first pool is normally inactive due to phosphorylation, however it can be activated after DNA damage by phosphatase Cdc14b. This results in the degradation of Plk1 and Claspin, where specific deubiquitylation of Claspin by Usp28 can normalize its expression level. The second pool that targets Geminin and Cyclins is inactive during G2 and remains inactive after DNA damage is induced, likely by inhibition of Emi1.[4]

In contrast to studies of Sudo *et al.* and Basserman *et al.*, the study of Lee *et al.* reports that activation of APC/C$^{Cdh1}$ by inhibition of Emi1 is dependent on p21 and leads to degradation of different substrates such as Cyclin A2 and B1. Also Wiebusch and Hagemeier *et al.* attribute the activation of APC/C$^{Cdh1}$ to a p53/p21-dependent process, resulting in down-regulation of multiple APC/C targets. By degrading these targets, especially Cyclin B, the activation of Cdk1 is inhibited, preventing the cell from its transition to mitosis. (Figure 6)

Since the slower-acting p21 has a more robust and prolonged effect on the activation of the APC/C$^{Cdh1}$, this could contribute to a better G2 checkpoint. It was demonstrated that Cdh1-depleted cells fail to remain in G2 arrest and could initiate mitosis. Down-regulation of Emi1 inhibits the progression of the cell cycle, but this inhibition was not seen when Cdh1 was down-regulated as well.[78,79] This shows that the activation of APC/C$^{Cdh1}$ by down-regulating Emi1 is required to induce a prolonged G2 cell cycle arrest and that this process is p21-dependent.

However different mechanisms have been presented, both lead to the inhibition of Cdk1, resulting in a better G2 arrest.

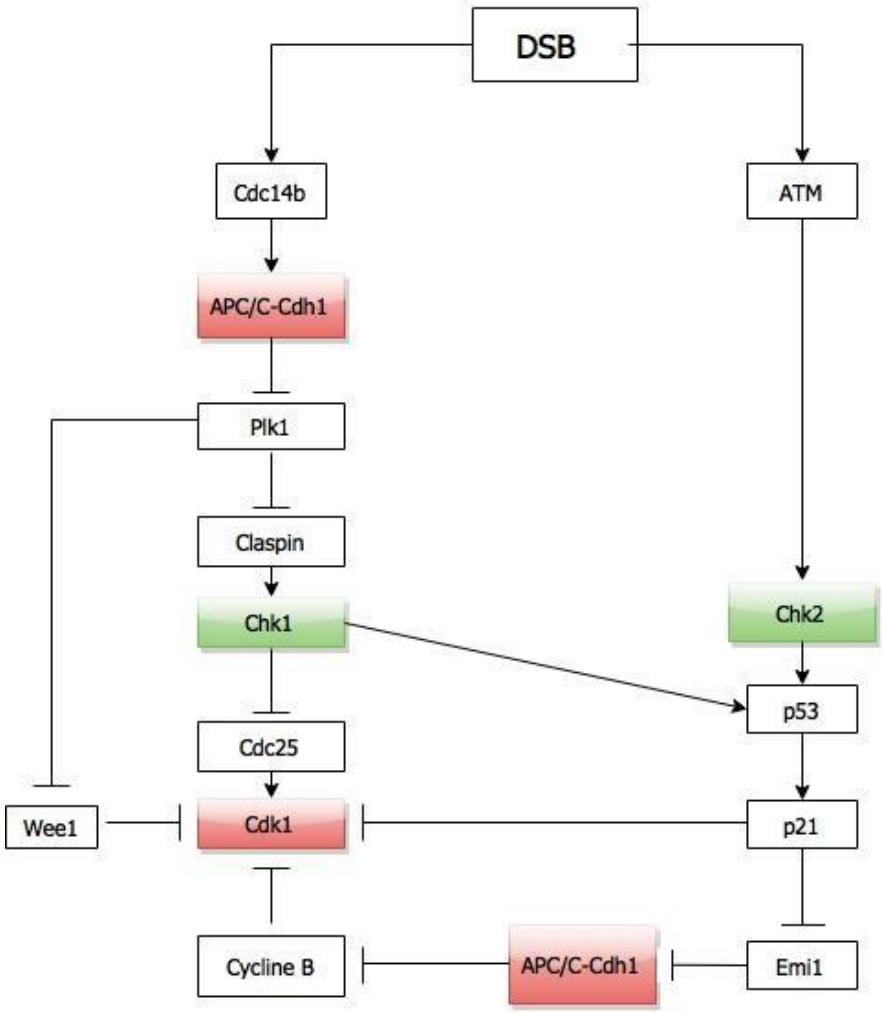


**Figure 6** DNA damage during G2 activates the APC/$C^{Cdh1}$ in two ways. Both pathways play a role in the inhibition of Cdk1, which is required for the maintenance of a stable G2 arrest. DNA damage can induce the ATM-Chk2-p53/p21 pathway resembling the normal response to errors in the DNA. Wiebusch *et al.* report a new mechanism that contributes the activation of APC/$C^{Cdh1}$ to an additional function of p21. P21 inhibits Emi1, which is an inhibitor of APC/$C^{Cdh1}$. Basserman *et al.* present a different model in which Cdc14b activates the APC/$C^{Cdh1}$ in G2, resulting in the degradation of Plk1, which leads to in the inhibition of Cdk1 via Chk1 activation. Plk1 also has a more direct function in inhibiting Cdk1 via Wee1.

### Exit from the cell cycle

DNA damage can lead to a transient cell cycle arrest, however permanent exit from the cell cycle can also be induced. By activating p53, levels of mitotic Cyclins decrease and are not sufficient to recover from G2 arrest. This could be the initiation of permanent withdrawal from the cell-cycle.[80]

The decision to withdraw from the cell cycle is rapidly provided, which will eventually lead to senescence. The induction of p53 and p21 is known to be required for the long-lasting G2 arrest in response to DNA damage[81] and also induces APC/$C^{Cdh1}$ activation.[78,79] APC/$C^{Cdh1}$ activity could play a role in the degradation of Cyclin B1, which could provoke the cell to a permanent cell cycle exit since nuclear translocation of Cyclin B1 precedes permanent cell cycle withdrawal.[82] However, the degradation of Cyclin B1 starts not until six hours after DNA damage, when the ability to return to G2 arrest is already lost. This implies that the induction of a senescent state is not dependent on the APC/$C^{Cdh1}$-mediated degradation of Cyclin B1. Rather, the p21-dependent nuclear translocation of

Cyclin B1 is the decisive step in the permanent exit of DNA damaged cells in G2. Yet, the APC/$C^{Cdh1}$-mediated degradation of Cyclin B1 can operate to enhance the withdrawal from the cell cycle. [82]

Activation of the DDR inhibits the CyclinB/Cdk1 complex via the p53-dependent pathway, removing the inhibitory effect on the APC/$C^{Cdh1}$. APC/$C^{Cdh1}$ activity results in degradation of Cyclin B1, which stops the negative feedback loop. This could trigger the transition from a cell capable of recovery to a cell in permanent cell cycle arrest.[82]

**Mitotic catastrophe**

When APC/$C^{Cdh1}$ is fully activated during G2 in response to DNA damage, it degrades mitotic proteins such as Cyclin B, which keeps the cell well protected against a premature initiation of mitosis and the cell remains in G2 arrest or can even leave the cell cycle permanently. However, it could occur that APC/$C^{Cdh1}$ is only activated partially. In that case, Cyclin B can accumulate and mitosis can be initiated, despite the DNA damage that the cell obtained. To prevent the cell from further cycling, a mechanism is known as 'mitotic catastrophe' to rescue the cell from genomic instability.

Mitotic catastrophe (MC) implies the failure to complete mitosis successfully after DNA damage. This process is caused by premature induction of mitosis, before earlier S or G2 phases have been completed.[83,84] This results in the formation of unviable cells with morphological abnormalities. The formation of nuclear envelopes around missegregated or fragmented chromosomes results in cells with multiple micronuclei. DNA damage can also induce failure of cytokinesis, which leads to multinucleated cells. [83,85] Mitotic catastrophe should not be seen as a separate form of cell death, but rather as a process that goes ahead of apoptosis or necrosis. [85,86]

A defect G2/M checkpoint can stimulate mitotic catastrophe. Several studies showed that the suppression of G2 checkpoint genes such as ATM/ATR, Chk1/Chk2, polo-like kinases and p53 can promote mitotic catastrophe.[81,83,87,88] (Premature) progression from G2 to mitosis is induced by CyclinB-Cdk1 complexes and it is assumed that the translocation of CyclinB-Cdk1 complexes to the nucleus can cause premature chromatin condensation (PCC) and subsequent cell death. In multiple experiments of MC cells an increased amount of Cyclin B has been found in the nucleus.[89,90,]

The inhibition or merely partial activation of the APC/$C^{Cdh1}$ can contribute to the accumulation of Cyclin B and thereby activates CyclinB-Cdk1 complexes. When a sufficient amount of CyclinB-Cdk1 complexes is generated, the premature transition to mitosis will be initiated, accompanied with several mitotic deficiencies as was shown in studies over-expressing APC/C inhibitor Emi1 or in cells that failed to degrade Emi1.[92] However, APC/$C^{Cdh1}$ activity is also responsible for the degradation of other mitotic regulators. The breakdown of proteins involved in the assembly of mitotic spindles and centrosome separation (e.g. Plk1 and AuroraA) results in abnormal chromosome segregation once the cell has been committed to mitosis.[83] To support this theory, characteristics such as centrosome aberrations, abnormal chromosome separation and the formation of micronuclei[43-46] that are comparable to characteristics of MC, are seen in Cdh1-depleted cells, resulting in polyploidy.[47]

During mitotic catastrophe, additional double-strand breaks are generated during anaphase, resulting in an increase of γH2AX foci. This increase of γH2AX is independent from the initial DNA damage the cell obtained before entering mitosis.[85] However these cells were unable to maintain a

G2/M arrest, they still showed an attempt to repair the double-strand breaks in the DNA. In MC cells, the chromatin is highly condensed, since γH2AX has a role in mitotic chromatin condensation[93,94], making accurate HR or NHEJ impossible. This will result in additional DNA damage by creating breakage-fusion-bridge (BFB) chromosome cycles. When DNA repair seems to fail, an ATM/p53-dependent apoptotic signal will be generated.[95,96] This mechanism eliminates MC cells after abnormal mitosis. The fact that Cdh1-depleted cells show more γH2AX foci[97], reinforces the concept that partial activation of APC/$C^{Cdh1}$ contributes to the γH2AX-ATM-p53 pathway that leads to apoptosis following mitotic catastrophe.[85]

Concluding; instead of maintaining a proper G2 arrest, the APC/$C^{Cdh1}$ can also contribute to a planned catastrophe. Partial activation of APC/$C^{Cdh1}$ will not be sufficient for complete degradation of Cyclin B, leading to the accumulation of CyclinB-Cdk1 complexes. This defect in the G2/M arrest will trigger the G2 to mitosis transition. However, by degrading other mitotic regulators such as Plk1 and Aurora A, the cell will be incapable to correctly assemble mitotic spindles and the proper duplication of centrosomes. This will lead to mitotic catastrophe, characterised by the formation of aberrant cells, followed by apoptosis or necrosis to eliminate cells after abnormal mitosis. This mechanism could act as a back-up or additional mechanism to avoid genomic integrity.

**DNA repair**

Besides the roles in arresting the cell cycle or induce mitotic catastrophe, APC/$C^{Cdh1}$ activity could execute a different function after DNA damage is induced. The APC/$C^{Cdh1}$ is thought to enhance the repair of DNA during the DNA damage response (DDR). The APC/$C^{Cdh1}$ is known to be involved in the DDR in G2 and might have a role in the maintenance of a robust G2 cell cycle arrest, as stated before. Since activation of APC/$C^{Cdh1}$ depends on Cdc14B, further experiments were done in Cdc14B-depleted cells. These cells showed a defective DSB repair mechanism, even if G2 arrest was possible.[98] Also $Cdh1^{-/-}$ cells and mouse models showed an increase of DNA damage and chromosomal abnormalities. The Cdh1-depleted cells were also found to be more sensitive to agents that induce a DSB in the genome.[42,99] These findings could indicate a role of APC/$C^{Cdh1}$ in the DNA repair mechanism.

When DSBs are found in the DNA, two possible mechanisms can repair this. HR is based on the use of sister chromatids as a template for repair, and can therefore only be executed when the DNA is replicated, which is after cells entered the S-phase.[100] During G0 and G1 of the cell cycle, the DNA repair relies on non-homologous end joining (NHEJ), which makes the DNA more prone to errors since the ends of the DNA are joined without the homologous sequence.[101] The choice to repair DNA by HR or NHEJ is thus dependent on the phase of the cell cycle. Many proteins that are essential for HR are cell cycle dependent, being higher in expression in S and G2 than in G1.[102] Also Cdks are involved in this process, by phosphorylating key players in the HR repair mechanism.

CtIP is a protein involved in homologous repair (HR) of DSBs, performing a function in DNA-end resection, where strands of ssDNA are generated at the site of the DSB.[103,104] Whether a cell repairs DSBs using HR depends on the possibility to execute DNA-end resection, which is a process that is regulated carefully during the cell cycle.[105] NHEJ is a mechanism that is only executed when cells are not able to accomplish DNA-end resection and is not suitable to repair DNA that shows extensive resection.[106] Recently, CtIP has been confirmed as a APC/$C^{Cdh1}$ substrate, and interacts with Cdh1

through one of its KEN-box motifs during G1 but also in G2 up to mitosis after DNA damage is induced.[107] Hence, the APC/$C^{Cdh1}$ regulates CtIP availability and with that, DNA repair.

In cells containing CtIP with a mutated KEN-box, repair by HR could not be achieved, suggesting that CtIP needs interaction with Cdh1 to induce HR.[107] Even though the processes of HR and NHEJ exclude each other, this mutant does not show an increase in NHEJ. This indicates that NHEJ could not be accomplished, because resection had already occurred in these cells. Based on these findings, a model is generated that shows a function for APC/$C^{Cdh1}$ in the degradation of CtIP. Extensive DNA damage induces HR, which depends on DNA-end resection and thus the presence of CtIP, since phosphorylation of CtIP is needed to initiate DNA-end resection. After this has occurred, NHEJ can no longer be a mechanism to repair the DNA. At this stage, APC/$C^{Cdh1}$ could be required to negatively influence CtIP availability. This results in a limitation of end-resection to assure CtIP activity will prevent an excessive amount of ssDNA, within the limits of HR repair.[107]

## Discussion

To maintain healthy cell proliferation, accurate regulation of the cell cycle is needed. The engine driving the cell cycle is formed by Cyclin-Cdk complexes that can initiate the transition to the next phase of the cell cycle. The abundance of Cyclins fluctuates over time, by transcription as well as degradation by the proteasome. To successfully degrade proteins, ubiquitylation is needed for recognition by the proteosome. For the process of ubiquitylation three important enzymes are needed, of which the E3 ligase marks the substrate protein for its degradation. The anaphase-promoting complex (APC/C) is a highly conserved E3 ubiquitin ligase that targets proteins involved in the cell cycle.

The APC/C needs the interaction of an activator protein to fulfil its duty. These activators are Cdc20 and Cdh1 and are members of the WD-repeat protein family. With this repeat domain, the activating protein can recognize the KEN-box of its substrates. Only one activator protein can interact with the APC/C at one time. Both activator proteins have different substrate specificity and are activated in different phases of the cell cycle. Cdc20 is active during the first phase of mitosis and is responsible for the degradation of Securin and Cyclins, leading to the metaphase to anaphase transition, whereas Cdh1 degrades Cyclins and a few other substrates, resulting in mitotic exit and the initiation of G1 phase. The APC/C is thus crucial for normal cell proliferation.

The APC/C is known to be activated only during mitosis and early G1 phase. However, in response to DNA damage in G2, cells show APC/$C^{Cdh1}$ activity as well. This seems to contradict the fact that during G2, Cdk activity is high and phosphorylates Cdh1, thereby inactivating it. Other mechanisms have been revealed to explain the activation of the APC/$C^{Cdh1}$ in response to DNA damage.

The study of Basserman showed that APC/$C^{Cdh1}$ is activated through the Cdc14b-Cdh1-Plk1 pathway. Basserman observed a rapid activation of the APC/$C^{Cdh1}$ after DNA damage, indicating a fast-acting mechanism. Resembling the activation of Cdh1 during the normal cell cycle, phosphatase activity is needed to dephosphorylate the inactive Cdh1. Cdc14b was identified as the phosphatase that is involved in this process. In contrast to this study, Lee *et al.*, and Wiebusch *et al.*, found another mechanism explaining the activation of APC/$C^{Cdh1}$. As in a normal response to DNA damage, cell cycle arrest is initiated by the inhibition of Cdc25 through the ATR/ATM-Chk1/Chk2 pathway. Yet, to maintain this arrest, the slower acting p53 and p21 are induced to suppress Cdk activity. Additionally, p21 was found to execute another function, which is the downregulation of Emi1, an APC/$C^{Cdh1}$ inhibitor.

The fast-acting Cdc14b response was not seen by studies of Lee *et al.* and Wiebusch *et al.* A different treatment for the synchronization of cells (before the DNA damage was induced) could have evoked a fast-acting DNA damage-like response[108] resulting in the rapid APC/$C^{Cdh1}$ activation in HeLa and U2OS cells in the experiments of Sudo and Basserman respectively. Furthermore, differences were seen in the substrates of the APC/$C^{Cdh1}$. Basserman *et al.* reported only the ubiquitylation of Plk1 and therefore suggested that two pools of APC/$C^{Cdh1}$ exist. One pool only targets Plk1 and can be activated in response to DNA damage, and a second pool that targets many substrates, but is inactive during G2 even in the presence of DNA damage. However, Lee and Wiebuch observed the degradation of many types of Cyclins.

An explanation for these different observations in the degradation of APC/$C^{Cdh1}$ targets might be due to cell type-specific responses. In U2OS and HeLa cells APC/C activity might be reduced since most APC/C substrates resisted ubiquitylation and thus degradation. This could be due to the lack of other APC/C subunits, the presence of deubiquitylating proteins[109] or a lower interaction with the proteasome[110] could explain the reduced degradation by the APC/C.

The question remains why APC/$C^{Cdh1}$ is activated in G2, because a well-functioning G2/M checkpoint would prevent cells from initiating mitosis without the intervention of APC/$C^{Cdh1}$. A few theories have suggested how the activation of APC/$C^{Cdh1}$ could be relevant.

The activation of APC/$C^{Cdh1}$ could have a function in maintaining a better G2/M checkpoint. However studies of Basserman et al. and studies of Lee and Wiebusch et al. present different mechanisms of activating APC/$C^{Cdh1}$, both hypothesize the function of this activation is to maintain a better G2 arrest. In the normal cell cycle, the DNA damage response will activate phosphatase Cdc25 and p53/p21, that inhibit Cdks. The activation of APC/$C^{Cdh1}$ could enhance the inhibition of Cdks by degrading Cycling B, thereby preventing the cell from initiating mitosis.

Krenning et al. hypothesised that the activation of APC/$C^{Cdh1}$, and with that the degradation of Cyclin B, could provoke the cell to a permanent exit from the cell cycle. The decision to withdraw from the cell cycle is rapidly provided and the cell loses its ability to return to G2 arrest within a few hours. However, experiments showed that the degradation of Cyclin B started not until six hours after the induction of DNA damage. This implies that the APC/$C^{Cdh1}$-mediated degradation of Cyclin B1 is not the decisive step in the permanent exit for the cell cycle, but rather the p21-dependent nuclear transition of Cyclin B1. Therefore, the permanent withdrawal from the cell cycle seems not to be the main goal of activation of APC/$C^{Cdh1}$, but it can enhance degradation of Cyclin B which can contribute to the permanent cell cycle exit.

When DNA damage is too extensive, DNA repair may not be helpful anymore. Instead of realizing a better G2/M arrest or permanent withdrawal from the cell cycle, cell death may be a better option to eliminate aberrant cells permanently. This process, mitotic catastrophe, occurs when cells fail to complete mitosis properly. The APC/$C^{Cdh1}$ contributes to this process when it is not fully activated. This allows accumulation of Cyclin B on the one hand, realising the G2 to mitosis transition. On the other hand, the degradation of other mitotic regulators such as Plk1 and Aurora A is detrimental for the correct cytokinesis, since the assembly of spindles, centrosome duplication and chromosome segregation are highly dependent on these proteins. Yet, it can be discussed how the activation of APC/$C^{Cdh1}$ must be regulated, since it cannot be activated entirely: this would lead to the degradation instead of accumulation of Cyclin B, which could not induce mitotic catastrophe.

Besides the proposed theories about the role of the APC/$C^{Cdh1}$ in cell cycle arrest, withdrawal or mitotic catastrophe, the APC/$C^{Cdh1}$ could play a role in the DNA repair. It is known that cell cycle arrest is strongly interconnected with DNA repair and that Cdh1-depleted cells show significantly more DNA damage and chromosomal instability. Lafranchi *et al*. reported a role for APC/$C^{Cdh1}$ in the degradation of CtIP, which is a protein involved in the DNA-end resection, that is essential for homologous repair. In response to DNA damage, CtIP has initially a role in the DNA-end resection. However, excessive amounts of ssDNA are not desirable in the process of HR repair. Therefore, the APC/$C^{Cdh1}$ could interfere by ubiquitylating CtIP, leading to its degradation.

Much experiments were done to clarify the role of the APC/C in response to DNA damage. In most of these experiments cells were irradiated with doses of 10 Gy, which is an excessive amount compared with the amount of irradiation that cells would encounter in a normal environment. It is remarkable that cells are equipped with a mechanism to handle such large amounts of DNA damage. This could be reported to the fact that an accurate DNA damage response is absolutely crucial and may be the most important mechanism for healthy cell proliferation and maintaining genomic integrity. The DNA damage response is known to be highly conserved in eukaryotes from yeast to human[111], for example the checkpoint kinases that are present in essentially all studied eukaryotes.[112] Cells might have adapted to high amounts of DNA damage in earlier times when exposed to higher amount of irradiation. The DNA damage response now consists of hundreds of genes and is regulated very carefully by enzymatic activity, post-translational modifications and interactions between proteins. The activation of the APC/C after DNA damage in G2, additionally to the normal G2/M checkpoint, could be seen as a backup system or last effort to save the cell from genomic instability.

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