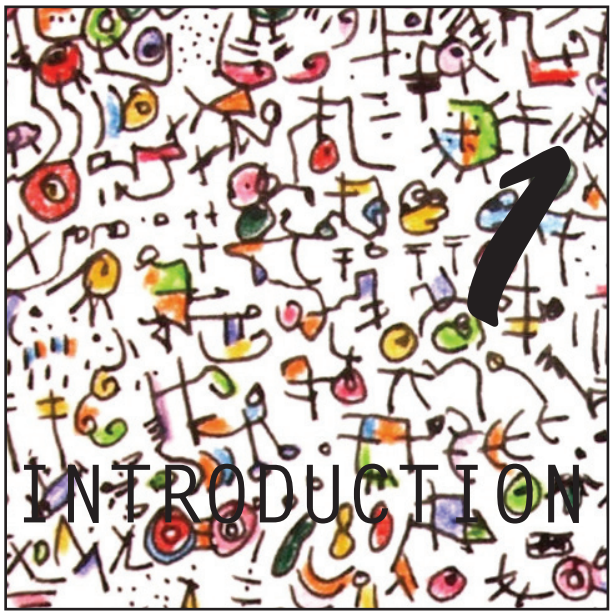


1 INTRODUCTION

DNA damage

The genetic information contained in the DNA dictates the structure, the organization and function of the cell. Thus, it is of major importance for every cell in a tissue or organ that compose an organism to protect the integrity of its genetic information. Especially due to its chemical composition, DNA is a fragile molecule, susceptible to DNA damage formation, when exposed to various genotoxic threats. In the environment, ionizing and ultraviolet radiation (IR and UV) as well as certain chemicals are examples of such genotoxic threats corrupting the chemical structure of DNA. Additionally, byproducts of normal cellular metabolic reactions such as oxygen radicals can interact with and damage the DNA molecule. As a consequence of these numerous attacks, the frequency of DNA damage induced in human cells is estimated to be around 1,000 to 1,000,000 lesions per cell per day [1, 2]. If left unrepaired or repaired inaccurately these lesions can lead to chromosomal aberrations and mutations, which in turn can lead to genome instability, cancer development or cell death [3]. In addition, during replication, DNA duplication by polymerases, although tightly regulated, leaves errors that modify the original information and can also result in mutations.

DNA damage responses

To combat DNA damage, cells have evolved an intricate system known as the DNA damage response (DDR), which senses DNA lesions and activates downstream pathways such as chromatin remodeling, cell cycle checkpoints and DNA repair [4]. Primarily, the DDR was defined as a cascade of reactions transmitting the signal from sensor proteins to downstream effectors via transducers that altogether coordinate gene expression, cell cycle progression and repair. However, it becomes apparent that this signaling pathway is not as linear as thought. Sensors can be part of effector or transducer complexes (e.g component of replication fork) or repair factors can feedback to sensors and thus play roles of transducer.

Importantly, dysfunctions in the DDR have been linked to human diseases. For example, defects in repair and signaling were found to result in chromosome aberrations that are hallmarks of multiple cancers such as lymphomas or osteosarcomas [5]. Human syndromes such as Ataxia telangiectasia (AT and AT-like) and Nijmegen breakage syndrome are caused by mutations in the central checkpoint kinase, ATM and Nbs1 respectively. The latter is a component of the MRN complex involved repair of DNA double stranded breaks. These are examples that stress the importance of the DDR for human health.

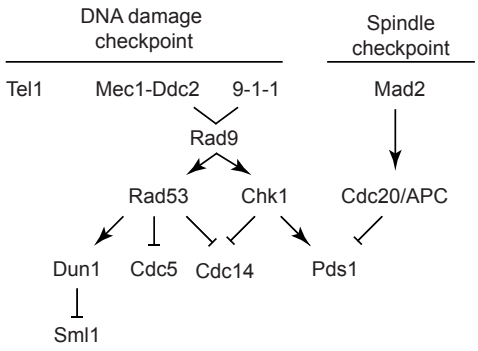
Most of these DDR pathways and factors are conserved from yeast to mammals. Since my thesis work dealt with the budding yeast Saccharomycces cerevisiae, I will focus on this model organism to give an overview of the different processes composing the DDR and will occasionally refer to the mammalian DDR.

Checkpoint signaling pathways

One essential and early component of the DDR is the DNA damage checkpoint. Its role is to delay the G1/S transition, arrest cells at the G2/M boundary or slow down S-phase progression upon induction of DNA damage to allow time for repair. In S-phase, in addition to the DNA damage checkpoint, the replication checkpoint operates to slow down replication and inhibit firing of late origins of replication when cells experience a replicative stress.

Mec1 and Tel1 kinases activate checkpoint-signaling cascades

The key components of these checkpoint pathways are the two phosphoinositol-3-kinase related (PI3K) kinases Mec1 and Tel1 (Box 1). ATM and ATR are the mammalian



Box 1: Schematic representation of the DNA damage and spindle checkpoint pathways and their combined roles in the regulation of the cell cycle (9-1-1 complex: Mec3-Rad17-Ddc1; see text for details).

homologues of Tel1 and Mec1, respectively. Mec1/ATR is crucial for signaling ssDNA at DNA lesions and stalled replication forks while mainly Tel1/ATM signals DSBs. DNA-PKcs, another mammalian PI3K kinase, which has no homologue in yeast, is involved in the detection and repair of DSB by non-homologous end-joining [6].

Recruitment of Tel1 and Mec1 to sites of DNA damage is essential for the activation of downstream signal transduction pathways. The recruitment of these kinases involves different complexes that recognize the DNA lesions. Mec1 binds to its partner Ddc2, which recognizes ssDNA coated with replication protein A (RPA) complex at stalled or collapsed forks and DSBs [7]. On the other hand, Tel1 is recruited to sites of DSB by the Mre11-Rad50-Xrs2 (MRX) complex, which tethers the ends [8].

Another group of proteins is required for the Ddc2-independent recruitment of Mec1 to sites of DNA damage [9, 10]. The Rad24-RFC complex, normally sliding on the DNA during replication, loads the Mec3-Rad17-Ddc1 complex (9-1-1 complex; Box 1) upon detection of aberrant DNA structures [11]. The 9-1-1 complex was then suggested to attract and stimulate Mec1 activity at the lesion by direct interaction with the 9-1-1 component, Ddc1 [12].

At last, the replication initiation and S-phase checkpoint factor Dpb11 was also found to physically and genetically interact with Ddc2-Mec1 [13]. The current evidence suggests that Dpb11 and the 9-1-1 complex independently recruit and activate Mec1 at DNA lesions.

Chk1 and Rad53 kinases control DNA damage-induced cell cycle arrest

Mec1 and Tel1 are the two kinases that are at the top of checkpoint signaling cascade. They activate by phosphorylation a number of downstream DDR factors. Two of these are the downstream checkpoint-transducing kinases, Chk1 and Rad53 (Box 1). Chk1 and Rad53 form two parallel pathways that amplify the checkpoint signal and promote cell cycle arrest by phosphorylation of a multitude of DDR and cell cycle regulators.

Chk1 becomes only activated by Mec1 in a process that necessitate the adaptor kinase Rad9 [14] (Box 1). Its main target is the anaphase inhibitor Pds1. Hyper-phosphorylated-Pds1 is stabilized and provokes cell cycle arrest before anaphase. Indeed, Pds1 phosphorylation prevents its ubiquitylation by the anaphase promoting complex in conjunction with Cdc20 (Cdc20/APC) and its subsequent degradation which is needed for sister chromatid separation during normal cell cycle [15, 16]. Chk1 also contributes to the inhibition of mitotic exit by inactivating the Cdc14 early anaphase release (FEAR) pathway (Box 1). Cdc14 desphosphorylates the targets of the cyclin-dependent kinase 1 (Cdk1) and thereby allows mitotic exit [17]. Thus, it is thought

that Chk1 either directly or via stabilization of Pds1 prevents release of the phosphatase Cdc14 from the nucleolus.

Although both Tel1 and Mec1 can activate Rad53, Mec1 seems to be the prime kinase for this process. Rad53 activation depends on two adaptor proteins Rad9 and Mrc1 that are also phosphorylated by Mec1 and Tel1. Rad53 has two FHA domains (FHA1 and FHA2), which mediate the interactions with the phosphorylated adaptor protein leading to activation and autophosphorylation of Rad53 [18]. Mrc1 and Rad9 are partially redundant in transducing replication stress signals [19]. However, Mrc1 as part of the replisome functions in the replication checkpoint while Rad9 signals DNA damage in S-phase [20].

Rad53's most-studied target is the Dun1 kinase (Box 1). Dun1 also has an FHA domain that mediates its interaction with phosphorylated-Rad53 leading to Dun1 activation [21]. Dun1 is required for the DNA damage-induced transcription of numerous genes, some of which promote cell cycle arrest in G2/M. Sml1, the ribonucleotide reductase (RNR) transcription inhibitor, is Dun1's best-characterized target. Upon phosphorylation, Sml1 is targeted for degradation, which results in increased RNR-dependent dNTP synthesis [22, 23]. Rad53 also affects Pds1 stability by preventing the Cdc20-Pds1 interaction thereby inhibiting recruitment of the APC complex as well as Pds1 degradation [24]. Finally, Rad53 was also proposed to inhibit the mitotic exit network (MEN) in two ways. First, similar to Chk1, it prevents Cdc14 release from the nucleolus and dephosphorylation of Cdk1 targets required for mitotic exit. Secondly, it inhibits the Cdc5 polo-like kinase, which consequently suppresses the MEN [16] (Box 1). However, the exact mechanism by which Rad53 abolishes the MEN is unclear and needs further investigation.

The spindle checkpoint collaborates with the DNA damage checkpoint to regulate cell cycle progression.

The spindle checkpoint controls the accurate segregation of the chromosomes by inspecting the attachment of microtubules to the kinetochores that are complexes of proteins associated with the centromeres. This checkpoint seems to sense the tension present at the kinetochores upon bipolar attachment [25]. Normally activated upon microtubule damage, the spindle checkpoint also contributes to DNA damage checkpoint-induced G2/M arrest. The spindle checkpoint stabilizes Pds1 by inhibiting the APC complex (Box 1). However, it is not known whether the spindle checkpoint is activated upon DNA damage induction. Nevertheless, cells deleted for the spindle checkpoint protein Mad2 are sensitive to DNA damaging agents (MMS and HU). Moreover, loss of Mad2 in *rad53*Δ*chk1*Δ double mutant eliminates residual cell-cycle arrest after UV treatment suggesting that the spindle and the DNA damage checkpoints work redundantly [26]. On the other hand, Rad9 and Rad53 were also found regulated by the spindle checkpoint. They are both phosphorylated after nocodazole-induced microtubule damage in Mad2-dependent and Mec1-independent fashions, which means that the DNA damage and the spindle checkpoints interplay with each other [27]. Yet, the exact roles of Rad9 and Rad53 in the spindle damage checkpoint are not clear.

Checkpoint recovery and adaptation

Cells need to inactivate the DNA damage checkpoint in order to re-enter the cell cycle. Conceivably, checkpoint inactivation

should occur either when repair of the DNA damage has been completed or when cells adapt to an irreparable lesion.

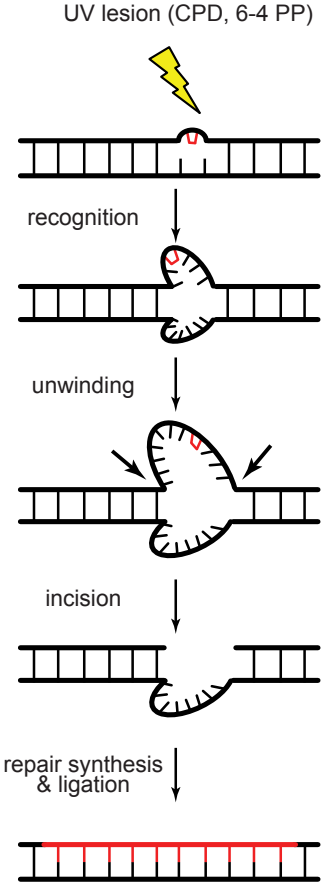
Checkpoint inactivation due to completion of repair is called checkpoint recovery and is initiated by the disappearance of DNA lesions. Then, constitutive inhibitors of the checkpoint can revert each step of the signal transduction cascade. For example, work from Keogh and coworkers showed that the phosphatase complex Pph3-Psy2 dephosphorylates γH2AX, a histone phosphorylated by Mec1 and Tel1 during the early steps of the DDR. Indeed, persistent γH2AX results in prolonged checkpoint activation even when repair has occurred [28]. Moreover, Pph3-Psy2 and two other phosphatases, Ptc2 and Ptc3 were also found to dephosphorylate Rad53 thereby allowing cell cycle resumption after completion of repair [29, 30]. In human cells, on the other hand, Wip1 and the phosphatase PP2A were found to dephosphorylate both Chk1 and Chk2 (the mammalian homolog of Rad53).

In yeast, inactivation of the checkpoint due to an irreparable DSB is called adaptation. Cells restart progression through the cell cycle while the damage persists. Many factors involved in repair (e.g Rad54, Rad51, Sae2, yKu70, Srs2), cell cycle regulation (e.g CKII, Cdc5) or checkpoint inhibition (Ptc2, Ptc3, Pph3) have been implicated in adaptation. Whereas adaptation is a process that leads to genomic instability [31], it appears to promote viability in cells carrying an irreparable DSB. Importantly, under these conditions, mammalian cells likely activate senescence or apoptosis. However, work from Yoo et al. showed that in xenopus egg extracts treated with the replication inhibitor aphidicolin, Chk1 is deactivated and the nuclei enter in mitosis while DNA replication is incomplete [32]. This suggests that other organisms than yeast may adapt to irreparable DNA damage.

DNA repair

Nucleotide excision repair

UV induces two main types of photolesions: 6-4 photoproducts (6-4 PP) and cyclobutane pyrimidine dimers (CPDs), which are repaired by nucleotide excision repair (NER). In humans, mutations in NER factors result in Xeroderma Pigmentosum (XP) and Cockayne syndrome (CS) diseases.

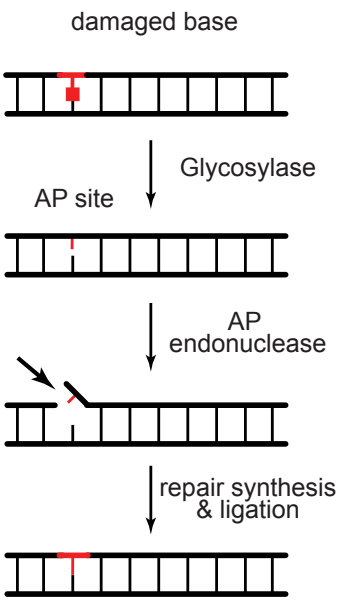


Box 2: Schematic representation of the nucleotide excision repair (NER) pathway (see text for details)

XP patients show an extreme sensitivity towards sunlight and develop skin cancers in sun-exposed parts of the body with an abnormally high incidence. CS patients also display photohypersensitivity (which does not give rise to cancer development) but more prominently mental and psychomotor retardation among other severe developmental and neurological disorders. NER is composed of two sub-pathways called transcription-coupled (TC-NER) and global genome repair (GG-NER). While the first pathway operates on lesions in transcribed strands and is activated by stalling of RNA polymerase II, GG-NER takes care of removing UV lesions from the rest of the genome. Generally, TC-NER and GC-NER follow a 3-steps mechanism starting from the detection of the photolesion followed by excision of the oligonucleotide-containing lesion and completion of repair by a gap-filling step recovering the lost information (Box 2) [33]. Although they differ at the DNA damage recognition step, the two pathways remove the lesion by a common mechanism using a core set of repair factors. In yeast Rad4/Rad23 (XPC/hRad23), Rad7/Rad16 (functional equivalent of mammalian UVDDB1/2) and Rad26-RNA Pol II (CSB/RNA pol II) are the factors that detect helix-distorting lesions during GG-NER and TC-NER respectively. The Rad4/Rad23 complex works in both GG-NER and TC-NER while Rad7/Rad16 is specific to GG-NER. Rad14-RPA (XPA/RPA) is another complex that acts in the two NER subpathways. TFIIH helicases subunits Rad3 and Rad25 help to unwind the DNA before the incision step, which is carried out by the two structure dependent endonucleases Rad1-Rad10 and Rad2 at the 5' and 3' side, respectively, of the damage. The DNA binding complexes Rad14-RPA and Rad4-Rad23 are also essential for the incision of the damage. In the last step, the replication machinery fills the gap and completes repair [34].

Base excision repair

Base-excision repair (BER) removes damaged bases such 8-Oxoguanine or apurinic/pyrimidinic sites (AP) from the DNA (Box 3). First, the damage is recognized by enzymes called N-glycosylases each having specific substrates, that cleave the DNA to remove the damaged bases from the DNA backbone leaving an AP site. Next, cleavage of AP sites by AP endonucleases or AP lyases lead to the formation of 5' and 3' blocked single strand break (SSB), respectively. In most cases, BER reactions are initiated by 5' incision of the AP site by either Apn1 or Apn2 AP endonuclease. Then, Polymerase ε can use the 3' end to fill the gap, which generates a 5' single strand overhang further removed by the flap endonuclease Rad27. The final step is the ligation by the Cdc9 ligase [35]. This long-patch BER pathway is the most common in yeast. Higher eukaryotes possess the multifunctional DNA polymerase β, which

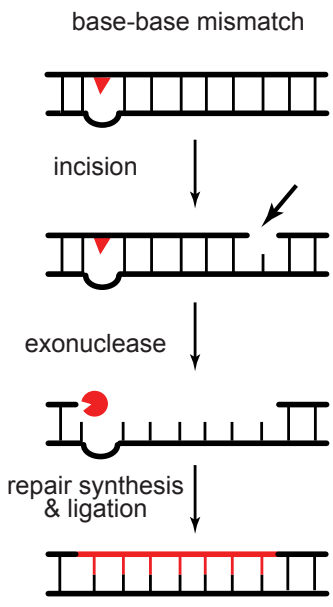


Box 3: Schematic representation of the base excision repair (BER) pathway (see text for details)

favors a short-patch repair mechanism. This polymerase is able to insert one nucleotide and removes the 5' extremity through lyase activity. The following ligation step is performed by XRCC1-Lig3 [36]. In the case of 3' blocked SSBs, resulting from 3' incision of the AP site by AP lyases such as Ntg1, Ntg2 or Ogg1, the BER reaction involves the Rad1-Rad10, a structure-specific flap endonuclease that cleases the 3' extremity of the AP site.

Mismatch repair

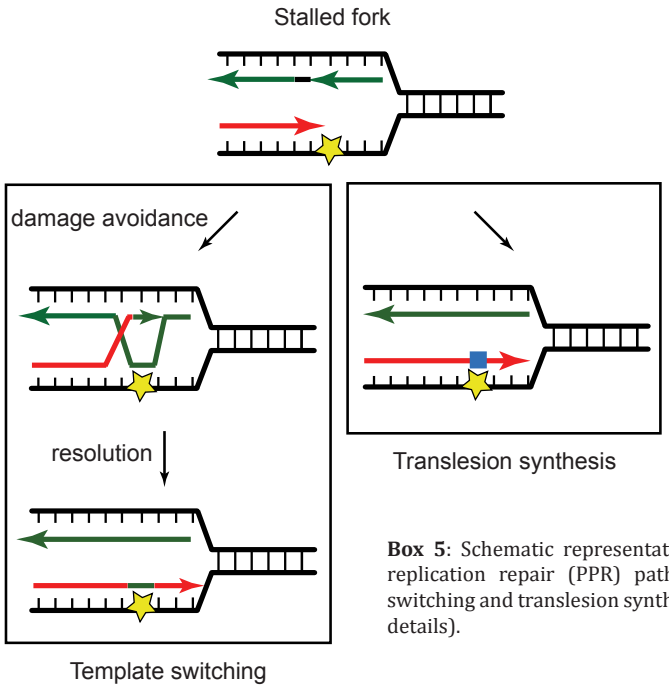
Mismatch repair (MMR) is particularly important to correct errors that arise from misparing during replication such as base mismatches, small insertions or deletions (Box 4). In humans, defects in mismatch repair lead to microsatellite instability, which is notably observed in hereditary non-polyposis colorectal cancer (HNPCC) [37]. Msh2, together with Msh3 or Msh6 forms two heterodimers that recognize the errors in the DNA. Msh2-Msh6 recognizes base mismatches and insertions (1-2 nucleotides) or deletion loops while Msh2-Msh3 detects insertion/deletion loops only. The binding of either of the two complexes induces a conformational change that attracts the Mlh1-Pms1 complex to the lesion. A nick on either side of the mismatch allows further processing by exonucleases such as Exo1. To date, no process has been found responsible for this nick. Whereas, in the lagging strand, nicks are likely due to the formation of Okazaki fragments, it is not clear how they are introduced in the leading strand. The 5' to 3' exonuclease activity was suggested to be performed by Exo1 and Rad27 while the only 3' to 5' exonucleases activity have been associated with the replication polymerases δ and ε. In fact, the proliferating nuclear cell antigen (PCNA) binds multiple components of the recognition complexes and likely recruits Polδ and ε to the mismatch [38]. Then, the polymerases are thought to complete the repair process by performing the DNA synthesis and ligation steps [39]. It is important to note that NER and BER factors genetically and physically interact with MMR components, which suggests extensive crosstalks between these pathways.



Box 4: Schematic representation of the mismatch repair (MMR) pathway (see text for details)

Post-replication repair

Lesions that are not removed by the earlier mentioned repair pathways before resumption of DNA replication, can interfere with the replication machinery and block its progression (Box 5). Two post-replication repair (PRR) mechanisms can bypass these lesions namely translesion synthesis (TLS) and template switching. PCNA is responsible for the initiation of PRR and for the choice of the bypass pathway. When replication forks are blocked by DNA lesions, PCNA is ubiquitylated by the Rad6-Rad18, an ubiquitin E2 conjugating-E3 ligating enzyme complex. Monoubiquitilation of



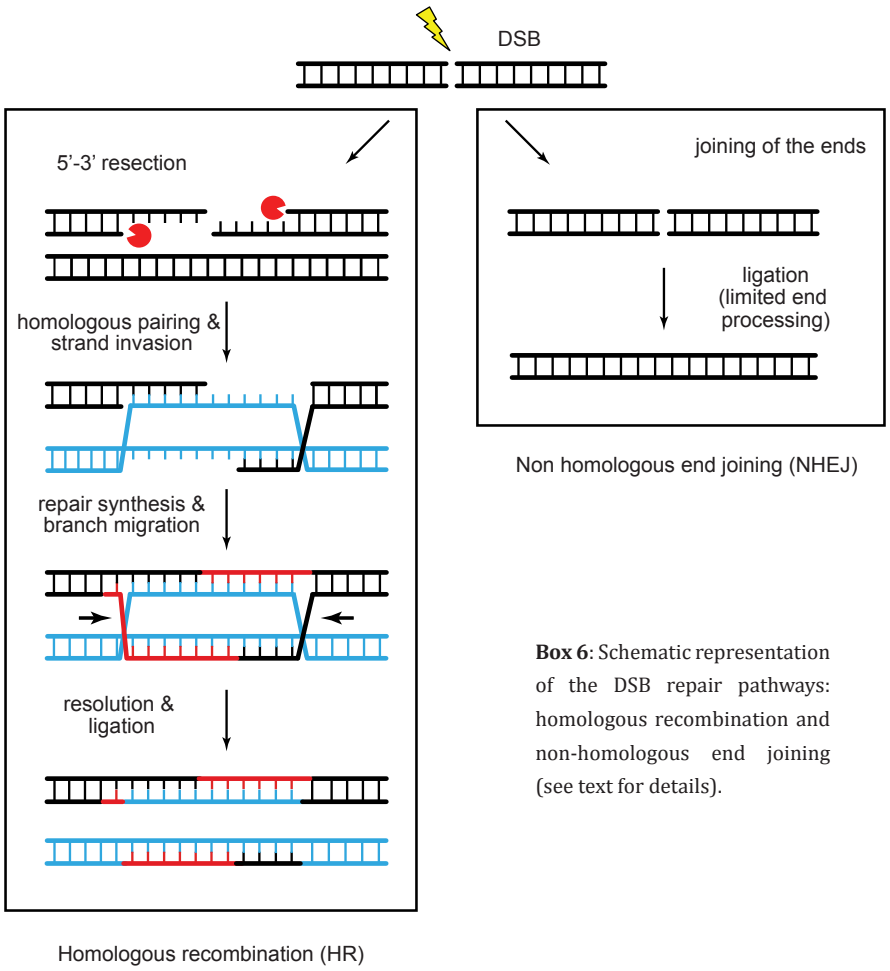
Box 5: Schematic representation of the post-replication repair (PPR) pathways: template switching and translesion synthesis (see text for details).

PCNA promotes bypass by TLS, while its polyubiquitilation by the ubiquitin Mms2-Ubc13 E2-conjugating and the Rad5 E3-ligase complex favors the template switching pathway. TLS involves specialized polymerases such as Rev1, Polζ or Polη that can incorporate correct or incorrect nucleotides opposite the lesion and as such may be mutagenic.

On the other hand, template switching, a largely unknown mechanism for DNA damage bypass, is thought to be error-free. One of the accepted models starts with reinitiation of replication downstream the blocking lesion and is followed by a gap-filling event that uses the newly synthesized complementary strand as template in a recombination-like reaction (Box 5). Although Rad52 is possibly involved in this process, the other factors implicated remain to be discovered [40].

Double stranded break repair

Double stranded breaks are repaired by two mechanisms, homologous recombination (HR) and non-homologous endjoining (NHEJ)(Box 6). The choice of these two repair pathways depends on the phase of the cell cycle. HR occurs mainly in S and G2 phase due to the fact that it uses a sister-chromatid to copy the information required to seal the break. NHEJ on the other hand, is active during the whole cell cycle but is predominantly used in G1 phase. The key step of HR is the 5' to 3' resection of the DSB, which is initiated by the Mre11-Rad50-Xrs2 (MRX) complex and its associated partner Sae2. Then, the exonuclease Exo1 or the helicase Sgs1 and the helicase/nuclease Dna2, in a second resection step called long-range resection, further process the ends. The resulting 3' single strand overhangs (ssDNA) are rapidly coated by replication protein A (RPA). Next, RPA proteins are replaced by Rad51 proteins in a Rad52 and



Box 6: Schematic representation of the DSB repair pathways: homologous recombination and non-homologous end joining (see text for details).

Rad51 paralogs Rad55-Rad57 dependent manner, to form the Rad51 filament. This filament in concert with the Swi/Snf Rad54 protein catalyzes the search for a homologous sequence and proceeds with strand invasion. Annealing of the filament with the homologous template initiates DNA synthesis and branch migration that leads to formation of a joint molecule and structures called Holiday junctions (HJ). In the end of HR, the Sgs1-Top3-Rmi1 complex resolves both the joint molecule and the HJ, a step that is followed by a final ligation step [41, 42].

NHEJ involves the direct religation of the broken ends. The yKu70-yKu80 and MRX complexes form the core set of NHEJ proteins. Human Ku is part of a complex of which catalytic subunit DNA-PKcs is required for efficient NHEJ. Yeast cells lack a functional DNA-PKcs homolog. MRX appears early after DSB formation to tether the ends. Although it is not clear which of the two complexes is the first to bind, it seems likely that the yKu complex occupy these extremities of the ends while the MRX complex can bind further away from the extremities. Then, DNA ligase IV and its associated partner Lif1 are recruited to the DSB through direct interaction with the MRX and Ku complexes and proceed with ligation

of the ends. In the context of clean breaks, ligation can occur without end-processing thereby being an error-free mechanism. However, in some cases, end-processing events must occur before ligation and necessitate the activity of enzymes such as the flap endonuclease Rad27, Polymerase Pol4 or lyases [43]. This type of NHEJ is thus error-prone.

DDR-induced chromatin modifications

In eukaryotic cells, DNA is packaged together with histone proteins into a sophisticated structure called chromatin. This structure acts as a natural barrier that restricts the access to DNA. To enable enzymes that function in DNA metabolic processes such as replication, transcription or DNA repair to access DNA, chromatin has to be highly dynamic. Histone proteins are the main components regulating chromatin flexibility. They are modified by various post-translational modifications that alter DNA packing and as such ease or limit its accessibility. Here are examples of histone modifications and their functions in the DDR.

Phosphorylation

Tel1/Mec1 kinases phosphorylate many DDR substrates preferentially on a consensus SQ motif. Tel1/Mec1 dependent phosphorylation of the C-terminal SQ motif of histone H2AX (γH2AX) is an important and conserved event during the DDR [44]. This modification spreads around and up to100 kilobases away from a DSB. H2A-S129A mutants that cannot be phosphorylated by Tel1 or Mec1 are sensitive to DNA damaging agents and have a mild DSB repair defect [45]. In addition, γH2AX helps the recruitment of chromatin remodelers to DBS sites such NuA4, INO80 or SWR1. These complexes respectively, acetylate histones at the damaged site, facilitate end-resection by the MRX complex or favor the binding of the Ku complex thus stimulating repair by NHEJ [46, 47].

Methylation

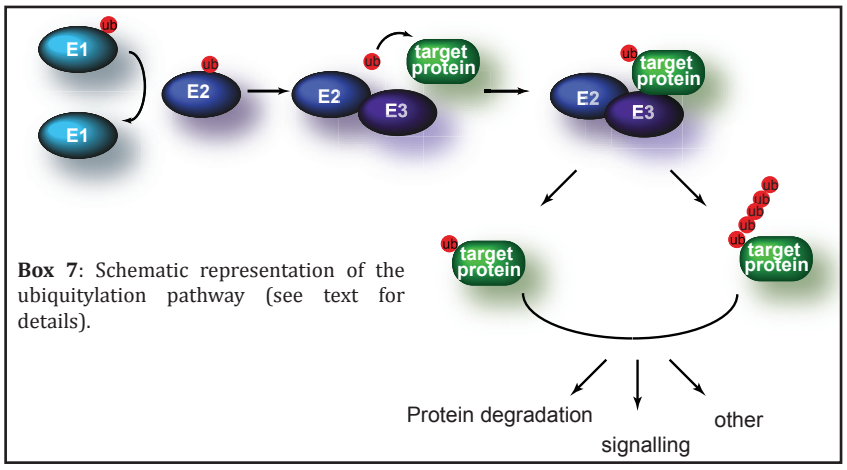
Methylation of lysine 79 on histone H3 (H3-K79me) also contributes to checkpoint activation and DNA repair. Dot1 is the histone methyltransferase responsible for the H3K79 mono-, di- or tri-methylation. Moreover, Rad9 is thought to be recruited to the DNA damage through its tudor domain, which recognizes and binds such methylated histones. Deletion of Dot1 causes defects in the G1/S and DNA intra-S damage-induced checkpoints. It was proposed that Dot1 loss perturbs Rad9 recruitment and subsequent Rad53 activation [48]. In addition, mutations in Rad9 tudor domain or failure to methylate H3K79 lead to a G1/S and intra-S checkpoint defect [48].

Acetylation

Acetylated histones H3 on lysine 56 (H3K56ac), by the histone H3 acetyltransferase Rtt109, are deposited during S-phase. In late S and G2 phases, the acetyl-groups on H3K56 are removed by the histone deacetylases (HDACs) Hst3 and Hst4 to allow cells to progress through mitosis. While H3K56ac is a mark for completion of S-phase, it was also found to affect checkpoint signaling in response to a DSB [49]. H3K56 acetylation by Rtt109 at site of DSB signals that repair has been completed; in turn H3K56ac removal is required for checkpoint recovery [49].

Ubiquitylation

Ubiquitylation is a cascade of reactions that results in the covalent attachment of a small peptide called ubiquitin to targeted proteins. This process involves E1 ubiquitin-activating, E2 ubiquitin conjugating and E3 ubiquitin-ligating enzymes (Box 7). Protein-ubiquitylation was at first associated with protein degradation. Now, it becomes



Box 7: Schematic representation of the ubiquitylation pathway (see text for details).

apparent that ubiquitylation of proteins is involved in various cellular pathways such as the DDR. For example, Histone H2B is ubiquitylated by the Rad6-Bre1 ubiquitin E2-conjugating E3-ligating complex, which is a prerequisite for methylation of H3K79 by Dot1. These two modifications affect cell survival in response to IR and the G1/S checkpoint in the presence DNA damage [48, 50]. In mammalian cells, ubiquitylation has recently been found to be an important component of the DSB response. The two ubiquitin ligases RNF8 and RNF168 are responsible for histone H2AX and H2A polyubiquitylation and thereby affect the recruitment of the downstream repair and signaling factors such as BRCA1 through its associated partner RAP80 that binds ubiquitin. The adaptor 53BP1 is also recruited via the RNF8 and RNF168 ubiquitylation cascade yet in an unclear mechanism [51].

Post-translational modifications at the interface between checkpoint signaling and repair

Sumoylation

Sumoylation is a cascade of reactions similar to ubiquitylation at the exception that it results to covalent binding of an ubiquitin-like moiety called SUMO to protein-targets. At the difference with ubiquitylation, sumoylation is not implicated in protein degradation but rather in other processes such as nucleocytoplasmic trafficking [52] or gene expression regulation [53]. In the DDR, sumoylation was found to modify Rad52 and impact on two events. Firstly, it likely protects Rad52 from proteasomal degradation when cells accumulate DNA intermediates in the process of HR. Secondly, it may determine the type of HR pathway by promoting gene conversion to the detriment of break induced replication and single-strand annealing. Srs2 and Sgs1 are other targets of sumoylation. Sumoylated-Srs2 seems to prevent unscheduled HR at replication forks, while Sgs1 modification stimulates HR at telomere and as such promotes telomere maintenance. PCNA is also sumoylated by the E2/E3 complex Ubc9/Siz1. Sumo-modified PCNA promotes Srs2 recruitment to replication forks, which prevents HR by disrupting Rad51 filaments 40]. In mammalian cells, sumoylation was recently implicated in the DSB response. The two SUMO E3 ligases, PIAS1 and PIAS4 are recruited to DSB sites and may regulate subsequent recruitment of repair factors like BRCA1 and 53BP1 [51].

Neddylation

Neddylation is a process similar to ubiquitylation or sumoylation by which the ubiquitin-like Rub1 protein (NEDD8 in humans) is conjugated to target proteins. The cellular processes that involve neddylation remain largely unknown due to the limited number of neddylation substrates that have been identified, namely the cullin-RING ubiquitin ligases (CRL) [54]. In yeast, the 3 CRL Rtt101, Cdc53 and Cul3 are neddylated in vivo, however only Rtt101 has been implicated in the DDR [55]. Moreover, absence of Rub1 does not seem to affect Rtt101 activity [56, 57]. Thus far, there is no evidence for a role of neddylation in the yeast DDR. On the other hand, in human cells, p53 is neddylated under unchallenged conditions, which triggers its proteasomal degradation [58]. In addition, neddylated forms of p53 transiently appear after UV exposure, which strongly suggests that neddylation regulates p53 levels and activity in response to DNA damage [58].

DNA damage response networks

Over the past ten years, the development of technologies allows genome-wide measurements of cellular organization, processes and responses to stressors. These technologies turned out to be powerful tools to study complex cellular responses including DDR.

Genome-wide analysis tools to study the DDR

Microarray studies have been used to monitor the transcriptional changes after various stress conditions including DNA damage. These studies helped to classify genes in functional categories. For example, yeast cells exposed to various DNA damaging agents including IR and the alkylating agent MMS, were used to identify a group of transcriptionally responsive genes which included DNA repair genes such as MAG1, NTG1, RAD7, RAD54, RAD51 and the RNR subunits [59, 60]. In addition, the combination of microarray data enabled the creation of large databases [61]. These databases have been used to characterize unknown genes or drugs and helped to define molecular targets of known drugs based on the following observations. First, mutants that affect the same cellular process are likely to display similar transcriptional profiles. Secondly, if a gene mutation induces a transcription profile that correlates with that of wild-type cells treated with a particular drug, it is likely that the protein encoded by this gene, is a target of that drug. For example, Hughes et al. found that functionally related genes had profiles that matched such as ribosomal or histone deacetylase genes. They also showed that wild-type cells treated with an inhibitor of the HMG-CoA reductase had a similar transcription profile as cells deleted for the gene coding one of isoenzyme of the HMG-CoA reductase [61]. In human studies, transcription profiling is used to characterize and classify tumor types. The ultimate goal is to use expression profiling to determine the disease state and the response of patients to a pharmaceutical treatment.

Transcription profiling also helped to identify transcription factors driving the response to DNA damaging agents. Work from Jelinsky et al. showed that genes transcriptionally regulated similar to MAG1, have a consensus regulatory element, which is almost identical to that of MAG1. They found that this regulatory element was identical to the proteasome associated control element (PACE) and that the genes containing this element as well as MAG1 were regulated by the transcription factor Rpn4 [62]. They proposed that regulation of DDR genes possessing this regulatory

element is linked to protein degradation.

Genomic phenotyping has been another useful tool to identify gene products that affect particular phenotypes such as sensitivity or resistance to DNA damaging compounds [63]. These studies were used to characterize the mechanism of action of DNA damaging compounds and classify unknown genes in functional categories. For example, Lee et al. exposed the whole yeast deletion library to 12 different DNA damaging compounds, among which some are used in cancer treatment. Surprisingly, they found more genes required for protection against DNA damaging agents than it was previously anticipated. Among them were genes without functional annotations or not related to the DDR. They also showed that while genes involved in DNA repair pathways such NER, HR or PRR were typically required for protection against the different compounds, their relative importance was variable depending on the compound. PRR genes as well as the previously characterized PSO2 gene were particularly found to promote resistance to interstrand cross-linking agents [64]. Interestingly, genes encoding proteins known to physically interact were found to display similar responses to the different compounds. In human cells, genomic phenotyping (that identify gene products that affect particular phenotypes such as sensitivity or resistance to DNA damaging compounds) may be used in the clinic to understand the sensitivity of patients to chemotherapeutic agents.

Genome-wide protein-protein interaction and yeast two-hybrid studies have revealed the extent to which gene products are organized in complexes to perform cellular processes [65]. These data were used in combination with previously described genomic-phenotyping data to identify protein sub-networks that may drive the cellular response to DNA damage. These sub-networks were found to contain proteins involved in various molecular processes including DDR, chromatin remodeling, RNA and protein metabolism. This interactome-genome phenotyping integration showed that the interplay between multiple and functionally unrelated cellular processes is necessary to cope with DNA damage [63].

Genome-wide genetic interaction technologies such as synthetic genetic array have also been used to functionally group genes. Pan and coworkers used a synthetic lethality screen to interrogate the functional interactions between cellular processes participating to DNA integrity [66]. In a synthetic lethality screen, query mutants are crossed to an array of mutants each carrying a single gene deletion, to generate a library of double mutants that are then scored for cellular growth defects such as cell death or reduced fitness. Based on the assumption that genes working in the same pathway tend to interact similarly with other genes, they were able to define functionally distinct groups of interacting genes or modules. They also found that these modules were interacting with each other and as such predicted new function for known modules. For example, the fact that the sister chromatid cohesion module CTF18/CTF8/DCC1 interacted with the DNA checkpoint module RAD9, led Pan and coworkers predict and confirm a role for the CTF18 module in the S-phase checkpoint. Based on their interaction profiles, they showed that new genes such as DIA2, HST3 or HST4 are involved in DNA replication. Finally, synthetic lethal interactions between modules led them to suggest that DNA oxidation and errors occuring during replication are likely to be the major source of spontaneous DNA damage [66].

The EMAP approach

Collins et al. developed a technology called Epistatic Mini Array Profiling (EMAP), which allows the precise measurement of both negative (synthetic lethality) and positive interactions (synthetic fitness) between pairs of genes [67]. This approach is based on synthetic genetic array technology [68], which is a high-throughput technique that explores genetic interactions through the systematic construction of double mutant yeast strains. Briefly, query strains carrying a single gene deletion are mated against an array of different gene deletion strains resulting in heterozygous diploids. Following sporulation and a series of selection steps haploid double-gene deletion strains are obtained. Growth rates are determined by measuring colony sizes. Size measurements are then normalized and statistically analyzed to assign each double mutant a quantitative S score [69]. This score reports on the extent to which it grew better (positive S score or interaction) or worse (negative S score or interaction) than expected [69]. Positive interactions (i.e., epistasis) typically occur among genes involved in the same complex or pathway, while negative interactions (i.e., synthetic sickness or lethality) usually identify genes in compensatory pathways.

Collins and colleagues generated the first EMAP, which focused on chromatin metabolism [67]. This map exhibited a modular organization, which allowed the authors to predict with high confidence protein complexes and to dissect large protein complexes in functional sub-complexes. Importantly, focusing on epistatic (positive) relationships between genes, they characterized a whole new pathway involved in the DDR that is driven by the Rtt109-dependent H3K56 acetylation [67]. The next step using this technique is to ask whether genetic interactions change under particular DNA damaging conditions. Recently, Bandyopadhyay et al. have developed a technology called differential epistatic mapping (dE-MAP), which allows to measure the genetic interaction changes in response to a perturbation such as DNA damage induction [70].

From pathway analysis to network

Computational data analysis of transcription profiling and genetic or protein interaction studies not only identified new components of cellular pathways but also revealed extensive interconnections between them. The integration of these different datasets has led to the new notion of cellular networks in which cellular pathways are only components connected to each other and regulated at different levels. Integrating other data such as post-translation modifications (e.g. ubiquitylation or phosphorylation) are likely to give a more dynamic view of the cellular networks and predict their rewiring under stress conditions such as the presence of DNA damage. The next step to understand the cellular responses to genotoxic stresses is to collect transcription data including microRNAs, protein and genetic interaction data both gathered under the same DNA damaging conditions since most of these datasets have been collected under unchallenged conditions or vastly differing exposure conditions to stressors.

In this thesis

The number of factors engaged in the global responses to DNA damage and the multiple layers of regulation, make the coordination of various responsive pathways and underlying mechanisms a complex task for the cell. The aim of this thesis is to improve our understanding of the crosstalks between cellular processes that are necessary for the cells to respond to specific types of DNA damage. To reach this goal, we used a high-throughput genetic approach called EMAP. To assess the genetic interaction changes that are induced by specific types of DNA damage, we generated EMAPs under three different DNA damaging conditions. A recently developed algorithm that allows quantifying the genetic interaction changes in response to a perturbation helped us in the analysis of this new type of genetic data [71].

In **chapter 2**, we describe the set-up and the analysis of our genetic screen and show that it is an extremely powerful method to highlight associations between DNA damaging drugs and DNA repair pathways. Four different novel interactions defined in our genetic map that is presented in this chapter, are studied at the molecular level and presented in the following chapters.

In **chapter 3**, we describe a new role for Rtt109, the histone H3 acetyltransferase, in regulation of the mutagenic bypass of DNA lesions.

In **chapter 4**, we show that the neddylation machinery affects genomic stability and cell cycle control in response to the Top1-inhibitor camptothecin, most likely by regulating the steady state level of DDR factors including Nhp10 and Mms22.

Chapter 5 describes the identification and the characterization of a new DDR factor, Irc21. We demonstrate that Irc21 influences genomic stability, DNA damage checkpoint and repair.

Chapter 6 reports on the coordination between the Sae2 endonuclease and the Pph3-Psy2 phosphatase complex in regulation of the DNA damage checkpoint.

In **chapter 7**, we discuss the implication of our findings in future research and in possible therapeutic outcomes.

The functional analysis of genetic interactions found in our genetic screen confirms that our approach was successful in the investigation of the interconnection between factors and pathways to mediate an appropriate cellular response to various types of DNA damage. We unraveled new factors and connections between cellular pathways and show how they act in the DDR. While we functionally investigated only a piece of this genetic network, we hope that it will initiate further studies leading to a better understanding of the DDR in yeast and in higher eukaryotes

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