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Review

The DNA Damage Response: Making It Safe to Play with Knives

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Damage to our genetic material is an ongoing threat to both our ability to faithfully transmit genetic information to our offspring as well as our own survival. To respond to these threats, eukaryotes have evolved the DNA damage response (DDR). The DDR is a complex signal transduction pathway that has the ability to sense DNA damage and transduce this information to the cell to influence cellular responses to DNA damage. Cells possess an arsenal of enzymatic tools capable of remodeling and repairing DNA; however, their activities must be tightly regulated in a temporal, spatial, and DNA lesion-appropriate fashion to optimize repair and prevent unnecessary and potentially deleterious alterations in the structure of DNA during normal cellular processes. This review will focus on how the DDR controls DNA repair and the phenotypic consequences of defects in these critical regulatory functions in mammals.

Since the discovery of the DNA structure more than 50 years ago, the remarkable mechanisms that preserve the genetic information encoded by DNA and guarantee its faithful transmission across generations have been the subject of extensive investigation. To maintain genomic integrity, DNA must be protected from damage induced by environmental agents or generated spontaneously during DNA metabolism. Spontaneous DNA alterations can be due to dNTP misincorporation during DNA replication, interconversion between DNA bases caused by deamination, loss of DNA bases following DNA depurination, and modification of DNA bases by alkylation (Table 1) (Lindahl and Barnes, 2000). Additionally, oxidized DNA bases and DNA breaks can be generated by reactive oxygen species (ROS) derived from normal cellular metabolism. Altogether, it has been estimated that every cell could experience up to 10^5 spontaneous DNA lesions per day (Hoeijmakers, 2009).

Environmental DNA damage can be produced by physical or chemical sources. Examples of physical genotoxic agents are ionizing radiation (IR) and ultraviolet (UV) light from sunlight, which can also induce up to 10^5 DNA lesions (pyrimidine dimers and 6–4 photoproducts) per cell per day (Table 1) (Hoeijmakers, 2009). IR (from, e.g., cosmic radiation and medical treatments employing X-rays or radiotherapy) can induce oxidation of DNA bases and generate single-strand and double-strand DNA breaks (SSBs and DSBs, respectively) (Table 1). Chemical agents used in cancer chemotherapy can cause a variety of DNA lesions: alkylating agents such as methyl methanesulfonate (MMS) and temozolomide attach alkyl groups to DNA bases, while crosslinking agents such as mitomycin C (MMC), cisplatin, psoralen, and nitrogen mustard introduce covalent links between bases of the same DNA strand (intrastrand crosslinks) or of different DNA strands (interstrand crosslinks or ICLs). Other chemical agents, such as the topoisomerase inhibitors camptothecin (CPT) and etoposide, which inhibit topoisomerase I or II, respectively, induce the formation of SSBs or DSBs by trapping topoisomerase-DNA covalent complexes. Cigarette smoking, one of the most common mechanisms of self-inflicted DNA damage, causes a wide variety of adducts and oxidative damage in lung and other tissues. The measurement of lesions in smokers in Table 1 is likely to be a vast underestimate of the total damage produced per day, since smoke-induced DNA adducts present in normal tissues adjacent to tumors are measured as a single snapshot at the time of tumor removal, presumably hours or days after the last cigarette exposure.

To counteract DNA damage, repair mechanisms specific for many types of lesion have evolved. Mispaired DNA bases are replaced with correct bases by mismatch repair (MMR), and small chemical alterations of DNA bases are repaired by base excision repair (BER) through excision of the damaged base (Jiricny, 2006; Lindahl and Barnes, 2000). More complex lesions, such as pyrimidine dimers and intrastrand crosslinks, are corrected by nucleotide excision repair (NER) through the removal of an oligonucleotide of approximately 30 bp containing the damaged bases, while ICLs are excised by ICL repair with the assistance of proteins involved in the genetic syndrome Fanconi anemia (Hoeijmakers, 2009; Moldovan and D'Andrea, 2009). SSBs are repaired by single-strand break repair (SSBR), whereas DSBs are processed either by nonhomologous end joining (NHEJ) or homologous recombination (HR) (Caldecott, 2008; West, 2003). While NHEJ promotes the potentially inaccurate religation of DSBs, HR precisely restores the genomic sequence of the broken DNA ends by utilizing sister chromatids as template for repair.

DNA repair is carried out by a plethora of enzymatic activities that chemically modify DNA to repair DNA damage, including nucleases, helicases, polymerases, topoisomerases, recombinases, ligases, glycosylases, demethylases, kinases, and phosphatases. These repair tools must be precisely regulated,

Table 1. DNA Lesions Generated by Endogenous and Exogenous DNA Damage

Endogenous DNA Damage	DNA Lesions Generated	Number Lesions/Cell/Day	
Depurination	AP site	10000[a]	
Cytosine deamination	Base transition	100–500[a]	
SAM-induced methylation	3meA	600[a]	
	7meG	4000[a]	
	O^6meG	10–30[b]	
Oxidation	8oxoG	400–1500[c]	
Exogenous DNA Damage	Dose Exposure (mSv)	DNA Lesions Generated	Estimated Number Lesions/Cell
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day[d]
Cigarette smoke	—	aromatic DNA adducts	45–1029[e]
Chest X-rays	0.02[f,g,h]	DSBs	0.0008[i]
Dental X-rays	0.005[f,g,h]	DSBs	0.0002[i]
Mammography	0.4[f,g,h]	DSBs	0.016[i]
Body CT	7[f]	DSBs	0.28[i]
Head CT	2[f,g]	DSBs	0.08[i]
Coronary angioplasty	22[h]	DSBs	0.88[i]
Tumor PET scan (^{18}F)	10[h]	DSBs	0.4[i]
^{131}I treatment	70–150[h]	DSBs	2.8–6[i]
External beam therapy	1800–2000[j]	DSBs	72–80
Airline travel	0.005/hr[f]	DSBs	0.0002/hr[i]
Space mission (60 days)	50[k]	DSBs	2[i]
Chernobyl accident	300[l]	DSBs	12[i]
Hiroshima and Nagasaki atomic bombs	5–4000[k]	DSBs	0.2–160[i]

Type and number of DNA lesions are indicated. The number of lesions/cell has been estimated as described.
[a] Lindahl and Barnes (2000)
[b] Rydberg and Lindahl (1982)
[c] Klungland et al. (1999)
[d] Hoeijmakers (2009)
[e] DNA adducts detected in the lung of smokers following 1–2 cigarette packs per day for ~40 years (Phillips et al., 1988). Higher number of cigarettes consumed correlates with higher number of aromatic DNA adducts. Up to 6000 adducts per cell could be present in smokers, if all the types of DNA adducts generated by cigarette smoke carcinogens are taken into account. This does not include oxidative damage.
[f] http://www.merck.com/mmhe/sec24/ch292/ch292a.html
[g] http://www.fda.gov/Radiation-EmittingProducts/RadiationSafety/RadiationDoseReduction/ucm199994.htm#ft6
[h] Hall and Giaccia (2006)
[i] Based on the effective dose received by the whole body. Dose absorbed by the specific tissue irradiated may be higher. The number of DSBs has been calculated assuming that mammalian cells irradiated with 1 Sv (corresponding to 1 Gy for X- and γ rays) experience approximately 40 DSBs (Elkind and Redpath, 1977). Approximately 1000 SSBs/cell are generated following 1 Gy irradiation (Elkind and Redpath, 1977).
[j] Typical single dose administered per day in the treatment of cancer. The number of DSBs has been calculated as described above.
[k] Task Group Report of the Committee 1 of the International Commission on Radiological Protection (http://www.icrp.org/downloadDoc.asp?document=docs/Low-dose_TG_rept_for_web.pdf). The radiation dose exposure for Hiroshima and Nagasaki atomic bombs survivors is based on the Life Span Study (LSS) mortality data set (2003). The average dose for the survivors of this study is 200 mSv.
[l] Average dose for people living near the Chernobyl plant (http://www.merck.com/mmhe/sec24/ch292/ch292a.html).

because each in its own right can wreak havoc on the integrity of DNA if misused or allowed to access DNA at the inappropriate time or place. Thus, eukaryotic cells have developed strategies to recruit and activate the right factors in the right place at the right time. Here, we describe the cellular mechanisms that regulate the recruitment of DNA repair factors to sites of DNA damage, activate those factors, and coordinate the choice of the pathways to employ for efficient DNA repair. Moreover, we describe the pathological consequences that result from a defective response to DNA damage in humans.

Signal Transduction: Sending an SOS to Repair

The DNA damage response (DDR) is a signal transduction pathway that senses DNA damage and replication stress and sets in motion a choreographed response to protect the cell and ameliorate the threat to the organism (Harper and Elledge, 2007; Jackson and Bartek, 2009). The DDR is primarily mediated by proteins of the phosphatidylinositol 3-kinase-like protein kinase (PIKKs) family—ATM, ATR, and DNA-PK—and by members of the poly(ADP-ribose) polymerase (PARP) family. ATM and DNA-PK are activated by DNA-damaging agents (e.g., IR)

that create DSBs (Figure 1A) (Harper and Elledge, 2007; Meek et al., 2008). Unlike ATM, which has hundreds of substrates, DNA-PK primarily regulates a smaller group of proteins involved in DSB end joining. ATR, in complex with its partner protein ATRIP, is activated following recruitment to RPA-coated ssDNA regions generated at stalled replication forks (Figure 1B) and DSBs (Cimprich and Cortez, 2008). The PARP family has 16 members, but only PARP1 and PARP2 have been implicated in the DDR (Schreiber et al., 2006). PARP1 and PARP2 are activated by SSBs and DSBs and catalyze the addition of poly (ADP-ribose) chains on proteins to recruit DDR factors to chromatin at breaks (Figure 1A) (Schreiber et al., 2006).

Much of the current understanding of the DDR is based on the study of the ATM and ATR kinases. Following the recognition of DNA lesions by sensor proteins, ATM and ATR initially phosphorylate mediator proteins, which can amplify the DDR by acting as recruiters of ATM/ATR substrates (Zhou and Elledge, 2000). Effector proteins of the DDR are either directly phosphorylated by ATM/ATR or by the CHK1 and CHK2 kinases as well as other kinases such as CK2, p38, and MK2 (Harper and Elledge, 2007). The stability of ATM and ATR—and other PIKKs—is dependent on the TEL2-TTI1-TTI2 (Triple T) complex, which has been reported to associate with the heat shock protein HSP90 and possibly promote the maturation of newly synthesized PIKKs (Hurov et al., 2010; Takai et al., 2007, 2010).

The DDR regulates physiological processes that involve multiple layers of decisions. These include the determination to undergo apoptosis or enter terminal differentiation through senescence, the activation of heightened immune surveillance, DNA damage prophylaxis through tanning, as well as DNA repair itself (Cui et al., 2007; Gasser and Raulet, 2006; Zhou and Elledge, 2000). ATM and ATR are required for NHEJ, HR, ICL repair, and NER, as well as replication fork stability during unperturbed DNA replication and in response to replication blocks. While primarily mediated through relatively fast post-translational modifications—such as phosphorylation and inhibition of the cell-cycle phosphatase CDC25 required for CDK activation—a significant portion of the decision processes are mediated through slower transcriptional responses that allow integration of information over time. The most extensively studied component of this response is p53, which is regulated by ATM and CHK2 in response to DSBs (Figure 1A) (Zhou and Elledge, 2000). p53 induces cell-cycle arrest, apoptosis, or senescence in response to DNA damage by transcriptionally regulating, among others, the CDK inhibitor p21 and the proapoptotic BAX and PUMA proteins (Riley et al., 2008). Moreover, p53 directly activates repair pathways such as NER through regulation of the NER factors XPC and DDB2 and induces dNTP synthesis as described below (Ford, 2005). Importantly, following DSB formation, p53 is activated by ATM in a cyclically periodic manner through a transcriptional circuit involving the WIP1 phosphatase and the MDM2 E3 ubiquitin ligase, both p53 targets, which turn off ATM and p53, respectively (Batchelor et al., 2009). This provides the cell with a time-measurement mechanism that activates p53 transcriptional pulses in an oscillating fashion, depending on whether the initiating damage has been repaired. This raises the interesting possibility that each succeeding pulse occurs in a different proteome environment and could impart distinct information to the cell on the persistence of damage, directing the cell to make different decisions, such as apoptosis or senescence.

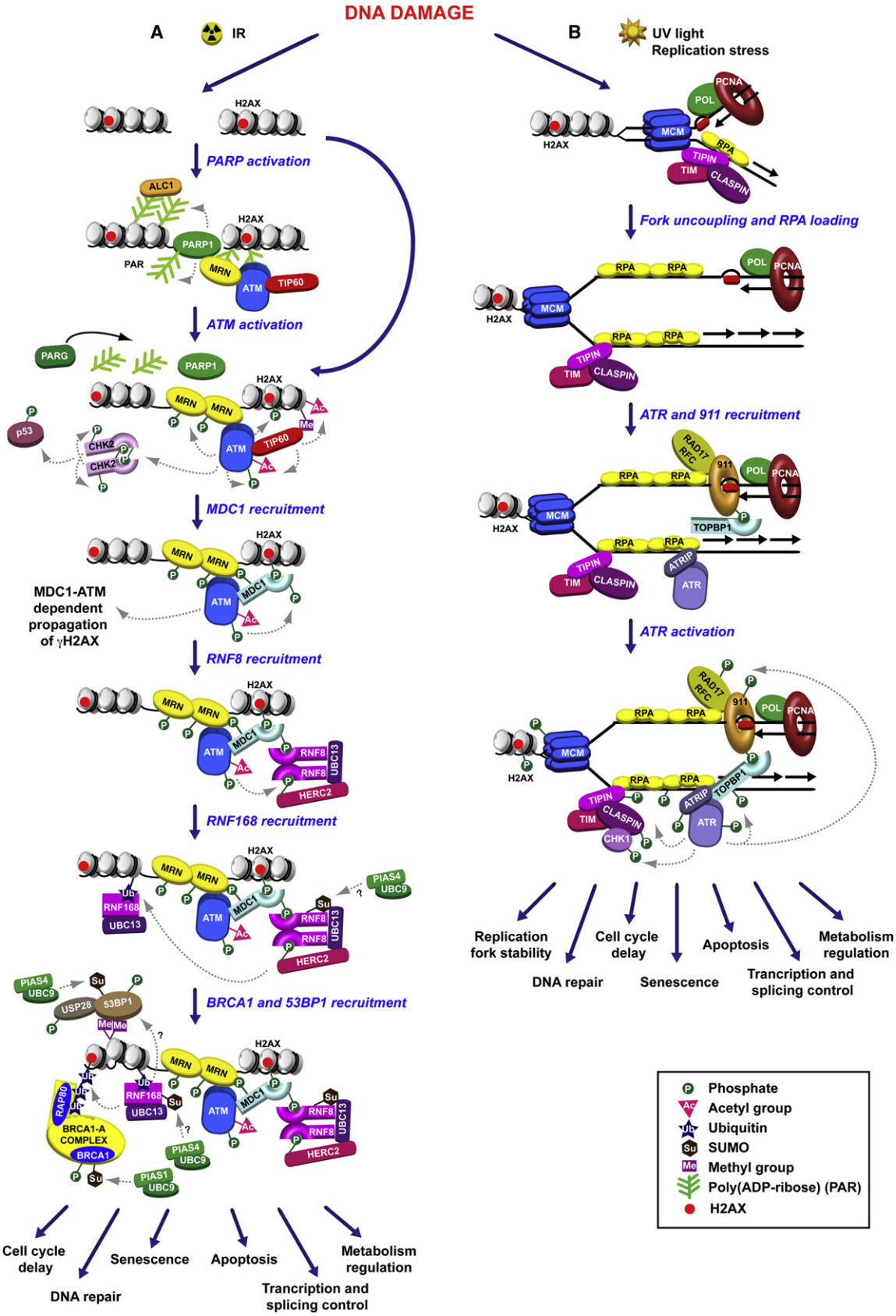
It is now clear that ATM and ATR coordinate a much wider variety of cellular activities than initially anticipated, from DNA replication and repair to transcription, metabolic signaling, and RNA splicing (Bennetzen et al., 2010; Matsuoka et al., 2007; Paulsen et al., 2009). Defective regulation of any of these activities results in genomic instability after DNA damage. In the next sections, we will focus our attention on the mechanisms employed by the DDR to regulate DNA repair in order to preserve genomic integrity.

Spatiotemporal Regulation of DNA Repair: Sensing the Damage

Localization of DDR factors to sites of DNA damage is initiated by sensor proteins that directly recognize specific DNA lesions and activate the DDR (Zhou and Elledge, 2000). Interestingly, experiments performed both in yeast and mammalian cells have demonstrated that forced tethering of sensor proteins to chromatin is sufficient to elicit the DDR cascade even in the absence of DNA damage (Bonilla et al., 2008; Soutoglou and Misteli, 2008). The next level of regulation of DNA repair resides in the DDR-regulated recruitment of factors to sites of DNA damage, which can be visualized as discrete nuclear foci by microscopy. DNA damage-induced foci are highly dynamic structures subjected to precise spatiotemporal regulation (Bekker-Jensen et al., 2006). The precise order and timing of recruitment is thought to provide a kinetic choice of repair options, presumably in an optimized order. The assembly of the DDR cascade is dependent on a broad spectrum of posttranslational modifications—phosphorylation, ubiquitination, sumoylation, poly(ADP-ribosylation), acetylation, methylation—induced by the activation of the DDR (Bergink and Jentsch, 2009; Harper and Elledge, 2007; Kleine and Lüscher, 2009; Misteli and Soutoglou, 2009). These post-translational modifications are specifically recognized by a wide variety of protein domains, many of which mediate the recruitment to DNA damage sites. In the following sections, we will highlight the different mechanisms by which different lesions are sensed, the different mechanisms by which the DDR factors are recruited to sites of DNA damage, and the consequences of DDR signaling on repair.

Single-Strand Break Repair

SSBs generated by IR and ROS or arising indirectly during BER of abasic sites and altered DNA bases, such as 8oxoG and 3meA, activate PARP family members (Caldecott, 2008). PARP1 and PARP2 act as molecular sensors of SSBs and DSBs, which are recognized by two PARP1 zinc finger motifs. Activation of PARP1 and PARP2 and subsequent synthesis of poly(ADP-ribose) (PAR) chains occurs within seconds at damage sites and is one of the earliest events of the DDR. PAR chains are rapidly disassembled by the PAR hydrolyzing enzyme PARG to provide a quick transient response lasting minutes (Schreiber et al., 2006). Upon DNA binding, PARP1/2 assembles PAR moieties from NAD^+ on target proteins, including histones H1 and H2B, and PARP1 itself (Schreiber et al., 2006). Histone PARylation is thought to contribute to chromatin reorganization and


DNA DAMAGE
A
IR
B
UV light
Replication stress
H2AX
PARP activation
ALC1
PARP1
PAR
MRN
ATM
TIP60
ATM activation
PARG
p53
CHK2
MDC1 recruitment
MDC1-ATM dependent propagation of γH2AX
RNF8 recruitment
RNF8
UBC13
HERC2
RNF168 recruitment
RNF168
UBC13
PIAS4
UBC9
BRCA1 and 53BP1 recruitment
USP28
53BP1
RAP80
BRCA1-A COMPLEX
BRCA1
PIAS1
Cell cycle delay
Senescence
Apoptosis
Metabolism regulation
DNA repair
Trancription and splicing control
PCNA
POL
MCM
RPA
TIPIN
TIM
CLASPIN
Fork uncoupling and RPA loading
ATR and 911 recruitment
RAD17
RFC
911
TOPBP1
ATRIP
ATR
ATR activation
CHK1
Replication fork stability
Phosphate
Acetyl group
Ubiquitin
SUMO
Methyl group
Poly(ADP-ribose) (PAR)
H2AX

recruitment of DNA repair and chromatin modifying complexes, such as polycomb and histone deacetylase (HDAC) complexes, at DNA damage sites (Polo et al., 2010; Schreiber et al., 2006; Chou et al., 2010).

PAR Recruitment

PAR structures act as platforms upon which to recruit factors to promote DNA repair. Three PAR-binding motifs have been described: the macrodomain, PAR-binding zinc finger (PBZ), and an 8 amino acid basic residue-rich cluster (Kleine and Lüscher, 2009). Ten human proteins contain macrodomains, including PARP9, PARP14, PARP15, the histone variant macroH2A1.1, and the chromatin remodeling factor ALC1 (Kleine and Lüscher, 2009; Schreiber et al., 2006). Recent studies have shown that macroH2A1.1 and ALC1 are recruited in a PAR-dependent manner to sites of DNA damage, where they contribute to the reorganization of chromatin structure (Ahel et al., 2009; Gottschalk et al., 2009; Timinszky et al., 2009).

Several DDR factors contain the acid basic residue-rich cluster, including p53, XRCC1, LIG3, MRE11, and ATM, whereas PBZ motifs have recently been identified in the nucleases APLF and SNM1 and in the cell-cycle checkpoint protein CHFR (Ahel et al., 2008; Gagné et al., 2008). XRCC1 and LIG3 are recruited to SSBs in a PARP1-dependent manner and promote SSB repair following DNA end processing by XRCC1-interacting proteins, such as DNA polymerase β, PNK, and the nucleases APE1, APTX, and APLF (Caldecott, 2008). APLF is dependent on the PBZ motif for its recruitment to DNA damage sites (Bekker-Jensen et al., 2007; Kanno et al., 2007; Rulten et al., 2008).

Double-Strand Break Repair

DSBs are life-threatening lesions whose repair is promoted by an intricate network of multiple DNA repair pathways. At least four independent pathways can repair DSBs: HR, NHEJ, alternative-NHEJ (alt-NHEJ), and single-strand annealing (SSA) (Figure 2). A main factor influencing the pathway choice is the extent of DNA end processing. Classical NHEJ does not require DNA end resection whereas alt-NHEJ (also known as microhomology-mediated end joining or MMEJ), HR, and SSA are dependent on DSB resection, which is limited for alt-NHEJ (5–25 nt) and more extensive for HR and SSA (Hartlerode and Scully, 2009). In addition, at least four partially independent sensors can detect DSBs: PARP, Ku70/Ku80, MRN, and with DSB processing, RPA.

DNA End Joining Promoted by Ku70/Ku80 and PARP

Double-strand DNA breaks are rapidly bound by the Ku heterodimer (Ku70 and Ku80), which has a toroidal structure with a hole through which it loads onto DSB ends (Figure 2A) (Mahaney et al., 2009). It also possesses a DNA end processing activity (Roberts et al., 2010). Ku localizes within seconds to DSBs, where it loads and activates the catalytic subunit of DNA-PK (DNA-PKcs) to initiate NHEJ (Mahaney et al., 2009).

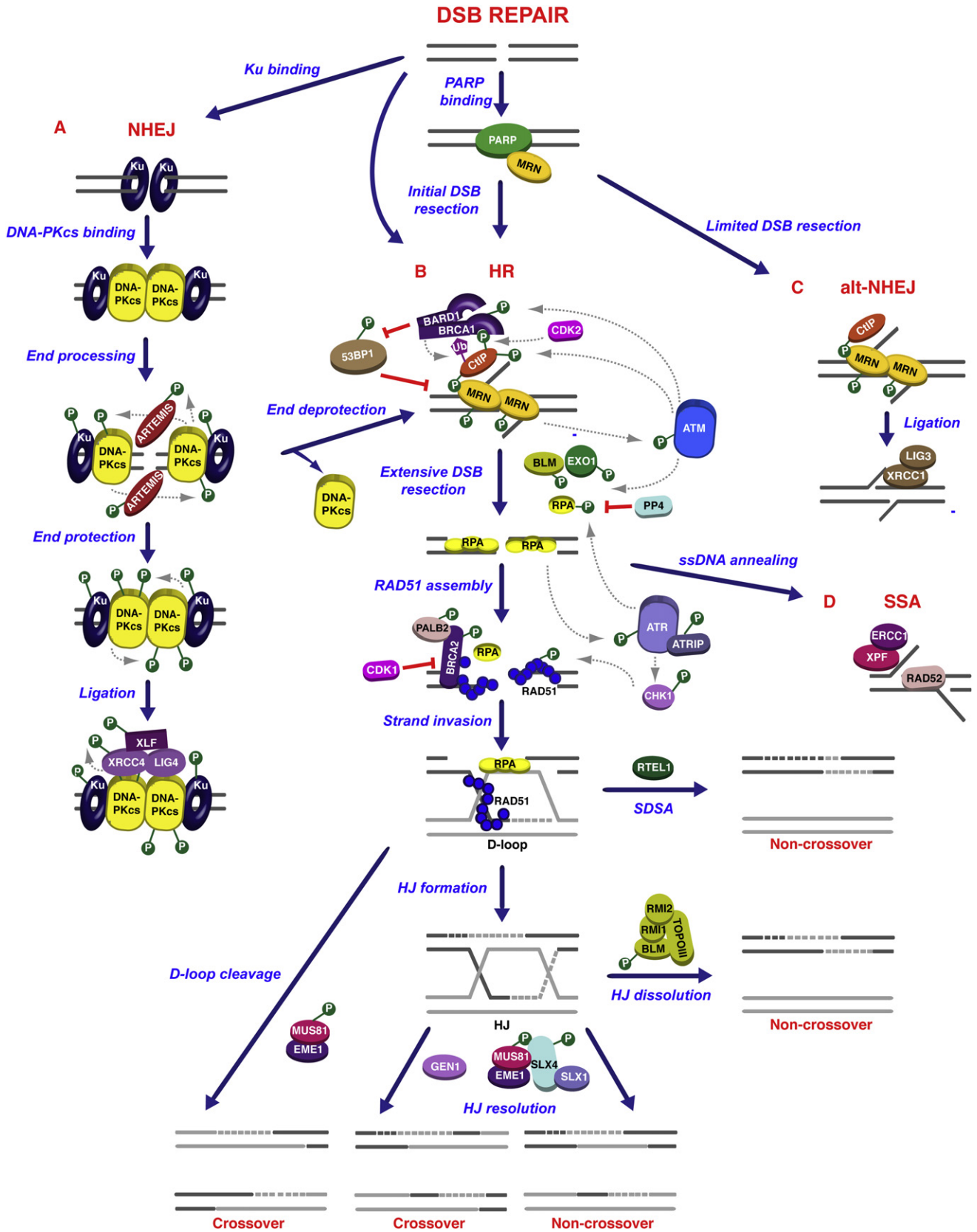
DNA-PKcs. During NHEJ, DNA-PKcs plays a critical role in stabilizing DSB ends and preventing end resection through a series of phosphorylation reactions (Figure 2A) (Meek et al., 2008). Following DSB binding, DNA-PKcs autophosphorylation on the six-residue ABCDE cluster (also known as the T2609 cluster) results in destabilization of the DNA-PKcs interaction with the DNA ends, thus providing access to end processing enzymes, such as ARTEMIS (Meek et al., 2008). Excessive end processing is then prevented by DNA-PKcs autophosphorylation on the five-residue PQR cluster (also known as the S2056 cluster), which helps protect the DNA ends (Meek et al., 2008). Interestingly, ABCDE phosphorylation, which can also be induced by ATM, has been shown to facilitate the access of DNA ends to DSB resecting enzymes in order to promote HR when NHEJ fails (Shrivastav et al., 2008). Conversely, PQR phosphorylation has an inhibitory effect on HR by preventing end resection (Meek et al., 2008). After DNA-PKcs is loaded, XRCC4/LIG4 is recruited, which promotes the religation of the broken ends with the help of the stimulatory factor XLF (Figure 2A) (Mahaney et al., 2009). DNA termini that contain nonligatable end groups are processed by the ARTEMIS and APLF nucleases and the PNK kinase/phosphatase prior to DNA ligation (Mahaney et al., 2009). All three factors are phosphorylated in an ATM-dependent manner, and ARTEMIS is a substrate for DNA-PKcs (Macrae et al., 2008; Mahaney et al., 2009; Matsuoka et al., 2007). ATM has been shown to play a role in 10% of NHEJ through ARTEMIS (Jeggo and Löbrich, 2005). This may be an underestimate because ATM and ATR often play redundant roles. Supporting this, telomeres deprotected by loss of TRF2 and POT1 resemble a DSB and undergo end-to-end fusion via NHEJ (Denchi and de Lange, 2007). This process requires either ATM or ATR as the double mutant abrogates end fusions (Denchi and de Lange, 2007).

PARP. As noted above, PARP1/2 also senses DSBs. PARP acts to promote alt-NHEJ, which functions as backup to the classical pathway of NHEJ described above (Figure 2C) (Wang et al., 2006). PARP1 also competes with Ku binding to DNA ends to promote HR (Figure 2B) (Hochegger et al., 2006). During DSB repair, PARP1 is thought to mediate the initial accumulation of the MRN complex to DSBs in a γH2AX- and MDC1-independent manner (Haince et al., 2008). Recruitment of ATM by MRN and PARP1 could then contribute to the activation of the γH2AX cascade and stabilization of DDR factors at sites of damage as discussed below (Figure 1A) (Haince et al., 2007). Indeed, PARP1 plays an initial role in the DDR by facilitating ATM activation, as indicated by the delayed phosphorylation of ATM substrates observed in the absence of PARP1 following

Figure 1. Schematic Model for ATM and ATR Activation in Response to DNA Damage

(A) Formation of DSBs following IR treatment activates PARP1, which mediates the initial recruitment of the MRN/ATM complex at DSBs. Activation of the ATM kinase activity by MRN and TIP60 leads to the phosphorylation of CHK2 and p53, in addition to a large number of other DDR factors, and the induction of the γH2AX-dependent signaling cascade, which results in the recruitment of MDC1, RNF8, RNF168, BRCA1, and 53BP1 to DSBs, as described in greater detail in the main text.

(B) DNA lesions induced by UV light or replication stress (denoted by red rectangular shapes) lead to replication fork stalling and accumulation of RPA-coated ssDNA regions, which recruit the ATR/ATRIP and the RAD17/RFC2-5 complexes. Loading of the 9-1-1 complex by RAD17/RFC2-5 and stimulation of the ATR kinase activity by the 9-1-1-associated protein TOPBP1 result in the activation of the ATR signaling cascade and CHK1 phosphorylation. Posttranslational modifications of the DDR factors depicted here are represented by different colored shapes, as indicated by the legend at the bottom of the figure.


DSB REPAIR
Ku binding
PARP binding
A
NHEJ
Ku
PARP
MRN
Initial DSB resection
Limited DSB resection
DNA-PKcs binding
DNA-PKcs
B
HR
C
alt-NHEJ
End processing
ARTEMIS
BARD1
BRCA1
CDK2
53BP1
Ub
CtIP
End deprotection
ATM
BLM
EXO1
RPA
PP4
Extensive DSB resection
Ligation
LIG3
XRCC1
End protection
ssDNA annealing
RAD51 assembly
D
SSA
PALB2
BRCA2
CDK1
ATR
ATRIP
CHK1
ERCC1
XPF
RAD52
Strand invasion
RAD51
D-loop
RTEL1
SDSA
Non-crossover
XLF
XRCC4
LIG4
HJ formation
RMI2
RMI1
TOPOIII
BLM
HJ dissolution
D-loop cleavage
MUS81
EME1
HJ
GEN1
SLX4
SLX1
HJ resolution
Crossover
Crossover
Non-crossover

treatment with DNA-damaging agents (Haince et al., 2007). However, PARP1/2 and ATM also have independent functions, as shown by the synthetic lethality of *PARP1* (or *PARP2*) deletion with *ATM* deficiency in mice (Huber et al., 2004).

Homologous Recombination Repair through MRN-ATM and RPA-ATR

DSBs can also be recognized by the MRE11-RAD50-NBS1 (MRN) complex, which promotes the activation of ATM and the preparation of DNA for HR (Figures 1 and 2) (Williams et al., 2007). RAD50, a member of the SMC family, contains ATPase domains that interact with MRE11 and associates with the DNA ends of the DSB (Williams et al., 2007). In addition to stabilizing DNA ends, MRE11 has endonuclease and exonuclease activities important for the initial steps of DNA end resection that is essential for HR, as described below (Williams et al., 2007). The third subunit of the MRN complex, NBS1, interacts with MRE11 and contains additional protein-protein interaction domains important for MRN function in the DDR. NBS1 associates with ATM via its C-terminal region, which promotes the recruitment of ATM to DSBs, where ATM is activated by the MRN complex by yet to be defined mechanisms, possibly involving the formation of ssDNA oligos during end resection (Figure 1A) (Jazayeri et al., 2008; Kanaar and Wyman, 2008; Lee and Paull, 2005).

Resection Control. DNA end resection is regulated by ATM through CtIP, which interacts with BRCA1 and MRN in the BRCA1-C complex (Figure 2B) (Huen et al., 2010b). DSB resection is primarily induced in the S and G2 phases of the cell cycle, when sister chromatids can be used for HR (You and Bailis, 2010). Limited DSB resection is carried out by CtIP in G1 in a BRCA1-independent manner to promote alt-NHEJ, which is mediated by the annealing of ssDNA microhomology regions, followed by LIG3-dependent DNA end ligation (Figure 2C) (You and Bailis, 2010; Yun and Hiom, 2009). In S and G2, CtIP associates with BRCA1, which ubiquitinates CtIP and facilitates its association with damage sites (Huen et al., 2010b). CtIP recruitment is also dependent on MRN and ATM kinase activity, helping explain ATM's role in DSB resection (You and Bailis, 2010). Two and seven ATM phosphorylation sites have been identified in CtIP and BRCA1, respectively, but their precise function remains to be elucidated (Ouchi, 2006; You and Bailis, 2010). In addition, EXO1, which is involved in the processive stage of DSB resection together with BLM following the initial resection carried out by CtIP, is also stimulated by ATM phosphorylation (Figure 2B) (Bolderson et al., 2010). Moreover, ARTEMIS, which is also regulated by ATM, has been suggested to play a role in DSB resection (Beucher et al., 2009).

Pathway Choice. The orderly progression of choices between alternative DNA repair pathways could be facilitated by negative regulation of one pathway by another. Indeed, DSB resection promoted by CtIP and ATM can be inhibited by 53BP1 (Figure 2B) (Bunting et al., 2010). 53BP1 has been suggested to promote NHEJ by increasing the stability and mobility of DSBs to find each other for productive ligation (Difilippantonio et al., 2008; Dimitrova et al., 2008). Loss of 53BP1 partially rescues the HR defect of BRCA1 mutant cells, suggesting that BRCA1 might somehow overcome 53BP1 function at DSBs in order to promote DSB resection (Bouwman et al., 2010; Bunting et al., 2010). Defective DSB resection in BRCA1 mutant cells results in NHEJ-dependent chromosomal rearrangements, whose formation could be prevented by 53BP1 loss (Bunting et al., 2010). Interestingly, DSB resection induced by *53BP1* deletion was shown to increase alt-NHEJ and decrease classical NHEJ during immunoglobulin maturation in G1 phase B cells (Bothmer et al., 2010). It is known that abnormal activity of alt-NHEJ in the absence of functional NHEJ induces chromosomal translocations in mammalian cells (Simsek and Jasin, 2010). Altogether, these observations indicate that alterations of the correct balance between DSB repair pathways can lead to genomic instability. Another case in point, the chromosomal instability defects and DNA damage sensitivity of Fanconi anemia (FA) mutant cells have recently been shown to be due to aberrant NHEJ, indicating that FA proteins might promote HR and suppress NHEJ (Adamo et al., 2010; Pace et al., 2010). Thus, in the absence of the proper repair pathway choice, incorrect pathway choices can be deleterious.

The RPA Platform. DSB resection and formation of 3′ ssDNA ends leads to RPA accumulation (Figure 2B). RPA is an essential heterotrimeric complex (RPA1, RPA2, RPA3) that stabilizes ssDNA regions generated during DNA replication and repair (Wold, 1997). RPA-ssDNA complexes play a critical role in activation of the ATR pathway, as described in greater detail below. In the presence of repetitive DNA sequences that are repaired by SSA, annealing of the resected 3′ ssDNA could be catalyzed by RAD52, followed by removal of DNA flaps by XPF/ERCC1 (Figure 2D) (Hartlerode and Scully, 2009; Motycka et al., 2004). Alternatively, assembly of RAD51 filaments on RPA-coated ssDNA mediated by BRCA2 can lead to HR (Figure 2B) (West,

Figure 2. Alternative DNA Repair Pathways Involved in the Repair of Double-Strand Breaks

(A) Rapid association of Ku to DSBs promotes NHEJ by recruiting DNA-PKcs. Sequential phosphorylation events on multiple DNA-PKcs amino acid clusters favors the initial processing of DNA ends by ARTEMIS, followed by DNA-PKcs-dependent protection of DNA ends required for DNA ligation.

(B) Alternatively to NHEJ, MRN, which is initially recruited to DSBs by PARP in competition with Ku, mediates the initial stages of DSB resection together with CtIP and BRCA1 to promote homologous recombination in S and G2. 53BP1 has an inhibitory role on DSB resection and is negatively regulated by BRCA1 by unknown mechanisms. The MRN/CtIP/BRCA1 complex can also promote DSB resection following deprotection of DNA ends when NHEJ fails. Extensive DSB resection and formation of RPA-coated 3′ ssDNA ends is induced by EXO1 and BLM. ATM plays a central role in the regulation of DSB resection, as described in the main text. Displacement of RPA from the 3′ ssDNA ends and assembly of RAD51 filaments mediated by BRCA2 leads to strand invasion into homologous DNA sequences. Recruitment of RAD51 to ssDNA ends is regulated by the ATR pathway, which is activated following DSB resection. D loop structures formed after strand invasion can be cleaved by MUS81/EME1 or displaced by RTEL1 during SDSA to generate crossover or noncrossover events, respectively. Noncrossovers are generated also by dissolution of Holliday junctions (HJs) by the BLM/TOPOIII complex, whereas HJ resolution by the nucleases GEN1 and SLX1/SLX4, which associates with MUS81/EME1, can generate both crossover and noncrossover events.

(C) Limited DSB resection carried out by CtIP and MRN in G1 results in alternative NHEJ.

(D) Following DSB resection, 3′ ssDNA ends with homologous sequences can be directly annealed by RAD52. Posttranslational modifications are indicated as in Figure 1.

2003). The interaction between RAD51 and BRCA2 C terminus, which is important for HR, is thought to be limited to S and G2 phases of the cell cycle by CDK-dependent phosphorylation of BRCA2 (Esashi et al., 2005). Further regulation of HR is provided by RAD51 phosphorylation mediated by CHK1, which is required for RAD51 recruitment to damage sites (Sørensen et al., 2005). BRCA2 is also phosphorylated by ATM/ATR (Matsuoka et al., 2007). Moreover, RPA2 undergoes ATM/ATR-mediated hyperphosphorylation followed by PP4-dependent dephosphorylation, which was shown to be important for HR (Lee et al., 2010a). Furthermore, sumoylation of RPA1 was recently suggested to promote HR by facilitating the recruitment of RAD51 (Dou et al., 2010).

Crossover Regulation. Following RAD51-dependent strand invasion into homologous sequences of the sister chromatid and formation of D loop structures, the 3′ invading strand could be extended by DNA polymerases and then reanneal to the processed second end of the break (West, 2003). This pathway, which is known as synthesis-dependent strand annealing (SDSA), is thought to be promoted by the RTEL helicase after displacement of the RAD51 filament and D loop dissociation (Figure 2B) (Barber et al., 2008). Alternative to this pathway, double Holliday junctions (dHJs) could be formed after ligation of the invading strand with the second end captured by D loop branch migration (West, 2003). HJ intermediates could be dissolved by the BLM/TOPOIII complex or cleaved by the endonucleases GEN1, MUS81/EME1, or SLX1/SLX4 to generate either crossover or noncrossover of the markers flanking the dHJ (Figure 2B) (Andersen et al., 2009; Ciccia et al., 2008; Fekairi et al., 2009; Ip et al., 2008; Muñoz et al., 2009; Svendsen et al., 2009). Crossover events, which could be generated by GEN1, MUS81/EME1, or SLX1/SLX4, are highly regulated, as they can lead to loss of heterozygosity and genomic rearrangements in mitotic cells. Indeed, the high increase in the number of crossover events, which can be visualized as sister chromatid exchanges or SCEs, displayed by BLM defective cells causes genomic instability (Chu and Hickson, 2009). It is known that BLM and SLX4 are ATM/ATR substrates and yeast MUS81 is inhibited by phosphorylation by the yeast CHK2 ortholog (Bachrati and Hickson, 2008; Kai et al., 2005; Svendsen et al., 2009). How phosphorylation of BLM, SLX4, and MUS81 might affect their activity on HJ intermediates has not been determined. Nonetheless, the importance of both ATM and ATR in HR is indicated by the strong recombination defects displayed by cells with ATM or ATR deficiency (Beucher et al., 2009; Wang et al., 2004).

γH2AX: Phosphorylation-Dependent Recruitment and Modification Cascades

ATM and ATR promote DSB repair in part through phosphorylation-dependent recruitment of DDR factors to sites of DNA damage. A critical aspect of this process involves the phosphorylation of Ser139 on the specialized histone H2AX called γH2AX (Figure 1A) (Rogakou et al., 1998). H2AX phosphorylation spreads for distances up to 1–2 megabases around DSBs in an ATM- and MDC1-dependent manner and initiates a cascade of factor assembly (Harper and Elledge, 2007). MDC1 directly binds the phospho-Ser139 of H2AX through its C-terminal BRCT repeats (Figure 1A) (Stucki, 2009). H2AX Tyr142 is constitutively phosphorylated by the kinase WSTF, a member of the BAZ/WAL family of chromatin remodeling enzymes, and blocks MDC1 recruitment (Xiao et al., 2009). Following DNA damage, Tyr142 is dephosphorylated by the tyrosine phosphatases EYA1 and EYA3 (Cook et al., 2009; Krishnan et al., 2009). Interestingly, MDC1 binding to γH2AX was shown to depend on Tyr142 dephosphorylation by EYA1/3, whereas the proapoptotic kinase JNK1 was reported to associate with H2AX phosphorylated on both Ser139 and Tyr142 (Cook et al., 2009). This observation has led to the proposal that Tyr142 phosphorylation of γH2AX might provide a molecular switch between JNK-mediated apoptosis and MDC1-dependent DSB repair (Stucki, 2009). In particular, MDC1 has been reported to facilitate both NHEJ and HR in H2AX-dependent manner (Hartlerode and Scully, 2009).

The MDC1 Platform. MDC1 associates with the FHA and BRCT motifs of NBS1 through multiple SDTD sites that are constitutively phosphorylated by CK2 (Chapman and Jackson, 2008; Melander et al., 2008; Spycher et al., 2008; Wu et al., 2008). Moreover, MDC1 binds ATM through its FHA domain to further propagate γH2AX spreading (Figure 1A) (Lou et al., 2006). Formation of extensive γH2AX regions is important for sustaining the DDR, as H2AX is not required for the initial localization of NBS1, BRCA1, and 53BP1 at DSBs via PARP but rather for the maintenance of these DDR factors at sites of damage (Celeste et al., 2002). Stabilization of DDR factor recruitment to γH2AX nucleosomes is achieved through the recruitment of an intricate network of chromatin modifying enzymes regulating ubiquitination, sumoylation, acetylation, and methylation, as described below. In addition, experiments in yeast and mammalian cells have shown that chromatin remodeling enzymes, such as the SNF2 family protein INO80 and SWI/SNF, are recruited to DSBs in a γH2AX-dependent manner (Lee et al., 2010b; van Attikum and Gasser, 2009). INO80 is thought to promote nucleosome eviction to facilitate DSB resection and HR. Similarly, SWI/SNF is known to stimulate chromatin relaxation at DSBs. Accumulation of SWI/SNF at DNA damage sites is facilitated by its interaction with the BRCT domain-containing protein MCPH1/BRIT1, which directly associates with γH2AX in an MDC1-independent manner (Lin et al., 2010).

Ubiquitin-Mediated Recruitment. DDR-dependent MDC1 phosphorylation and recruitment to γH2AX initiates a ubiquitination cascade at sites of DNA damage that primarily involve protein monoubiquitination or Lys63 (K63)-linked polyubiquitination (Messick and Greenberg, 2009). The ubiquitin ligase RNF8 associates to MDC1 phospho-TQ sites through an N-terminal FHA domain and activates a DDR-induced ubiquitination cascade by K63-linked ubiquitination of H2A and γH2AX (Figure 1A) (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007). The E3 ubiquitin ligase RNF168 then binds these chains through its motifs interacting with ubiquitin (MIU) to stimulate K63 ubiquitination (Doil et al., 2009; Stewart et al., 2009). A third E3 ubiquitin ligase, HERC2, interacts with the FHA domain of RNF8 in a phosphorylation-dependent manner and facilitates the assembly of the RNF8/UBC13 complex, further stimulating K63-linked ubiquitin ligase activity (Bekker-Jensen et al., 2010). The K63-ubiquitin chains generated by RNF8 and RNF168 are recognized by the ubiquitin-interacting motif (UIM) of RAP80, which recruits the BRCA1-A complex, itself an E3 ligase, through the interaction with the scaffold protein ABRA1

(Figure 1A) (Huen et al., 2007; Kolas et al., 2007; Wang and Elledge, 2007). The BRCA1-A complex additionally includes the ubiquitin-conjugating enzyme variant (UEV) motif containing protein BRE, the ubiquitin protease BRCC36, and the adaptor protein NBA1 and has structural similarities to the proteasome lid (Feng et al., 2009; Shao et al., 2009; Wang et al., 2009). Ubiquitin binding activity has been shown for several subunits of the BRCA1-A complex, including ABRA1, BRE, BRCC36, NBA1, and RAP80 (Wang et al., 2009). Interestingly, recent observations have shown that the deubiquitinating enzyme OTUB1 suppresses RNF168-dependent ubiquitination by direct inhibition of UBC13 (Nakada et al., 2010).

SUMO-Mediated Recruitment. It has recently been reported that the accumulation of the BRCA1-A complex at DSBs is also dependent on sumoylation (Galanty et al., 2009; Morris et al., 2009). Indeed, the SUMO ligases PIAS1 and PIAS4 have been shown to localize at sites of DNA damage, and PIAS4 was reported to stimulate the ubiquitin ligase activity of the RNF8/UBC13 complex, thus promoting the recruitment of RNF168 and BRCA1 to sites of DNA damage (Figure 1A). PIAS1 has instead been proposed to directly sumoylate BRCA1 and stimulate its ubiquitin ligase activity (Galanty et al., 2009; Morris et al., 2009). Therefore, PIAS1 and PIAS4 might function as regulators of the ubiquitin ligase activity of RNF8 and BRCA1, respectively. In addition, 53BP1 recruitment to DSBs depends on the SUMO ligase PIAS4, which is also thought to directly sumoylate 53BP1 (Figure 1A) (Galanty et al., 2009). Given that 53BP1 does not display ubiquitin binding activity, it has been proposed that RNF8/RNF168 ubiquitination might induce chromatin relaxation and subsequent exposure of dimethyl H4K20—and possibly dimethyl H3K79—which are recognized by 53BP1 tudor domains (FitzGerald et al., 2009). H3K79 and H4K20 dimethylation levels do not appear to change after DNA damage, indicating that de novo histone methylation is not promoting 53BP1 recruitment (FitzGerald et al., 2009). 53BP1 is known to associate with the deubiquitinating enzyme USP28 and PTIP, a six-BRCT motif-containing protein, in a phospho-dependent manner (Mohammad and Yaffe, 2009; Zhang et al., 2006). Despite the interaction with 53BP1, PTIP appears to be recruited to damage sites in a 53BP1-independent but RNF8/UBC13-dependent manner (Mohammad and Yaffe, 2009).

All of the complexities in modifications that are responsible for recruiting these many factors to sites of DNA damage or replication stress pose a significant conundrum for the field. Why do we need so many different modifications to build these structures? Is this an evolutionary accident or is there method in this madness? One possibility is that each layer of modification, i.e., phosphorylation, ubiquitination, sumoylation, and acetylation, recruits a different constellation of factors with distinct repair capabilities. Since the focus of proteins is built in layers, there could be a kinetic ordering of repair choices evident in each layer. Furthermore, since multiple E3 ligases (RNF8, RNF168, HERC2, BRCA1/BARD1) and deubiquitinating enzymes (USP28, BRCC36) are simultaneously recruited, phosphorylated, and modified themselves, significant regulation of modifications could occur within these structures, thereby directing repair choices as events unfold. For example, if a DSB is clean, it can be directly religated. If it is not, nucleolytic restructuring must occur. If the break is not held together, factors that promote end searching may be required to bring the ends into proximity for repair, as proposed for 53BP1. If this attempt fails, unwinding or resection of the ends might occur to allow a search for microhomologies for alt-NHEJ or more extensive homologies as for classical RAD51-mediated HR. These choices require different, often competing repair activities that must be temporally and structurally coordinated in a manner that optimally deals with the eventualities that occur with different repair events. Multiple modification layers may allow exquisite control of these choices. Clearly, it will be critical to dissect the contribution of each individual focus component to different repair pathways in order to understand how these dynamic structures influence repair choices.

DNA Replication Stress, Fork Stalling, and ICLs

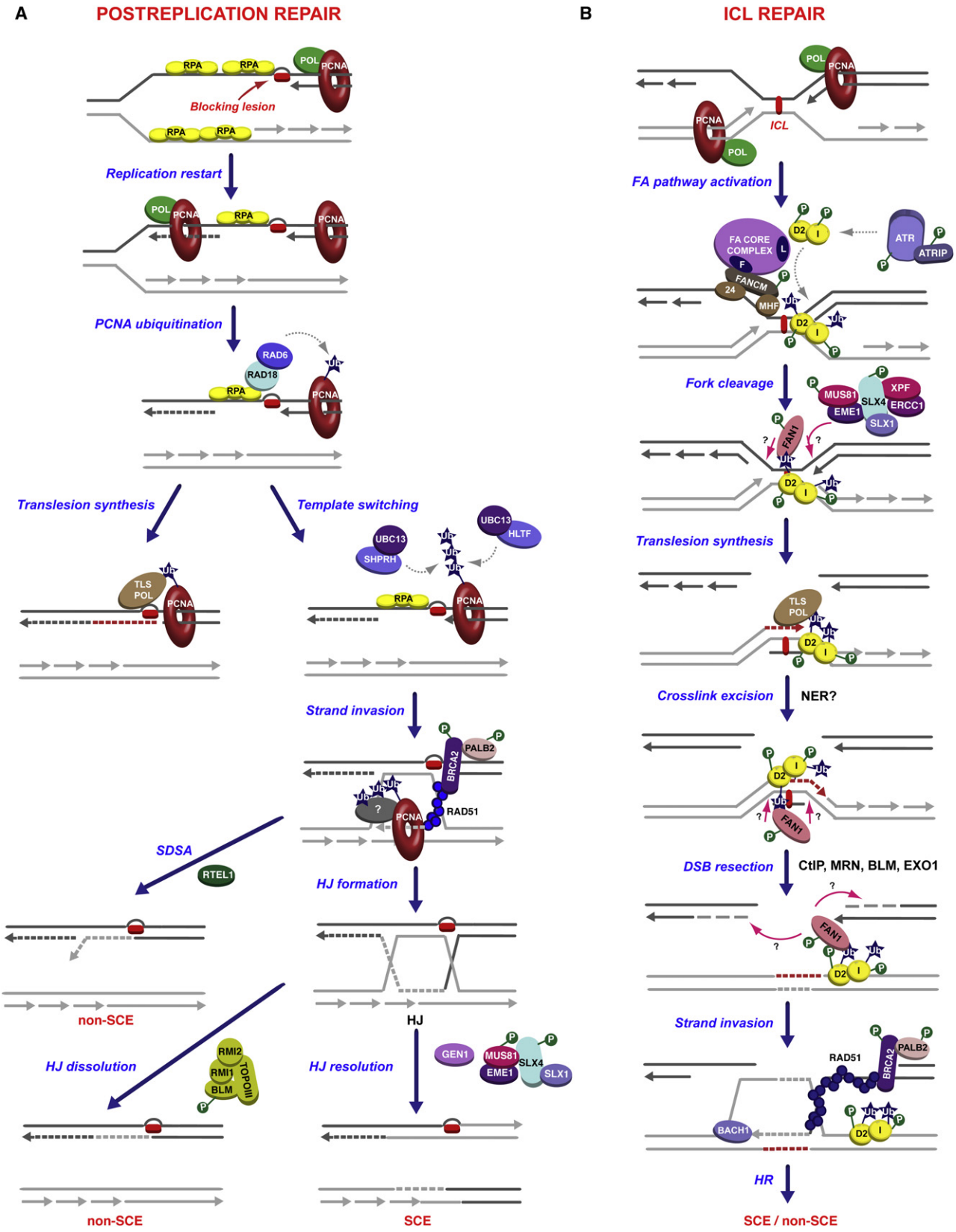
Perhaps the most dangerous lesion facing cells is the stalling of a replication fork. Failure to properly overcome such lesions leads to an inability to complete chromosome duplication and can lead to mitotic catastrophe, complex chromosomal rearrangements, and cell death. Cells have evolved multiple mechanisms to sense and respond precisely to these catastrophic types of lesions.

RPA: Sensor of DNA Replication Stress

Bulky DNA lesions can lead to arrest of leading strand synthesis at the replication fork and formation of extensive RPA-coated ssDNA regions due to the uncoupling between the MCM helicase and the DNA polymerase (Figure 1B) (Byun et al., 2005). RPA1 polymerizes on this ssDNA to generate a platform that activates the central signaling pathway orchestrating DNA replication responses, the ATR pathway. RPA-ssDNA complex recruits the ATR/ATRIP complex through direct interaction with ATRIP to localize it to the fork (Figure 1B) (Zou and Elledge, 2003). Furthermore, it stimulates binding and activation of the RAD17-RFC2-5 clamp loader, which loads the PCNA-related RAD9-HUS1-RAD1 (9-1-1) heterotrimer bound to the ATR-activating TOPBP1 protein and stimulates ATR kinase activity (Cimprich and Cortez, 2008; Ellison and Stillman, 2003; Kumagai et al., 2006; Mordes et al., 2008; Zou et al., 2003). The 9-1-1 complex is loaded onto 5′ or 3′ DNA ends adjacent to RPA-coated ssDNA regions (Cimprich and Cortez, 2008). It is the colocalization of these two RPA-dependent complexes that sets in motion the ATR signaling cascade, which results in the activation of CHK1 and CHK2 kinase signaling and phosphorylation of many chromatin bound factors to promote fork stability and restart of stalled or collapsed replication forks in order to complete chromosome replication (Cimprich and Cortez, 2008).

Restart of Stalled or Collapsed Replication Forks

Replication forks are fragile DNA structures that must be stabilized when fork progression is arrested by DNA lesions. Fork stability is promoted by the TIM/TIPIN complex and CLASPIN, both ATR targets (Errico and Costanzo, 2010). TIPIN and its partner protein, TIMELESS, associate with RPA2 to stabilize stalled forks and promote the accumulation of CHK1 and its regulatory protein CLASPIN to RPA-ssDNA regions where CHK1 can be activated by ATR (Figure 1B) (Kemp et al., 2010). Restart of stalled replication forks is dependent on several


A
POSTREPLICATION REPAIR
RPA
POL
PCNA
Blocking lesion
Replication restart
PCNA ubiquitination
RAD6
RAD18
Ub
Translesion synthesis
Template switching
TLS POL
UBC13
HLTF
SHPRH
Strand invasion
PALB2
BRCA2
RAD51
SDSA
RTEL1
HJ formation
non-SCE
HJ
HJ dissolution
RMI2
RMI1
BLM
TOPOIII
HJ resolution
GEN1
MUS81
EME1
SLX4
SLX1
non-SCE
SCE
B
ICL REPAIR
ICL
FA pathway activation
FA CORE COMPLEX
L
F
FANCM
24
MHF
D2
I
ATR
ATRIP
Fork cleavage
XPF
ERCC1
FAN1
Translesion synthesis
Crosslink excision
NER?
DSB resection
CtIP, MRN, BLM, EXO1
Strand invasion
BACH1
HR
SCE / non-SCE

DNA helicases or translocases, including BLM, WRN, FANCM, HLTF, and SMARCAL1, many of which are recruited to forks by RPA or interact with RPA at forks (Bachrati and Hickson, 2008; Driscoll and Cimprich, 2009; Luke-Glaser et al., 2010; Unk et al., 2010). In particular, SMARCAL1 interacts with RPA2 through an RPA2 interaction motif common to TIPIN, RAD52, XPA, and UNG2 (Bansbach et al., 2009; Ciccia et al., 2009; Postow et al., 2009; Yuan et al., 2009; Yusufzai et al., 2009). SMARCAL1, BLM, WRN, and FANCM undergo ATM/ATR-dependent phosphorylation after DNA damage (Bachrati and Hickson, 2008; Bansbach et al., 2009; Postow et al., 2009; Whitby, 2010; Yuan et al., 2009). BLM phosphorylation by ATR was shown to be important for recovery after replication stress, and FANCM phosphorylation results in its tighter association with chromatin (Bachrati and Hickson, 2008; Kim et al., 2008). BLM, WRN, FANCM, and HLTF have been suggested to regress replication by favoring the annealing of the leading- and lagging-strands and generating a HJ-like structure also known as "chicken foot," which could allow the restart of DNA synthesis by template switching and lesion bypass (Atkinson and McGlynn, 2009; Blastyák et al., 2010). SMARCAL1 might also promote replication fork regression, given the similarity between the helicase domain of SMARCAL1 and yeast Rad5, which was shown to regress replication forks (Atkinson and McGlynn, 2009; Blastyák et al., 2007). Future studies will be needed to uncover the coordination between these enzymes at the replication fork.

SSBs encountered by the replication fork can be converted into DSBs during DNA synthesis, thus inducing fork collapse. Replication fork collapse could also be induced by direct fork cleavage by the MUS81/EME1 endonuclease following replication arrest (Ciccia et al., 2008; Hanada et al., 2007). Different from DSBs formed in nonreplicating regions, collapsed replication forks contain one-ended DSBs. Repair of one-ended DSBs is carried out by the break-induced replication (BIR) pathway, which involves DSB resection, strand invasion, and reassembly of a new replication fork at RAD51-generated D loop intermediates (Llorente et al., 2008). PARP1/2 could facilitate processing of DSBs generated after replication stress by recruiting MRN (Bryant et al., 2009). Incorrect regulation of BIR could lead to multiple rounds of strand invasion and DNA synthesis at nonperfectly homologous DNA sequences, thus leading to chromosomal rearrangements (Llorente et al., 2008). BIR has been observed in yeast; however, its role in replication fork recovery in higher eukaryotes has not yet been visualized.

PCNA and Postreplication Repair of ssDNA Gaps

Replication restart of stalled replication forks could be accomplished by reinitiation of leading- and lagging-strand synthesis downstream of the DNA lesion, as demonstrated in bacteria and yeast (Figure 3A) (Branzei and Foiani, 2010). Replication restart would leave behind the replication fork ssDNA gaps, which in yeast have been shown to be subsequently repaired by either translesion synthesis (TLS) or error-free postreplication repair (PRR) in a manner dependent on the RAD6/RAD18 ubiquitin ligase complex (Branzei and Foiani, 2010; Daigaku et al., 2010; Karras and Jentsch, 2010). Whereas TLS involves the direct bypass of the DNA lesion using the 3′ end of the DNA filament arrested, error-free PRR is thought to promote strand invasion and repair of the ssDNA gaps by template switch and HR (Budzowska and Kanaar, 2009). TLS and error-free PRR are thought to be dependent, respectively, on monoubiquitination or K63-linked polyubiquitination of PCNA (Figure 3A) (Ulrich and Walden, 2010). PCNA monoubiquitination is promoted by the RAD6/RAD18 complex, which is recruited by RPA to unreplicated ssDNA regions (Davies et al., 2008). Monoubiquitinated PCNA can be further subjected to K63-linked polyubiquitination by the ubiquitin ligases UBC13, HLTF, and SHPRH (Unk et al., 2010). HLTF and SHPRH are the mammalian orthologs of yeast Rad5 (Unk et al., 2010).

The PCNA Platform. PCNA is a homotrimeric DNA sliding clamp that functions as processivity factor for DNA polymerases (Moldovan et al., 2007). PCNA, which is loaded by the clamp loader RFC at 3′ primer-template junctions, tethers DNA polymerases to DNA and serves as loading platform for proteins that operate in conjunction with DNA synthesis. PCNA accumulates rapidly at sites of DNA damage, where it recruits DDR factors containing PCNA interaction motifs (Moldovan et al., 2007). How PCNA accumulates on DNA and whether that is regulated by the DDR is not yet clear, although RFC is phosphorylated in response to DNA damage (Matsuoka et al., 2007). Three PCNA interaction motifs are known: the PCNA-interacting protein (PIP)-box, the AlkB homolog 2 PCNA-interacting motif (APIM), and the KA-box (Gilljam et al., 2009; Moldovan et al., 2007; Xu et al., 2001). More than 400 human proteins with putative PIP-box or APIM motif have been identified based on bioinformatic analyses (http://tare.medisin.ntnu.no/pcna/index.php). Approximately 30 PIP-box-containing proteins have currently been shown to directly interact with PCNA, including TLS polymerases and NER, BER, and MMR factors (Moldovan et al., 2007). Following DNA damage, the accumulation of PIP-box-containing proteins, such as TLS polymerases, was shown to

Figure 3. Repair of DNA Lesions Encountered during DNA Replication

(A) Postreplication repair of ssDNA gaps. Leading strand synthesis arrested at DNA lesions (red rectangular shapes) can be reprimed downstream of lesions, leaving ssDNA gaps behind the replication fork. Repair of ssDNA gaps is mediated by RAD6 and RAD18, which are recruited by RPA to ssDNA gaps, where they monoubiquitinate PCNA. Monoubiquitinated PCNA associates with translesion polymerases, which promote lesion bypass. Alternatively, polyubiquitination of PCNA by SHPRH, HLTF, and UBC13 induces template switching and strand invasion into homologous sequences of the sister chromatid. Template switching could possibly involve proteins interacting with polyubiquitinated PCNA. Resolution of Holliday junctions (HJs) formed after strand invasion can then result in sister chromatid exchanges (SCEs), whereas HJ dissolution and SDSA do not generate SCEs.

(B) Repair of interstrand crosslinks. Converging replication forks blocked by interstrand crosslinks (red rectangle) activate the FA pathway. The FA core complex associated to blocked replication forks through the FANCM complex promotes the monoubiquitination of the FANCD2/FANCI (ID) complex. Phosphorylation of FANCI by ATR regulates the ubiquitination of the ID complex and its subsequent relocalization to blocked replication forks. Monoubiquitinated ID complex promotes fork cleavage, probably through the interaction with FAN1 and possibly other nucleases, translesion synthesis, and crosslink excision. DSB resection, which could be dependent on FAN1, in addition to CtIP/MRN, BLM, and EXO1, leads to strand invasion and homologous recombination with or without formation of SCEs as described in (A). Posttranslational modifications are indicated as in Figure 1.

be dependent on a functional PIP-box (Moldovan et al., 2007). Given the intense competition among PIP-box-containing proteins for the same binding pocket of PCNA (the interdomain-connecting loop that connects the PCNA monomers), the PCNA binding affinity of specific DDR factors after DNA damage is regulated by DDR-induced PCNA posttranslational modifications, including RAD6/RAD18-dependent PCNA ubiquitination. Indeed, TLS polymerases associate with monoubiquitinated PCNA through UBZ or UBM ubiquitin binding motifs, which then provide an additional PCNA interaction surface required to target TLS polymerases to sites of DNA damage (Bienko et al., 2005; Ulrich and Walden, 2010). Monoubiquitination of the TLS polymerase η (Polη) has been proposed to promote the intramolecular interaction between ubiquitin and the UBZ domain of Polη, thus impairing its association with monoubiquitinated PCNA (Bienko et al., 2010). Whether PCNA K63-linked polyubiquitin chains might serve as a signal to recruit factors involved in error-free PRR is currently unknown.

A key question in this field for both RPA and PCNA, which have many, many interacting factors, is how does the cell decide which factor to recruit? It is possible that everything is recruited simultaneously as a repair toolkit, and then the proper tool is selected based on geometrical constraints at the damage site. One parameter that could influence the selection of the proper tool might be the amount of ssDNA present or whether the recruitment is occurring in the context of a replication fork. This remains a significant challenge to unravel in the future.

The Fanconi Anemia Pathway for Interstrand Crosslink Repair

ICLs that covalently connect the two strands of DNA are a formidable bidirectional barrier to replication fork progression and require no less than four incision events, TLS polymerases, and recombination events to be circumvented (Figure 3B). Central components of the ICL repair pathway are 13 genes mutated in the genetic syndrome Fanconi anemia (FA) (Moldovan and D'Andrea, 2009). Eight of the FA proteins (FANCA, B, C, E, F, G, L, and M) form the FA core complex, an E3 ubiquitin ligase, with the addition of four associated factors (FAAP24, FAAP100, and the heterodimer MHF1/MHF2) (Thompson and Jones, 2010). The histone-fold heterodimer MHF1/MHF2 has recently been shown to stimulate FANCM fork reversal activity, whereas FAAP24 could target the FANCM complex to ssDNA regions (Ciccia et al., 2007; Singh et al., 2010; Yan et al., 2010). FANCM has DNA binding activity and has been implicated in targeting the core complex to DNA (Figure 3B) (Kim et al., 2008). Moreover, FANCM has been suggested to associate with the BLM complex and also contribute to the activation of the ATR pathway at blocked replication forks (Collis et al., 2008; Deans and West, 2009; Huang et al., 2010; Schwab et al., 2010).

The FANCI-FANCD2 Platform. The FANCI-FANCD2 (ID) complex lies at the heart of the FA pathway and becomes monoubiquitinated on both subunits by the FANCL ubiquitin ligase within the FA core complex (Moldovan and D'Andrea, 2009). Once monoubiquitinated, the ID complex accumulates at sites of crosslinks and colocalizes with three additional FA proteins, BRCA2/FANCD1, PALB2/FANCN, and BACH1/FANCJ (Figure 3B) (Moldovan and D'Andrea, 2009). The DDR tightly regulates the ID complex through ATR-dependent phosphorylation of both FANCI and D2 and several other components of the FA core complex, like FANCM (Smogorzewska et al., 2007; Sobeck et al., 2009). Phosphorylation of FANCI was recently shown to play a critical role in promoting the monoubiquitination of FANCD2 and itself, thus acting as a molecular switch of the FA pathway (Ishiai et al., 2008). ID phosphorylation might facilitate the recruitment of the ID complex to the FA core complex for monoubiquitination. Monoubiquitinated ID complex is required for the incision and translesion synthesis steps of ICL repair (Figure 3B) (Knipscheer et al., 2009). The monoubiquitinated ID complex promotes the recruitment of DDR factors required for ICL repair. These include the newly identified FAN1 endo- and exonuclease, which was shown to associate with the monoubiquitinated ID complex through a UBZ domain, and possibly the MUS81/EME1 and XPF/ERCC1 nucleases (Kratz et al., 2010; Liu et al., 2010; MacKay et al., 2010; Smogorzewska et al., 2010). Monoubiquitinated ID could also recruit the TLS polymerases REV1 and Polζ for the translesion step as they contain ubiquitin interaction domains (Moldovan and D'Andrea, 2009). Factors involved in HR are also likely to be recruited subsequent to these steps to repair the DSBs created in this process. Once the lesion has been repaired, the ID complex is deubiquitinated by the USP1-UAF1 complex, which also acts on monoubiquitinated PCNA when associated to the RFC subunit ELG1 (Cohn et al., 2007; Lee et al., 2010c). The FA pathway is one of the clearest examples of how the DDR, through ATR/ATRIP localization and activation at the sites of replication stress, controls the enzymatic toolbox of repair at the right time and location to promote the appropriate repair event.

Additional DDR-Regulated Pathways that Promote DNA Repair

dNTP Biosynthesis for DNA Replicational Repair Processes

Deoxyribonucleotides (dNTPs) are required for many aspects of DNA repair, and their levels are tightly regulated. In yeast through humans, dNTP synthesis is increased in S phase to meet the demands of DNA replication. However, occasionally, levels of dNTPs are insufficient, in particular for DNA repair outside of S phase. The enzyme ribonucleotide reductase (RNR), which controls the rate-limiting step in the synthesis of dNTPs, is one of the most highly regulated proteins in response to DNA damage. RNR is a tetramer containing a dimeric small and large subunit and is highly cell-cycle regulated (Nordlund and Reichard, 2006). The transcription of many RNR subunits is highly inducible in response to DNA damage and replication stress in eukaryotes. Mutations that decrease the activity of yeast RNR or the failure to induce it result in sensitivity to DNA damage (Elledge and Davis, 1989). Mammals induce a specialized small subunit, p53R2, through the ATM/ATR-p53 pathway in response to DNA damage (Nordlund and Reichard, 2006). While RNR is primarily cytoplasmic, in response to DNA damage it is acetylated by TIP60 and is localized to sites of DNA damage in the nucleus in a manner dependent on TIP60 (Niida et al., 2010). In theory, this produces dNTPs at the site of repair synthesis outside of S phase and may provide high local concentrations of dNTPs required for optimal function of TLS polymerases (Nordlund and Reichard, 2006).

DNA Repair of Transcribed Regions

Active transcription can provide obstacles to DSB repair because of possible collisions between the RNA polymerase and the HR machineries. Formation of DSBs in the highly repetitive nucleolar rDNA has been reported to induce transcriptional arrest and displacement of RNA polymerase I from rDNA in an ATM-, NBS1-, and MDC1-dependent manner (Kruhlak et al., 2007). The mechanism by which ATM regulates RNA polymerase transcription has not been defined. However, several nucleolar factors have been shown to be potential ATM/ATR substrates (Matsuoka et al., 2007). Recent observations have suggested that DSB formation leads to ATM-dependent repression of transcription in the vicinity of the DSB (Shanbhag et al., 2010). This could be reinforced by histone methyltranferase complexes, such as polycomb proteins, histone deacetylases (HDACs and sirtuins), and DNA methyltransferases, which have been shown to be recruited to sites of DNA damage (O'Hagan et al., 2008; Polo et al., 2010; Chou et al., 2010). Transcriptional repression could also be achieved by the RecQ helicase RECQ5, which prevents chromosomal rearrangements by both inhibiting RNA polymerase II and preventing excessive HR (Aygün et al., 2008, 2009; Islam et al., 2010).

Bulky DNA lesions, such as UV-induced pyrimidine dimers, in transcribed genes can lead to RNA polymerase II stalling (Hanawalt and Spivak, 2008). Removal of stalled RNA polymerase and repair of these lesions is promoted by transcription-coupled NER (TC-NER), which is initiated by the Cockayne syndrome proteins CSA and CSB. Once RNA polymerase has been removed, the repair of the DNA lesions is catalyzed by the Xeroderma pigmentosum (XP) proteins (Hanawalt and Spivak, 2008). TC-NER is distinct from global genome NER (GG-NER), which is initiated by XPC and operates throughout the genome (Hoeijmakers, 2009). Following UV radiation, XPC is phosphorylated by ATM/ATR and polyubiquitinated by the cullin ligase complex CUL4-DDB1-DDB2; however, the significance of these modifications is still unknown (Bergink and Jentsch, 2009; Matsuoka et al., 2007).

DNA Repair of Heterochromatic Regions

Heterochromatic regions, such as centromeric regions and inactive X chromosome in females, pose a particular problem to DNA repair because of extreme chromatin compaction. Establishment and maintenance of heterochromatin is dependent on H3K9me3 marks, which are recognized by the chromodomain of the heterochromatin protein HP1 (Fischle, 2009). Recent studies have shown that the chromodomain of HP1 is phosphorylated by CK2 after DNA damage, thus releasing HP1 from H3K9me3 (Ayoub et al., 2009). Association of TIP60 with exposed H3K9me3 through its chromodomain has been suggested to stimulate its acetyltranferase activity (Figure 1A) (Sun et al., 2009). TIP60 acetylation of histones H3 and H4 could then induce chromatin relaxation and facilitate the DSB repair of heterochromatic regions (Sun et al., 2010). TIP60 could also promote chromatin remodeling at DSBs by acetylating γH2AX-modified histones, which are then removed following ubiquitination by the UBC13 ubiquitin ligase (van Attikum and Gasser, 2009). TIP60 is also known to interact with ATM and stimulate its kinase activity by directly acetylating ATM (Sun et al., 2010). Activation of ATM leads to very rapid phosphorylation of the transcriptional repressor KAP1, which accumulates at heterochromatic DSBs in a 53BP1-dependent manner, where it contributes to chromatin relaxation (Noon et al., 2010). Moreover, 53BP1 interacts with the chromatin remodeling factor EXPAND1, which induces chromatin decondensation at sites of DNA damage (Huen et al., 2010a). Altogether, these observations point toward a critical role of the ATM signaling for the repair of DSBs in heterochromatin regions (Sun et al., 2010).

The DNA Damage Response and Human Disease

The central role of the DDR in human physiology is indicated by the broad spectrum of defects displayed by individuals carrying mutations in DDR genes. DDR genetic syndromes primarily affect the homeostasis of the nervous, immune, and reproductive systems and can also lead to premature aging or cancer predisposition (Table 2) (Jackson and Bartek, 2009). In this section, we will describe the pathologies associated with DDR defects.

Neurological Defects

The nervous system relies heavily on an intact DDR for functionality. Given that neurons display limited capacity of replacement, they must overcome DNA damage lesions often on a lifetime basis. Neurons exhibit high oxygen consumption by mitochondrial respiration, which can result in oxidative stress and subsequent DNA damage, such as DNA base lesions and DNA breaks (Jackson and Bartek, 2009). Consistent with this observation, defects in the regulation of oxidative stress and repair of DNA lesions often result in neuronal death and neurodegeneration. Several DDR syndromes affect primarily the cerebellum, which is responsible for motor coordination (Table 2) (Katyal and McKinnon, 2008). The cerebellum is composed of three classes of neurons—granule cells, Purkinjie cells, and interneurons—which account for approximately 50% of the neurons of the whole brain (Katyal and McKinnon, 2008). Degeneration of cerebellar neurons often results in ataxia (impaired motor coordination), oculomotor apraxia (eye movement defect), and dysarthria (speech disorder). One of the most extensively studied neurodegenerative diseases is ataxia telangiectasia (A-T), which is caused by mutations in the *ATM* gene (Biton et al., 2008). A-T patients develop profound ataxia due to the progressive loss of granule and Purkinjie cells and are confined to a wheelchair before 10 years of life. Similar symptoms, although characterized by later onset and slower progression, are developed by patients with the ataxia telangiectasia-like syndrome (A-TLD), which is caused by mutations in *MRE11*. The similarity between ATM- and MRE11-deficient syndromes is consistent with the previously described role of MRE11 in ATM activation. The mechanism by which ATM deficiency causes cerebellar degeneration is still the object of extensive debate. ATM has been shown to regulate oxidative stress, as indicated by the increased ROS levels in the absence of ATM (Biton et al., 2008). Neurodegeneration could then be caused by excessive ROS-induced DNA damage in ATM-deficient cerebellar neurons. Interestingly, treatment with antioxidant agents was reported to enhance the survival of $ATM^{-/-}$ Purkinjie cells in vitro (Biton et al., 2008). Moreover, A-T patients subjected to steroid therapy showed improved cerebellar functions associated with reduction of ROS levels (Russo et al., 2009).

Table 2. Human Genetic Diseases Associated with DDR Defects

Syndrome	Mutated Gene	DDR Defect	Phenotype				
			Neurological Disorder	Immunodeficiency	Progeria	Cancer	Other
Cerebro-oculo-facio-skeletal syndrome (COFS)	*CSB*, *XPD*, *XPG*, *ERCC1*	TC-NER	Brain calcification, hypomyelination, microcephaly, neurodegeneration	—	Cataracts, hearing loss, optic atrophy, osteoporosis	—	Facial dysmorphism, joint contractures, photosensitivity, growth defects
Cockayne syndrome (CS)	*CSA*, *CSB*, *XPB*, *XPD*, *XPG*	TC-NER	Microcephaly, neurodegeneration, neuronal demyelination	—	Cachexia, cataracts, hearing loss, retinopathy	—	Photosensitivity, growth defects
Trichothiodystrophy (TTD)	*XPB*, *XPD*, *TTDA*	TC-NER	Hypomyelination, neurodegeneration	—	Cachexia, cataracts, osteoporosis	—	Brittle hair and nails, photosensitivity, scaly skin
Xeroderma pigmentosum (XP)	*XPA-G*, *POLH*	NER	Microcephaly, neurodegeneration	—	—	Squamous and basal cell carcinoma, melanoma	Photosensitivity, scaly skin
XPF-ERCC1 (XFE) syndrome	*XPF*	NER, ICL repair	Microcephaly	—	Cachexia, osteoporosis, scoliosis	—	Photosensitivity, liver and renal dysfunction
Fanconi anemia (FA)	*FANCA-C*, *FANCD1*, *D2*, *FANCE-G*, *FANCI*, *J*, *L-N*	ICL repair, HR	Microcephaly	Pancytopenia	Bone marrow failure	AML, myelodysplasia, squamous cell carcinoma	Abnormal skin pigmentation, infertility, limb deformities, renal dysfunction
Fanconi anemia-like disorder	*RAD51C*	ICL repair, HR	—	—	Growth defects	—	Hypogonadism, limb deformities, renal dysfunction
Familial breast cancer	*ATM*, *BRCA1*, *BRCA2*, *BRIP1*, *CHK2*, *NBS1*, *PALB2*, *RAD50*, *RAD51C*	HR, damage signaling	—	—	—	Breast cancer, ovarian cancer (*BRCA1, BRCA2, RAD51C*)	—
Bloom syndrome (BS)	*BLM*	HR	Microcephaly, mild mental retardation	Immunoglobulin deficiency	—	Carcinomas, leukemias, lymphomas	Abnormal skin pigmentation, facial dysmorphism, infertility, growth defects
Rothmund Thomson syndrome (RTS)	*RECQL4*	BER, HR?	—	—	Cataracts, gray hair	Osteosarcoma, skin cancers	Skin and skeletal abnormalities, growth defects
Werner syndrome (WS)	*WRN*	HR, BER, telomere maintenance	—	—	Atherosclerosis, cataracts, gray hair, osteoporosis	Sarcomas	Type II diabetes, growth defects
Dyskeratosis congenita (DKC)	*DKC1*, *TERC*	Telomere maintenance	Microcephaly, mental retardation	Pancytopenia	Bone marrow failure, osteoporosis	Carcinomas	Abnormal skin pigmentation, nail dystrophy, growth defects
Ataxia with oculomotor apraxia 1 (AOA1)	*APTX*	SSB repair	Ataxia, neurodegeneration, oculomotor apraxia	—	—	—	Hypercolesterolemia
Ataxia with oculomotor apraxia 2 (AOA2)	*SETX*	SSB repair?	Ataxia, neurodegeneration, oculomotor apraxia	—	—	—	Hypercolesterolemia
Spinocerebellar ataxia with axonal neuropathy (SCAN1)	*TDP1*	SSB repair	Ataxia, neurodegeneration, muscle weakness	—	—	—	Hypercolesterolemia

Table 2. *Continued*

Syndrome	Mutated Gene	DDR Defect	Phenotype				
			Neurological Disorder	Immunodeficiency	Progeria	Cancer	Other
Ligase I syndrome	*LIG1*	SSB repair, NER	—	Immunoglobulin deficiency	—	—	Growth defects, photosensitivity
MYH-associated polyposis (MAP)	*MYH*	BER, oxidative damage repair	—	—	—	Colorectal cancer	—
Hereditary nonpolyposis colorectal cancer (HNPCC)	*MSH2*, *MSH6*, *MLH1*, *PMS2*	MMR	—	—	—	Colorectal cancer, carcinomas	—
Immunodeficiency with microcephaly	*XLF*	NHEJ	Microcephaly	Hypogamma-globulinemia, lymphopenia	—	—	Growth defects
Ligase IV syndrome	*LIG4*	NHEJ	Microcephaly	Hypogamma-globulinemia, lymphopenia	—	ALL, lymphomas	Growth defects
Radiosensitive severe combined immunodeficiency (RS-SCID)	*ARTEMIS*	NHEJ	—	Agamma-globulinemia, lymphopenia	—	Lymphomas	Growth defects
Severe combined immunodeficiency (SCID)	*RAG1*, *RAG2*	NHEJ	—	Agamma-globulinemia, lymphopenia	—	—	Growth defects
Microcephaly, intractable seizures, and developmental delay syndrome (MCSZ)	*PNKP*	NHEJ, SSB repair	Microcephaly	—	—	—	Seizures, growth defects
Hyper-IgM syndrome	*AID*, *UNG*	CSR	—	Increased IgM levels, lymphoid hyperplasia	—	—	—
Aicardi Goutieres syndrome (AGS)	*RNASEH2*, *TREX1*	Damage signaling, immunological response	Cerebral atrophy, intracranial calcifications, microcephaly, neurodegeneration	—	—	—	—
Ataxia telangiectasia (A-T)	*ATM*	Damage signaling, DSB repair, oxidative stress	Ataxia, cerebellar degeneration, oculomotor apraxia	Immunodeficiency	—	Lymphomas, leukemias, breast cancer	Dilated blood vessel, infertility, metabolic defects, growth defects
Ataxia telangiectasia-like disorder (A-TLD)	*MRE11*	Damage signaling, DSB repair, oxidative stress	Ataxia, cerebellar degeneration, oculomotor apraxia	Immunodeficiency	—	—	—
Li-Fraumeni syndrome (LFS)	*TP53*	DNA damage signaling, DSB repair	—	—	—	Brain and breast cancer, sarcomas	—
Nijmegen breakage syndrome (NBS)	*NBS1*	Damage signaling, DSB repair, repl. fork repair	Microcephaly	Immunodeficiency	—	B cell lymphoma	Facial dysmorphism, growth defects
Nijmegen breakage syndrome-like disorder (NBSLD)	*RAD50*	Damage signaling, DSB repair, repl. fork repair	Microcephaly	—	—	—	Facial dysmorphism, growth defects
Riddle syndrome	*RNF168*	Damage signaling, DSB repair	—	Immunodeficiency	—	—	Facial dysmorphism, growth defects
Seckel syndrome (SS)	*ATR*, *PCTN*, *SCKL2*, *SCKL3*	Damage signaling, DSB repair, repl. fork repair	Microcephaly, mental retardation	—	—	AML?	Facial dysmorphism, growth defects
Primary microcephaly 1	*MCPH1*	Damage signaling, DSB repair, repl. fork repair	Microcephaly, mental retardation	—	—	—	—

(*Continued on next page*)

Table 2. *Continued*

Syndrome	Mutated Gene	DDR Defect	Phenotype				
			Neurological Disorder	Immunodeficiency	Progeria	Cancer	Other
Schimke immuno-osseous dysplasia (SIOD)	*SMARCAL1*	Repl. fork repair	Microcephaly	T cell deficiency	—	—	Nephritis, skeletal dysplasia, growth defects
Roberts syndrome (RBS)	*ESCO2*	Cohesion, repl. fork repair	—	—	—	—	Cleft palate, phocomelia
Hutchinson-Gilford progeria syndrome (HGPS)	*LMNA*	Nuclear organization, damage signaling, DSB repair	—	—	Alopecia, atherosclerosis	—	Adipose tissue deficiency
Restrictive dermopathy (RD)	*LMNA*, *ZMPSTE24*	Nuclear organization, damage signaling, DSB repair	—	—	—	—	Facial dysmorphism, tight skin, growth defects
Amyotrophic lateral sclerosis (ALS)	*SOD1*, *SETX*	Oxidative stress, SSB repair?	Degeneration motor neurons	—	—	—	Muscular atrophy
Charcot-Marie-Tooth syndrome (CMT)	*PMP22*, *GJB1*, *EGR2*, *SH3TC2*, *MTMR2*, *MTMR13*	Damage processing? Oxidative stress?	Motor and sensory neuropathy, neuro-demyelination	—	—	—	Muscular atrophy
Spino-cerebellar ataxia-epilepsy syndrome (SCAE)	*POLG*, *TWINKLE*	mtDNA maintenance, oxidative stress?	Ataxia, dysarthria, neuropathy	—	—	—	Epileptic seizures
Progressive external ophtalmoplegia (PEO)	*POLG*, *POLG2*, *TWINKLE*, *RRM2B*	mtDNA maintenance, oxidative stress?	—	—	—	—	Eye, limb, and facial muscle weakness

Defects in repair of SSBs, one of the primary ROS-induced lesions, have also been associated with cerebellar degeneration and ataxia (Table 2). The neurodegenerative disorders ataxia with axonal neuropathy (SCAN1) and ataxia with oculomotor apraxia 1 (AOA1) are caused by mutations of the DNA end-processing enzymes TDP1 and APTX, respectively (Caldecott, 2008). TDP1 processes 3′ ends linked to TOP1, which are generated by abortive release of the topoisomerase from DNA, and other nonligatable 3′ ends induced by ROS and other DNA-damaging agents, whereas APTX is a 5′ end processing enzyme that removes abortive ligation intermediates from ssDNA and dsDNA ends (Rass et al., 2007). The helicase SETX, which is mutated in ataxia with oculomotor apraxia 2 (AOA2), has also been linked to the repair of SSBs generated by oxidative damage, but its precise role is still unknown (Rass et al., 2007).

Accumulation of mutations in mitochondrial DNA (mtDNA), which can lead to defects in oxygen metabolism and increased ROS levels, has also been associated with neurodegenerative disorders (Table 2). Indeed, mutations in the mitochondrial polymerase Polγ and its DNA helicase TWINKLE have been identified in the spinocerebellar ataxia-epilepsy syndrome (SCAE), which is characterized by neuropathy, dysarthria, and epileptic seizures (Copeland, 2008). Defects in Polγ, TWINKLE, and the RNR subunit RRM2B have also been linked to progressive external ophthalmoplegia (PEO), a disorder leading to degeneration of the external eye muscle (Copeland, 2008). Mutations and deletions of mtDNA have also been found in Parkinson's, Alzheimer's, and Huntington's diseases and amyotrophic lateral sclerosis (ALS), and they have been correlated with an increase in oxidative damage in the brain (Druzhyna et al., 2008). ALS, which causes progressive degeneration of motor neurons, resulting in paralysis, can be induced by mutations of the ROS detoxifying enzyme SOD1 and the helicase SETX (Chen et al., 2004; Valdmanis and Rouleau, 2008). Recently, genes causing another motor neuron disorder, the Charcot-Marie-Tooth (CMT) syndrome, which leads to myelination defects and motor and sensory neuropathy, have been shown to prevent accumulation of DNA damage (Paulsen et al., 2009). Whether CMT genes might prevent oxidative damage or might regulate other DDR pathways has not been yet defined.

In addition to maintaining the homeostasis of the nervous system, the DDR plays a critical role during brain development. Indeed, a wide number of DDR syndromes display microcephaly, a reduced head circumference resulting from defective proliferation of neuroprogenitor cells during fetal development (Table 2) (O'Driscoll and Jeggo, 2008). Microcephaly is one of the typical phenotypes of Seckel syndrome (SS) patients, in addition to dwarfism and "bird-like" facial dysmorphism (Kerzendorfer and O'Driscoll, 2009). Mutations of four different loci—SCKL1 through SCKL4—have been found in SS patients. SCKL1 SS is caused by a hypomorphic *ATR* mutation that decreases ATR protein levels due to aberrant splicing of the ATR transcript, whereas the centrosomal protein PCNT, which has been shown to function in the ATR pathway, is mutated in SCKL4 patients (Kerzendorfer and O'Driscoll, 2009). *PCNT* mutations have recently been found in the microcephalic osteodysplastic primordial dwarfism type II (MOPDII), a disorder similar to SS

(Kerzendorfer and O'Driscoll, 2009). Given that SS appears to be a disorder of defective ATR pathway, the uncharacterized genes responsible for SCKL2 and SCKL3 are probably novel ATR pathway components. Another protein shown to mediate the activation of the ATR pathway, MCPH1/BRIT1, has been shown to be defective in patients with primary microcephaly (O'Driscoll and Jeggo, 2008). Moreover, defects of the ATR pathway have been proposed to cause the microcephalic phenotype of the blepharophimosis-ptosis-epicanthus inversus syndrome, Miller-Dieker lissencephaly syndrome, and the Williams-Beuren syndrome, which are haploinsufficient for *ATR*, *RPA1*, and *RFC2*, respectively (Kerzendorfer and O'Driscoll, 2009).

Microcephaly is also characteristic of the Nijmegen breakage syndrome (NBS) and the Nijmegen breakage syndrome-like disorder (NBSLD), which are caused by hypomorphic mutations of *NSB1* and *RAD50*, respectively (Katyal and McKinnon, 2008; Waltes et al., 2009). Similar to SS, NBS and NBSLD patients display growth retardation and "bird-like" face. These phenotypes are remarkably different from the A-T-like symptoms due to *MRE11* hypomorphic mutations in A-TLD patients, as mentioned above. This might reflect the complex functions of the MRN complex in both ATM activation and regulation of ATR-mediated processes, such as replication fork stabilization and restart. Therefore, the different impact of the hypomorphic mutations in *MRE11*, *NBS1*, and *RAD50* on the ATM or ATR pathways might give rise to the distinct phenotypes of A-TLD and NBS/NBSLD patients. Interestingly, it has been recently reported that A-TLD mutant mice display loss of ATM-dependent apoptosis in the nervous system after DNA damage, thus causing faulty incorporation of damaged cells in the brain, whereas NBS mutant neurons still exhibit ATM-dependent apoptosis, which could result in cell loss after damage and microcephaly (Shull et al., 2009). Therefore, the level of ATM residual activity in the brain might determine the outcome between neurodegeneration and microcephaly.

Syndromes with defective HR, such as Bloom syndrome, which is caused by mutations in the RecQ helicase BLM, or impaired ICL repair, like FA and the XFE syndrome, which is due to *XPF* mutations, lead to microcephaly (O'Driscoll and Jeggo, 2008). Moreover, microcephaly could also be caused by disorders that exhibit defective replication fork restart after DNA damage, as the Schimke immunosseous dysplasia (SIOD), which is caused by mutations of the SMARCAL1 helicase or by NHEJ defective disorders with *LIG4*, *XLF*, or *PNKP* mutations (Driscoll and Cimprich, 2009; O'Driscoll and Jeggo, 2008; Shen et al., 2010). Defective neuronal development is also observed in Ku-deficient mice (Gu et al., 2000). Altogether, these observations have led to the proposal that the developing brain, and especially neuroprogenitor cells, might be particularly sensitive to the presence of DSBs, which could be formed following replication stress or oxidative damage (O'Driscoll and Jeggo, 2008). Defective DSB repair could then lead to microcephaly as a consequence of ATM-dependent apoptosis of damaged neural cells. Interestingly, prenatal exposure to IR is known to cause microcephaly in humans, as indicated by the reduced brain size of the Hiroshima and Nagasaki atomic bomb survivors that were in utero at the time of the radiation exposure (Fernandez-Capetillo, 2010).

Infertility and Immunological Defects

The DDR plays an essential role in the generation of gametes and the development of the immune system. The generation of gametes during meiosis requires the exchange of genetic material between homologous chromosomes, which involves the formation of DSBs by the nuclease SPO11 and their subsequent repair by HR (Neale and Keeney, 2006). Defective DSB repair during meiosis results in infertility. A large number of DDR-deficient mice and various human DDR syndromes, such as A-T, BS, and FA, display aberrant meiotic progression and infertility (Table 2) (Biton et al., 2008; Bohr, 2008; Matzuk and Lamb, 2008). Given the requirement of a functional DDR for meiotic progression, a significant proportion of human infertility could be due to DDR defects (Jackson and Bartek, 2009).

The development of a functional immune system necessitates the generation of a wide number of unique immunoglobulins and T cell receptors. This is obtained by joining different combinations of variable (V), diversity (D), and junction (J) DNA sequences through V(D)J recombination (Gennery, 2006). V(D)J recombination is initiated by the nucleases RAG1 and RAG2, which introduce specific DSBs at the segments to be rearranged. The ends of the RAG1/RAG2 DSBs are generated as hairpin loops that are opened by the nuclease ARTEMIS prior to end joining by NHEJ factors (Gennery, 2006). Mutations of *RAG1* and *RAG2* have been identified in patients with severe combined immunodeficiency (SCID) caused by profound lymphopenia with diminished or absent immunoglobulins (Table 2) (Sobacchi et al., 2006). Similarly, *ARTEMIS* mutations give rise to SCID, which is, however, accompanied by radiosensitivity (RS-SCID) due to the role of ARTEMIS in the repair of radiation induced DSBs (Gennery, 2006). Hypomorphic mutations of *RAG1*, *RAG2*, or *ARTEMIS* have been found in the Omenn's syndrome, a less severe immunodeficiency disorder characterized by low levels of V(D)J recombination and clonal expansion of limited set of T lymphocytes (Sobacchi et al., 2006). Low immunoglobulin levels, lymphopenia, and radiosensitivity are also characteristic of the ligase IV and immunodeficiency with microcephaly syndromes, which are defective in the NHEJ factors LIG4 and XLF, respectively (Gennery, 2006). As mentioned above, LIG4 and XLF, unlike ARTEMIS, are also required for brain development, thus reflecting a more essential role of LIG4 and XLF in NHEJ. No mutations in Ku70, Ku80, and DNA-PKcs, which are all essential for V(D)J recombination, as indicated by the SCID phenotype of knockout mouse models, have yet been reported in human immunodeficiency syndromes (Brugmans et al., 2007; Gennery, 2006).

Diversification of V(D)J variable domains is further achieved by class switch recombination (CSR) and somatic hypermutation (Stavnezer et al., 2008). CSR promotes the switch between the constant regions of immunoglobulins, whereas somatic hypermutation introduces point mutations in the V(D)J variable domains. Both CSR and somatic hypermutation are triggered by the AID enzyme, which deaminates cytosines to uracil (Stavnezer et al., 2008). In CSR, DNA breaks are then generated by the combined action of the uracil DNA glycosylase UNG and the AP endonuclease APE1. Classical NHEJ or alt-NHEJ could then lead to the exchange of the antibody constant regions. Defects in AID and UNG have been shown to cause the Hyper-IgM

syndrome, which is characterized by normal IgM levels but few or no IgG, IgA, and IgE isotypes (Stavnezer et al., 2008).

Immunodeficiency has also been reported for A-T patients, who display lymphopenia and decreased levels of IgG, IgA, and IgE isotypes (Gennery, 2006). Indeed, it has been shown that ATM stabilizes DNA repair complexes during V(D)J recombination and facilitates CSR (Stavnezer et al., 2008). Defective CSR has also been reported for mice deficient for H2AX, MDC1, 53BP1, and NBS1 (Stavnezer et al., 2008). Given that other DDR syndromes with defects of the ATM pathway—A-TLD, NBS, and the Riddle syndrome, which is due to *RNF168* mutation—lead to immunodeficiency resulting from reduced efficiency of V(D)J recombination and/or CSR, the ATM pathway appears to play an important role in the development of the immune system in humans (Gennery, 2006; Stewart et al., 2007). Interestingly, the recently identified SIOD syndrome leads to deficiency of T but not B lymphocytes (Boerkoel et al., 2002). The specific role of the SIOD protein SMARCAL1 in T cell development or maturation awaits further investigation.

Premature Aging and Stem Cell Exhaustion

Accumulation of DNA damage can lead to premature aging, as indicated by several DDR syndromes with progeroid phenotypes (Table 2). Deficiency in multiple DDR pathways, including NER, BER, and DSB repair, has been linked to premature aging. The progeroid syndromes Cockayne syndrome (CS), cerebro-oculo-facio-skeletal syndrome (COFS), and trichotiodystrophy (TTD) are caused by defects in TC-NER, whereas the related cancer-prone syndrome Xeroderma pigmentosum (XP)—classified into eight complementation groups, XPA through XPG and XPV—is associated with defects in GG-NER or TLS (Hoeijmakers, 2009). CS, which can be caused by mutations of the TC-NER factors CSA and CSB, the TFIIH helicases XPB and XPD, and the NER endonuclease XPG, is characterized by hearing loss, cataracts, weight loss with muscle atrophy (cachexia), and retinal defects associated with microcephaly and neurodegeneration. Mutations in *XPD*, *XPG*, and *CSB* can also be found in COFS, an early-onset form of CS with severe symptoms apparent at birth (Garinis, 2008). Moreover, some *XPB* and *XPD* mutations are associated with TTD, which has symptoms similar to CS with the addition of brittle hair and nails, and with an XP and CS combined syndrome (XP-CS) (Schumacher et al., 2008).

Deficiency in the RecQ helicase WRN leads to the development of Werner syndrome (WS), one the best examples of progeroid syndromes for its remarkable resemblance to normal aging (Bohr, 2008). WS patients prematurely develop gray hair, osteoporosis, cataracts, type II diabetes, and atherosclerosis and generally die in the fifth decade of life because of cancer or cardiovascular diseases. Premature aging features, such as gray hair and cataracts, have also been associated with the Rothmund-Thomson syndrome (RTS), which is caused by mutations of the RecQ helicase RECQL4 (Chu and Hickson, 2009). It has been proposed that WRN and RECQL4 might participate in the removal of oxidative lesions by BER (Bohr, 2008). Moreover, WRN can promote HR and telomere maintenance (Chu and Hickson, 2009). Indeed, telomere shortening is known to lead to DDR activation and induction of senescence (Sahin and Depinho, 2010).

The impact of telomere dysfunction on organismal aging is further demonstrated by the progeroid syndrome dyskeratosis congenita (DKC), which is due to mutations of the telomerase-associated TERC RNA or its interacting protein DKC1 (Schumacher et al., 2008). DCK patients develop progressive bone marrow failure and pancytopenia, accompanied by growth defects, osteoporosis, and abnormal skin pigmentation due to telomere shortening, resulting in the deprotection of telomeres and the activation of the DDR. Bone marrow failure and pancytopenia are also found in FA patients, who additionally display skeletal abnormalities, renal dysfunction, abnormal skin pigmentation, sensitivity to ICL agents, and infertility, as mentioned above (Moldovan and D'Andrea, 2009). The bone marrow failure in DKC and FA patients is probably due to exhaustion of hematopoietic stem cells (HSC) caused by telomere shortening or DNA damage, respectively (Park and Gerson, 2005).

Depletion of HSCs has been observed in a variety of mouse models defective for DDR components, including ATM, BRCA2, ERCC1, FANCC, Ku80, LIG4, MSH2, XPD, and mouse telomerase RNA (mTR) (Niedernhofer, 2008; Park and Gerson, 2005; Sharpless and DePinho, 2007). Spontaneous DNA damage accumulated in mice deficient for ATM, LIG4, XPD, mTR, or Ku80 was shown to impair HSC self-renewal (Niedernhofer, 2008). In ATM-deficient mice, HSC failure was reported to depend on increased oxidative damage, which induces the accumulation of the CDK inhibitor $p16^{INK4A}$ (Sharpless and DePinho, 2007). $p16^{INK4A}$, which functions together with the tumor suppressor Rb to induce cell-cycle arrest and cellular senescence, has been shown to accumulate with age in adult stem cells, thus reducing their regenerative capacity (Collado et al., 2007). Deficiency of p53, which also regulates cellular senescence, was shown to rescue many of the progeroid features displayed in mTR-deficient mice due to defects of HSC and stem cells from the intestinal crypt and testis (Sharpless and DePinho, 2007). Similarly, the accelerated aging phenotypes of mice defective for BRCA1, Ku80, and ZMPSTE24, a nuclear membrane protease mutated in the Hutchinson-Gilford progeria syndrome, were rescued by *TP53* deletion (Sahin and Depinho, 2010). Deficiency of p53 in these mouse models led to increased cancer formation, indicating that p53 rescues aging at the expense of cancer. Interestingly, *TP53* deletion was shown to exacerbate the progeroid phenotypes of a SS mouse model with ATR deficiency (Fernandez-Capetillo, 2010). Given that the progeroid features of SS mice might be due to replication stress during embryonic development, the increased proliferation rate induced by p53 deficiency could further increase the amount of replication stress and therefore accelerate the aging phenotype. Deletion of *ATR* in adult mice was also shown to lead to stem cell loss and premature aging (Ruzankina et al., 2007).

Analysis of several DDR mouse models, including mouse models for SS, CS, and XFE progeria, have revealed a reduction of the growth hormone and insulin-like growth factor 1 (GH-IGF1) axis (Schumacher et al., 2008). Attenuation of the GH-IGF1 pathway is known to lead to extended life span and increased stress resistance and occurs during natural aging. The decreased function of the GH-IGF1 pathway after DNA damage might represent an attempt to reduce further damage

accumulation by shifting the organismal resources from growth to tissue maintenance (Schumacher et al., 2008).

Cancer Development and Therapy

Cancer is an evolutionary disease fueled by genomic instability (Negrini et al., 2010). The majority of cancers display chromosomal instability (CIN), which is characterized by alterations of chromosomal numbers and/or structure. Other forms of genomic instability include accumulation of DNA base mutations and microsatellite instability (MIN), which results in contraction or expansion of the number of repetitive microsatellite sequences (Negrini et al., 2010). Maintenance of genomic integrity by the DDR is critical to prevent tumorigenesis, as indicated by the cancer-prone phenotype of several DDR syndromes (Table 2). Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is caused by heterozygous mutations of MMR genes, such as *MLH1*, *MSH2*, *MSH6*, and in fewer cases, *PMS2* (Spry et al., 2007). HNPCC is associated with MIN, which predisposes primarily to the development of colorectal cancer, with additional possible carcinomas of the endometrium, ovary, stomach, and kidney. The risk of colorectal cancer is increased also in the MYH-associated polyposis (MAP), which is caused by defective repair of oxidative lesions due to mutations of the DNA glycosylase MYH (David et al., 2007). The mechanism by which deficiency of MMR factors and MYH preferentially affects intestinal cells has not been determined. Instead, deficiency of NER factors in the XP syndrome is known to greatly increase the risk of skin cancer and melanoma because of defective repair of UV lesions in skin cells following sun exposure (Hoeijmakers, 2009).

Familial breast cancer accounts for approximately 5%–10% of breast cancer cases (Fackenthal and Olopade, 2007). The most prevalent mutations leading to hereditary breast and ovarian cancer affect the HR genes *BRCA1* and *BRCA2*. Heterozygous individuals carrying mutations of the *BRCA1* or *BRCA2* genes have a 40%–80% risk of developing breast cancer (Fackenthal and Olopade, 2007). Patients with *BRCA2* mutations have increased incidence of male breast, pancreas, and prostate cancer (Moynahan and Jasin, 2010). Tumors with *BRCA1* or *BRCA2* mutations are significantly associated with low level of 53BP1, indicating that *53BP1* mutation might confer a survival advantage in the absence of BRCA1 and BRCA2 (Bouwman et al., 2010). This is probably due to partial restoration of HR in *BRCA1* mutant tumors, as previously described. Recently, mutations in three additional HR genes, *BACH1*, *PALB2*, and *RAD51C*, have been identified in approximately 3% of familial breast cancer patients and have been associated with a 2-fold increased risk of breast cancer (Levy-Lahad, 2010; Walsh and King, 2007). Mutations of *CHK2*, *ATM*, *NBS1*, and *RAD50* have also been associated with a doubled risk of breast cancer, indicating the importance of the ATM pathway, together with HR, in preventing breast cancer formation (Walsh and King, 2007).

The strong connection between familial breast cancer and FA syndrome has recently become apparent (Levy-Lahad, 2010). Indeed, *BRCA2*, *BACH1*, and *PALB2* mutations predispose carriers to breast cancer formation when monoallelic and lead to FA syndrome—complementation groups D1, J, and N, respectively—when biallelic. Similarly, biallelic mutations of *RAD51C* have been identified in an FA-like disorder (Levy-Lahad, 2010). FA is also associated with increased cancer risk, in particular for the development of myelodisplasia (preleukemic syndrome) and acute myeloid leukemia (AML) (Moldovan and D'Andrea, 2009). Hematological neoplasia is also found in A-T patients (lymphomas and leukemias) and NBS patients (B cell lymphomas) (Gennery, 2006; Spry et al., 2007). Some cases of hematological malignancies, such as EBV-associated lymphomas, have been associated with LIG4 syndrome and RS-SCID defective in ARTEMIS (Gennery, 2006). The preferential development of lymphoid tumors in A-T, NBS, and NHEJ defective syndromes is due to their critical role during lymphocyte development. Lymphoid tumors are often caused by chromosomal translocations, which can lead to aberrant expression of oncogenes, such as c-Myc, or generation of deregulated chimeric proteins with enhanced activity (Nussenzweig and Nussenzweig, 2010). Experiments in mice have shown that NHEJ defective mice in the absence of p53 develop B cell lymphomas harboring translocations between the Ig locus and c-Myc, which are dependent on the unrepaired breaks created by RAG1/RAG2 during V(D)J recombination (Nussenzweig and Nussenzweig, 2010). Similarly, ATM deficiency was shown to lead to persistent RAG-induced breaks, which could join other DSBs, such as breaks induced by aberrant activity of AID, to generate translocations and promote tumorigenesis (Nussenzweig and Nussenzweig, 2010).

A broad spectrum of malignancies is displayed by the DDR disorders Bloom syndrome (BS) and Li-Fraumeni syndrome (LFS). The high level of CIN of BS patient cells due to hyperrecombination can lead to the development of lymphomas, leukemias, and carcinomas (Chu and Hickson, 2009). LFS, which is primarily caused by germline *TP53* mutations, predisposes carriers to breast cancer, brain tumors, leukemia, sarcomas, melanomas, and gastrointestinal cancers (D'Orazio, 2010). Sporadic cancers arise from the accumulation of stochastic DNA lesions that increase the fitness of cancer cells. It is known that early stages of human tumors have elevated levels of DNA damage (Bartek et al., 2007). DNA damage could be generated by exposure to carcinogens, as confirmed by the complex mutational signature identified in the lung cancer genome of a smoker and in the genome of a malignant melanoma (Pleasance et al., 2010a, 2010b). Moreover, DNA damage can be induced by telomeric shortening, increased oxidative damage, or replication stress induced by oncogene activation (Bartek et al., 2007; Luo et al., 2009). Activation of the DDR could normally prevent tumorigenesis by inducing cellular senescence or apoptosis of early tumor cells (Bartek et al., 2007). However, mutation of DDR genes, such as *TP53* or *ATM*, could predispose carriers to cancer formation by facilitating senescence and apoptosis bypass and cellular proliferation despite accumulation of DNA damage (Luo et al., 2009). Activation of p53 occurs in response to multiple stimuli. However, it is likely to be its role in responding to DNA damage that provides the majority of its tumor suppressive function, as demonstrated by the observation that more than 50% of sporadic human cancers harbor somatic *TP53* mutations and another 15% have *ATM* mutations in a mutually exclusive manner, indicating that they function in the same pathway (Ding et al., 2008).

In an attempt to develop better cancer therapeutic approaches, the concept of targeting nononcogene addiction (NOA) for cancer therapy has recently been proposed (Luo et al., 2009). This idea is based on the observation that the cancer cells rely on many nononcogenic pathways, which are not essential for the survival of normal cells. Therefore, targeting NOA pathways would provide more selective cancer treatments. In particular, given the elevated levels of DNA damage in cancer cells compared to normal cells, further increasing the amount of DNA damage by inhibiting DDR components, in combination with other chemotherapeutic drugs, could lead to cancer cell death. Indeed, recent studies have shown that ATM, DNA-PK, and CHK1 inhibitors have preferential toxicity toward cancer cells following treatment with genotoxic agents (Bolderson et al., 2009). Interestingly, ATM and DNA-PK depletion have been shown to sensitize *TP53* and *ATM* mutant tumor cells to genotoxic agents, respectively (Jiang et al., 2009). Moreover, PARP1 inhibitors have been successfully used to treat tumors that carry mutations in HR genes, such as *BRCA1* and *BRCA2* (Jackson and Bartek, 2009). This effect has been proposed to be caused by the accumulation of unrepaired DNA breaks in the absence of both PARP-dependent SSBR and HR. Recently, methotrexate, an inhibitor of DNA base synthesis, has been shown to selectively kill MMR-deficient cancer cells by leading to accumulation of oxidative DNA lesions (Martin et al., 2010a). Interestingly, depletion of the BER polymerase Polβ and the mitochondrial polymerase Polγ was shown to be synthetic lethal in combination with MSH2 and MLH1 deficiency in tumor cells because of an increased number of oxidative lesions (Martin et al., 2010b).

In summary, the coordination of DNA repair processes plays a critical role in allowing the proper development and survival of organisms. They are responsible for preventing numerous human diseases and conditions, including cancer and aging. We envision that the further understanding of the molecular mechanisms through which the DDR operates, in combination with the elucidation of the genetic interactions between different DDR pathways and between DDR pathways and other cellular pathways, will provide therapeutic opportunities for many human diseases.

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