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Perspective

The DNA Damage Response: Ten Years After

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The DNA damage response (DDR), through the action of sensors, transducers, and effectors, orchestrates the appropriate repair of DNA damage and resolution of DNA replication problems, coordinating these processes with ongoing cellular physiology. In the past decade, we have witnessed an explosion in understanding of DNA damage sensing, signaling, and the complex interplay between protein phosphorylation and the ubiquitin pathway employed by the DDR network to execute the response to DNA damage. These findings have important implications for aging and cancer.

In a space in time of 10 years, advances in the DNA damage response (DDR) field have profoundly altered the conceptual overview of the field and brought several of the key mechanisms of signaling initiation and transduction into sharper focus. Until approximately 1996, the majority of what was known came from budding and fission yeast (Elledge, 1996). It was becoming clear that the DDR was a signal transduction pathway as it appeared to be a kinase cascade activated by DNA damage and replication stress. However, at this point there was no understanding how conserved these pathways were in mammals. Furthermore, many thought the pathway's central purpose was to regulate cell-cycle transitions, primarily due to the powerful conceptual appeal of the checkpoint idea that emerged out of the cell-cycle field. This was despite considerable evidence indicating these pathways had profound roles in responding to DNA replication stress and that many of the targets of the transcriptional branch of the response were enzymes involved in DNA repair and DNA replication.

The past decade has revealed that this regulatory pathway is highly conserved and elaborated in mammals, the subject of this review. Homologs exist for all components of these pathways discovered in yeast, including ATM, ATR, Chk1, and Chk2, as well as the mediator proteins. In addition, it is now clear that the DDR is multifaceted and that cell-cycle control is only one of many subroutines orchestrated by this sensory network whose central goals are the repair of DNA damage and the facilitation of DNA replication.

A Sense of the Problem

To properly protect the genome, all types of DNA structural alterations must be detected, including nicks, gaps, double-strand breaks (DSBs), and the myriad alterations that block DNA replication. At least five independent molecular complexes sense and signal different types of DNA damage (Figure 1). Of these, we have learned the most about the ATM and ATR complexes. ATM is activated in response to DSBs, but initially the mechanism was unclear. We have learned that the Mre11-Rad50-Nbs1 (MRN) mediator complex acts as a DSB sensor for ATM and recruits it to broken DNA molecules (Lee and Paull, 2005). ATM exists as inactive dimers that, when recruited to DSBs, dissociate and autophosphorylate on multiple residues thought to be important for maintaining ATM activation (Bakkenist and Kastan, 2003). The MRN complex is also a substrate of ATM whose phosphorylation is important for downstream signaling. In addition, MRN might recruit substrates to ATM. There are still important issues to be resolved, including precisely how damaged DNA activates ATM, the role of autophosphorylation in the dimer-monomer transition, how ATM returns to its off state, and how ATM can be activated by stimuli such as osmotic shock and chloroquine which do not obviously cause DNA damage (Bakkenist and Kastan, 2003). In addition, a number of other proteins, such as the histone acetyltransferase Tip60 and the PP5 phosphatase, have been implicated in ATM activation and their roles remain to be integrated into the biochemistry of activation.

Our understanding of how replication blocks are detected has also experienced significant advancement. We now know that when DNA polymerases stall, the MCM replicative helicases continue DNA unwinding ahead of the replication fork, leading to the generation of ssDNA, which is then bound by the single-strand binding protein complex RPA, and this unwinding is required for signaling (Byun et al., 2005). The ssDNA-RPA complex then plays two critical roles: it recruits the ATR protein through its regulatory subunit ATRIP (Zou and Elledge, 2003), and it recruits and activates the Rad17 clamp loader which then loads the PCNA-related 911 (Rad9-Rad1-Hus1) complex onto DNA (Yang and Zou, 2006). The colocalization of 911 and ATR-ATRIP allows interaction at damage sites. ATR phosphorylates Rad17 and 911, which is important for downstream signaling. ATR is activated during this process. Biochemical experiments with budding yeast proteins have indicated that 911 can

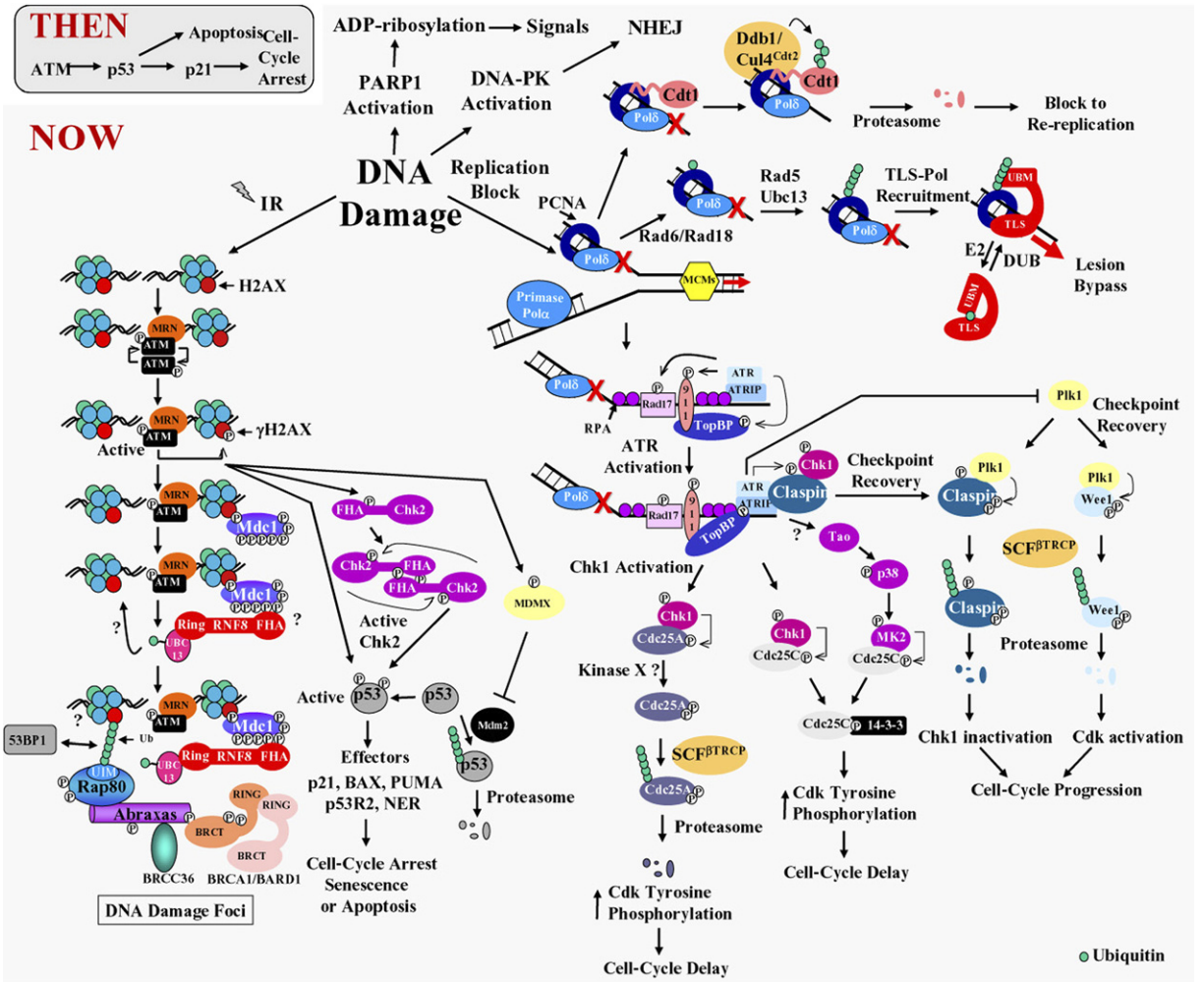

Figure 1. The DNA Damage Response Then and Now

A schematic of the DDRs discussed in this perspective. The red x's represent replication blocks, and the red arrows indicate the direction of movement of replication helicases and polymerases. Open circles containing "P" represent phosphate, while green filled circles represent ubiquitin.

activate the ATR kinase on DNA (Majka et al., 2006). This has not yet been established for human ATR. However, another important player has emerged in this context, TopBP1. TopBP1 is required for DNA replication and checkpoint signaling and is the ortholog of Cut5 and Dpb11 in yeast. In addition to its role in DNA replication, TopBP1 binds the 911 complex and contains a domain that can bind ATRIP and stimulate ATR kinase activity (Kumagai et al., 2006). TopBP1 is a substrate for ATR and ATM, and its phosphorylation appears to be required for its ability to function in checkpoint signaling. An appealing model for activation of this signaling pathway is shown in Figure 1. In this model, ssDNA-RPA generated at replication blocks recruits ATR-ATRIP and Rad17 to load 911. Rad17, 911, and TopBP1 are then phosphorylated by ATR and stimulate ATR kinase activity, although the order of these events is not entirely clear. In mammals, but not in yeast, ATR activation in response to DSBs appears to require ATM (Myers and Cortez, 2006). Precisely how each of these components contributes to ATR activation remains to be established, but our vision of this process has gained significant resolution in the last decade.

Mediators: A Focus on Foci

Mediators are proteins that act directly downstream of the ATM and ATR kinases as substrates that play various roles, acting both as recruiters of additional substrates and as scaffolds upon which to assemble complexes. The last 10 years have brought the discoveries of Mdc1, 53BP1, the MRN complex, Claspin, Brit1/Mcph1, and Brca1 as mediators. At the site of DNA damage, the variant histone H2AX becomes phosphorylated on Ser139 by ATM, ATR, and DNA PK (Rogakou et al., 1998). This phosphorylation then directly recruits Mdc1, which acts to amplify H2AX phosphorylation, possibly by tethering ATM or preventing H2AX dephosphorylation (Stucki and Jackson, 2006). Mdc1 and H2AX allow the recruitment of many additional factors to sites of damage leading to the generation of

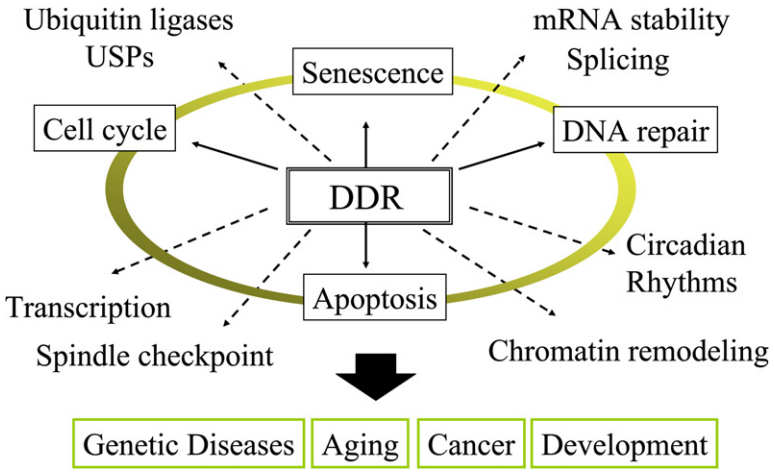

Figure 2. Biochemical and Physiological Consequences of the DNA Damage Response

IR-induced foci (IRIF). 53BP1, a mediator with roles in recombination, Chk2, and p53 activation, is recruited to IRIFs in an H2AX- and MDC1-dependent manner. Mdc1 phosphorylation also sets in motion polyubiquitination at sites of DSBs, which serves as a signal to further assemble IRIFs. Phosphorylation of Mdc1 recruits an E3 ubiquitin ligase, Ubc13-Rnf8, which ubiquitinates H2AX and possibly other proteins to then recruit 53BP1 and the Brca1 "A complex" (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007; Wang and Elledge, 2007), the latter through the UIM domains of its Rap80 component (Petrini, 2007). Ubiquitin foci at IRIFs depend upon Ubc13, Rnf8, and Brca1, itself a ubiquitin ligase. Currently it is not clear which ligase gives rise to the majority of the ubiquitin foci, nor what their roles are in cellular physiology. Importantly, elegant microscopic analyses have revealed IRIFs to be dynamic spatiotemporal regulated structures with layers of regulators woven into their structures in a precise order and position (Bekker-Jensen et al., 2006). Interestingly, irrespective of the type of DNA damage, multiple mediators and effectors may be called in (akin to the EMS, police, and fire department) although eventually only a subset will be required. It is likely that this diverse tool kit facilitates multiple types of repair processes.

The role of IRIFs in signal transduction and DNA repair is not yet fully understood. H2AX deletion prevents IRIF formation of most proteins with surprisingly mild effects on DNA damage sensitivity, genomic stability, and DNA damage signaling. However, one of the key findings facilitated by the IRIF studies has been that mediator foci serve as surrogates of the presence of DNA damage and have been detected in early-stage tumors, allowing us to visualize genomic instability in the evolution of cancer and helping us to understand the strong selective pressure to disable p53 (Bartek et al., 2007). The source of DNA damage appears diverse, including telomere shortening, oncogenic damage, or chemotherapy, but the effect is similar, resulting in activation of p53 leading to cell-cycle arrest, senescence, or apoptosis, thereby suppressing tumorigenesis. Interestingly, normal telomere function requires some of the proteins that normally participate in DDR, and elucidation of their roles promises to be an active area of future study.

Effectors: A Flood of Downstream Targets

The key to understanding the physiological significance of pathways regulated by protein kinases lies in its phosphorylation substrates. Over the past decade, single-protein analyses as well as unbiased screens for ATM and ATR substrates identified over 700 proteins phosphorylated in response to IR or UV (Matsuoka et al., 2007). These studies revealed a very strong enrichment for proteins involved in DNA replication, such as the MCMs, ORCs, RFC, GINS, TopBP1, and DNA polymerase complexes, which could go a long way toward explaining how fork stability and slowing of DNA replication are controlled in response to damage (Branzei and Foiani, 2007; Lambert et al., 2007). Enrichment was also observed for factors with known roles in direct DNA repair such as bypass polymerases, nucleotide metabolism, transcription coupled repair, global excision repair, mismatch repair, crosslink repair, and homologous recombination (HR). These connections underscore the critical role the DDR surveillance pathways play in directly controlling DNA repair and genomic stability beyond their roles in controlling the cell cycle. For example, Chk1 phosphorylation of Rad51 is important for HR, and ATR phosphorylation of FANCD2 controls crosslink repair. Most impressive were the large numbers of new connections to pathways not previously directly implicated in the DDR such as insulin signaling, RNA splicing, nonsense mediated decay, the spindle checkpoint, mitotic spindle and kinetochore proteins, tumor suppressors, chromatin remodeling, and a multitude of transcription factors. Furthermore, an intriguing series of connections to the circadian clock has emerged (Collis and Boulton, 2007). These findings point to a much broader role for the DDR in cellular physiology than previously appreciated (Figure 2). A key next step is to identify the substrates of other kinases in the DDR, including Chk1, Chk2, the newly implicated MK2 kinase

(Reinhardt et al., 2007), and casein kinases, which are known to be involved in this response, and to begin to decipher the significance that these substrates and their phosphorylation events have for aging, diabetes, and cancer.

Additional Key Findings

In addition to the general issues outlined above, important advances have occurred in our understanding of how Chk kinases are regulated. Chk2 is activated by direct phosphorylation by ATM on sites that lead to homodimerization via the FHA domains and autophosphorylation of the kinase activation loop (Ahn et al., 2004). Chk1 activation was illuminated by the discovery of the Mrc1 ortholog Claspin (Kumagai and Dunphy, 2000). Claspin is a protein involved in DNA replication that becomes phosphorylated by ATR at sites of replication stress and binds and activates Chk1, likely through recruitment to ATR.

Just as chromatin has taken a central stage in transcriptional control, so has an appreciation for chromatin regulation of and by the DDR. Many studies have identified roles for Kap1, a chromatin global regulator, and the INO80, NuA4, SWI/SNF, and RSC chromatin remodeling complexes in responding to DNA damage (Lee and Workman, 2007). In addition, the presence of tandem Tudor domains in 53BP1 (and yeast Rad9 and Crb2) has led to speculation that chromatin at DSBs is directly sensed, although whether it is a primary or secondary event with respect to signaling is not yet resolved (Huyen et al., 2004). Furthermore, cohesin activity and loading, which regulates interactions between chromatin regions, was found to respond to DNA damage.

The DDR Signals to Ubiquitin

Arguably one of the most far-reaching advances in the last decade has been the realization of the extensive roles in the DDR played by ubiquitin in both protein turnover and protein recruitment. To put this into perspective, in 1996 linkage of the ubiquitin system with the DDR was limited to the finding that budding yeast Rad6 was an E2 conjugating enzyme, whereas Rad23 contained a ubiquitin-like domain required for function (Bailly et al., 1994; Watkins et al., 1993). The major classes of ubiquitin ligases, including HECT domain and the SCF class of ubiquitin ligases, were only beginning to emerge, and it would be some time before the role of RING finger domains as E2 enzyme-targeting subunits of ubiquitin ligases would come to light (Tyers and Willems, 1999). The discovery of RING fingers as E3s had significant ramifications, as it led to an understanding of the role of the RING domains of BRCA1/BARD, FANCL, Rad18, Mdm2, and other E3s in the DDR. Likewise, with the exception of the Rpn10/S5a subunit of the proteasome, ubiquitin recognition motifs were unknown at this time. We now know that the DDR utilizes multiple classes of ubiquitin-binding motifs to coordinate signaling and repair (Bienko et al., 2005; Bertolaet et al., 2001).

Integrating the DDR with Turnover of Cell-Cycle Regulators

The cell cycle is regulated by oscillations in the activity of cyclin-dependent kinases (Cdks), which are positively regulated by cyclins and negatively regulated by Cdk inhibitors (CKIs) and by inhibitory tyrosine phosphorylation (Guardavaccaro and Pagano, 2006). One arm of the DDR is the G2/M checkpoint, which inhibits Cdk activity. We now know that the relevant DDR targets are the Wee1 kinase and the Cdc25 phosphatases, which regulate inhibitory tyrosine phosphorylation on Cdk1 and Cdk2 to control progression into S phase and mitosis. Chk1 and Chk2 play important roles in this process through multiple mechanisms. Cdc25C phosphorylation by checkpoint kinases leads to inhibitory sequestration by 14-3-3 proteins. In contrast, the major fate of Cdc25A in response to DNA damage is ubiquitin-mediated proteolysis. Cdc25A acts earlier in the cell cycle than Cdc25B and Cdc25C and is thought to be important for maximal Cdk activity during progression through S phase, and also contributes to passage through mitosis. During S phase and in response to DNA damage, Cdc25A is phosphorylated by Chk1 to create a phosphodegron leading to its ubiquitination by the $SCF^{\beta\text{-}TRCP}$ ubiquitin ligase and its rapid degradation (Guardavaccaro and Pagano, 2006). Failure to regulate Cdc25 leads to hyperactive Cdk activity and intra-S phase checkpoint defects.

During a normal G2/M transition, Plk1 phosphorylates Wee1 to create a phosphodegron that targets Wee1 for degradation via $SCF^{\beta\text{-}TRCP}$(Guardavaccaro and Pagano, 2006). Plk1 itself is negatively regulated by the DDR in an ATM/ATR-dependent manner. Inhibition of Plk1 prevents formation of the Wee1 phosphodegron, thereby stabilizing Wee1 in the face of DNA damage, and maintains Cdks in their inhibited form.

Important advances in understanding how adaptation to checkpoint signals allows slippage through a cell-cycle arrest came with the finding that in *Xenopus* extracts Chk1 was inactivated through Plk1 phosphorylation of Claspin (Yoo et al., 2004). This echoed earlier connections to adaptation by Cdc5, the budding yeast Plk1 ortholog. Plk1 phosphorylates Claspin and, like Wee1, targets it for destruction via $SCF^{\beta\text{-}TRCP}$, leading to reduced Chk1 signaling and cell-cycle progression (Gewurz and Harper, 2006). Thus, Plk1 and $SCF^{\beta\text{-}TRCP}$ have emerged as central components of the machinery controlling protein degradation and mitotic entry that are manipulated by the DDR.

A critical aspect of the DDR is to inhibit DNA replication during repair to prevent polymerases from encountering DNA damage. Part of this regulatory circuit is now understood to occur through regulation of Cdt1. Cdt1 and Cdc6 load the replicative helicase MCM2-7 onto origins, forming the prereplication complex (pre-RC) (Arias and Walter, 2007). The process of helicase translocation from the origin leads to disassembly of the pre-RC, thereby blocking reinitiation during S, G2, and M phase. During S phase and in response to DNA damage, Cdt1 is targeted for

ubiquitin-mediated destruction by an SCF-like ubiquitin ligase composed of Cul4, Rbx1, Ddb1, and the WD40-containing specificity factor Cdt2 (Arias and Walter, 2007). This reaction occurs on PCNA loaded onto chromatin at the sites of active origins. It is critical that origin firing be tightly controlled during the DDR, especially under conditions where late origins have yet to fire, as this could lead to further genomic instability. In contrast to Cdc25A turnover, Cdt1 turnover does not appear to require ATM/ATR. Understanding the biochemical pathways that coordinate Cdt1 degradation with DNA damage remains a challenge for the future.

Holding On to Ubiquitin

A decade ago, the best understood function for ubiquitin was promoting protein turnover through the proteasome, which we now know involves primarily lysine 48 (K48) polyubiquitin chain linkages. However, it is now evident that ubiquitin plays roles unrelated to protein turnover by binding to a variety of ubiquitin-binding domains, and its use in the DDR is no exception. Both K63 polyubiquitin chain linkages as well as monoubiquitin provide signals for recruitment of repair factors in the DDR. Early findings indicated that while K63 in ubiquitin was not required for bulk protein turnover, it was required for Rad6-dependent DNA repair (Spence et al., 1995). One role appears to be in facilitating PCNA-dependent alterations in polymerase choice for DNA replication (Moldovan et al., 2007). In response to stalling of replication forks due to bulky DNA adducts, PCNA is monoubiquitinated on K164 via the Rad6 (E2)/Rad18 (RING E3) complex. This modification is thought to be required for recruitment of translesion synthesis (TLS) polymerases, a group of error-prone polymerases that synthesize DNA past adducts. TLS polymerases contain ubiquitin-binding domains that recognize monoubiquitinated PCNA (Bienko et al., 2005). Because these reactions are untemplated, TLS polymerases act in an error-prone manner. An alternative error-free mode of repair, sometimes referred to as template switching (TS), requires the Rad5/Mms2(Uev2)/Ubc13 ubiquitin ligase complex, which assembles K63 polyubiquitin chains onto K164 of PCNA (Moldovan et al., 2007). The signals that are sensed by Rad6 and Ubc13 complexes to initiate ubiquitination are not yet known.

Interestingly, PCNA K164 is also modified by sumoylation in both yeast and mammals (Moldovan et al., 2007). In yeast, sumoylation of PCNA leads to recruitment of Srs2, which suppresses a Rad52-dependent sister chromatid recombination pathway acting on stalled replication forks. This recombinational approach for restarting stalled replication forks can be detrimental, and sumoylation of PCNA provides a mechanism to limit this process during the normal replication process. How the choice between ubiquitination and sumoylation is determined is not understood. A potential mechanism for this might employ the deubiquitinating enzyme Usp1, which can reverse monoubiquitination of PCNA (Huang et al., 2006).

In many cases, proteins containing ubiquitin-interacting domains are themselves ubiquitinated, often in a manner that requires their ubiquitin-interacting domain. A case in point is TLS polymerases, whose monoubiquitination requires their ubiquitin-binding UBZ or UBM domains (Bienko et al., 2005). Once monoubiquitinated, TLS polymerases are unable to efficiently interact with other targets via their ubiquitin-binding domains, suggesting that monoubiquitination plays an inhibitory role. Precisely how these monoubiquitination events are regulated is not known. Recent work has suggested that this type of monoubiquitination can occur in *cis* in an E2-dependent but E3-independent manner. Because E2s are largely charged in cells, it may be that ubiquitin-binding domain-containing proteins that are not engaged with a target protein are kept in an inactive form via *cis* monoubiquitination. If this is the case, signal-dependent deubiquitination may be required to reverse this inhibitory modification. Future studies will be directed at understanding how the dynamics and specificity of these processes are controlled.

Genome Integrity and Cancer

Defects in the ability to properly respond to and repair DNA damage underlie many forms of cancer. The last decade has brought an added appreciation of the roles of the DDR in genomic stability and cancer with the identification of roles for breast cancer genes Brca1 and Brca2 in the DDR, both in signaling and in DNA recombinational repair (Jasin, 2002). Direct links between ATM and ATR to these proteins followed. In addition, Chk2, along with ATM, was found to regulate the transcription factor p53 by preventing its ubiquitination by the RING E3 ligase Mdm2 (Ahn et al., 2004). Chk2 is mutant in a subset of patients with the Li Fraumeni cancer syndrome, the same syndrome caused by mutations in p53 (Ahn et al., 2004). The first DNA damage regulated signaling protein discovered, budding yeast Dun1, is a paralog of Chk2 and, like Chk2, regulates the transcriptional response to DNA damage. This satisfying connection underscores the deep conservation of this pathway throughout evolution. These connections demonstrate that not only are the direct DNA repair proteins such as the XP, Fanconi anemia, and MMR proteins important in cancer prevention, so are the higher order sentries of DNA damage sensing typified by ATM and Chk2.

Connections between ubiquitin and cancer have also come from several repair pathways that utilize ubiquitin signaling, such as the Fanconi anemia (FA) pathway, which promotes repair of interstrand crosslinks in DNA. FA is a developmental and cancer predisposition syndrome, and mutations in 13 complementation groups have been identified. Eight FA proteins form a multisubunit ubiquitin ligase in which the RING finger-containing FANCL protein catalyzes monoubiquitination of each subunit of the FANCI/FANCD2 (ID) complex, thereby promoting ID recruitment to sites of damage (Grompe and van de Vrugt, 2007). This event is required for subsequent recruitment of a BRCA2(FANCD1)/Rad51 complex that promotes

homologous recombination-mediated repair of the lesion. FANCD2 is directly phosphorylated by ATR, and this phosphorylation is required for FANCD2 ubiquitination, thereby forging yet another link between DDR signaling and direct DNA repair. A deubiquitinating enzyme, USP1, has been implicated in deubiquitination of the ID complex (Grompe and van de Vrugt, 2007). In response to DNA damage, USP1 is phosphorylated and undergoes an inactivating self-cleavage through recognition of an internal ubiquitin-like domain (Huang et al., 2006). USP1 inactivation in part contributes to ID complex ubiquitination.

The familial breast cancer gene BRCA1 contains an N-terminal RING finger and forms a heterodimer with the RING finger of BARD1 to generate an E3 complex critical for the DDR (Ruffner et al., 2001). Despite our understanding of the biochemistry of the BRCA1 E3 ligase, we know very little of its cellular targets and how these targets are chosen. The identification of ubiquitination substrates of BRCA1 and the elucidation of how BRCA1 controls the DDR remain two of the most important challenges for the field.

Nucleotide excision repair (NER) provides multiple routes for the removal of damaged DNA, both in a global genome-wide form (GG-NER) and in a pathway that specifically removes transcriptional blocks, transcription-coupled NER (TC-NER) (de Laat et al., 1999). Mutations in NER underlie the extreme photosensitivity and predisposition to skin cancer exhibited in patients with Cockayne syndrome (CS) and xeroderma pigmentosum (XP), although CS patients display developmental and neurological phenotypes not observed with XP patients. Mutations in the CSA and CSB proteins lead to specific defects in NER of the transcribed strand in transcriptionally active genes. Recent work has demonstrated that CSA is a member of the Ddb1- and Cul4-associated factor (DCAF) family of WD40 containing proteins that act as receptors for the Ddb1/Cul4 ubiquitin ligase (O'Connell and Harper, 2007). The Cul4-Ddb1^{CSA} complex promotes the degradation of the chromatin remodeling factor CSB at the latter stages of the repair process, thereby allowing recovery of transcriptional activity. Ironically both CSB activity and its destruction are required for function of this pathway. The signals that initiate CSB destruction are currently unknown. A subset of XP complementation group E patients harbor mutations in DDB2, a WD40-containing DCAF protein and candidate substrate recruitment factor. These Ddb2 mutations lie within the WDXR motif responsible for interaction of Ddb2 with Ddb1, implicating the Cul4-dependent ubiquitin ligase function of this complex in NER (O'Connell and Harper, 2007). Ddb2 appears to play a critical role in the recognition of particular types of cyclopyrimidine dimers. Recent data suggest that the role of Ddb1-Cul4 may be to promote Ddb2 degradation once the damage has been recognized, thereby facilitating the recruitment of the XPC-Rad23 complex to initiate NER (O'Connell and Harper, 2007). Cul4-Ddb1^{Ddb2} complexes also promote histone H2A and XPC ubiquitination at the site of damage, suggesting that Ddb2 serves multiple functions in this pathway (O'Connell and Harper, 2007). XPC ubiquitination promotes recruitment of other components of the XP repair system to chromatin.

Where Does the Future Lie?

The last 10 years have been a thrilling time in the DDR field. Several of the key mechanisms integral to the signal transduction pathway have come to light, with many more details left to be discovered. We are now in a position to drill very deep into the mechanisms of the regulation and orchestration of DNA repair and DNA replication control, the heart of this response. The next decade will yield many new advances as the genetic tools that have emerged, such as RNAi, give us the ability to perform more gene discovery and detailed analysis of protein function, especially the roles of the many kinase substrates identified. These advances will be complemented by escalation of structural information and biochemical reconstitution of these pathways. The current in vitro repair assays in *Xenopus* extracts are excellent systems in which to begin purification and reconstitution of active subcomplexes. We envision a time when we can add purified components into a reaction together with damaged chromatin and observe properly regulated and appropriate repair reactions. The systems level integration of these classes of knowledge will provide a rich picture of the DDR 10 years hence.

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