Review Article

The DNA Damage Response—Repair or Despair?

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The term ''the DNA damage response'' (DDR) encompasses a sophisticated array of cellular initiatives set in motion as cells are exposed to DNA-damaging events. It has been known for over half a century that all organisms have the ability to restore genomic integrity through DNA repair. More recent discoveries of signal transduction pathways linking DNA damage to cell cycle arrest and apoptosis have greatly expanded our views of how cells and tissues limit mutagenesis and tumorigenesis. DNA repair not only plays a pivotal role in suppressing mutagenesis but also in the reversal of signals inducing the stress response. If repair is faulty or the cell is overwhelmed by damage, chances are that the cell will despair and be removed by apoptosis. This final fate is determined by intricate cellular dosimeters that are yet to be fully understood. Here, key findings leading to our current view of DDR are discussed as well as potential areas of importance for future studies. Environ. Mol. Mutagen. 51:879–889, 2010. © 2010 Wiley-Liss, Inc.

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HISTORY OF THE DDR RESEARCH FIELD

Long before it was determined that DNA is the basic repository of the genetic material of all life, Hermann Muller realized that environmental agents, such as X-rays, induce mutations [Muller, 1927]. Subsequently, Alexander Hollaender discovered that cells have the innate ability to recover from damage induced by UV light and realized that cellular responses must exist that transiently arrest the growth of exposed cells, allowing them time to repair the damage before resuming growth [Hollaender and Curtis, 1935; Hollaender and Duggar, 1938]. Discoveries of various DNA repair pathways during the second part of the 20th century explained many of these early observations in mechanistic terms. However, it was not until the discovery of DNA damage-induced signal transduction pathways during the 1990s that we began to understand the full meaning of the DNA damage response (DDR).

In Figure 1, some of the key findings in the DDR field are summarized in a time line. It should be noted that there is never a single finding or publication that truly defines a new discovery but rather all discoveries build on the accumulated knowledge up to that point. Therefore, any attempts to single out individual publications as ''the discovery'' are inherently flawed and I apologize for leaving out many important contributions from this time line. Nevertheless, the intellectual building blocks of our field listed in this timeline are astonishing and one may ask what new discoveries are yet to be made?

COMPONENTS OF DDR

The DDR is a comprehensive and complex set of responses aimed at safeguarding the genomic integrity of cells [Jackson and Bartek, 2009]. DNA repair processes lay the foundation of this response with added layers of monitoring leading to the activation of cell cycle checkpoints or apoptosis (Fig. 2). The cellular fate of promoting either survival or death is governed by the severity of damage, the efficiency of repair, and is strongly dependent on cell type [Gudkov and Komarova, 2003].

DNA Repair

DNA repair is arguably the most important component of DDR. The DNA restoration task of the various DNA

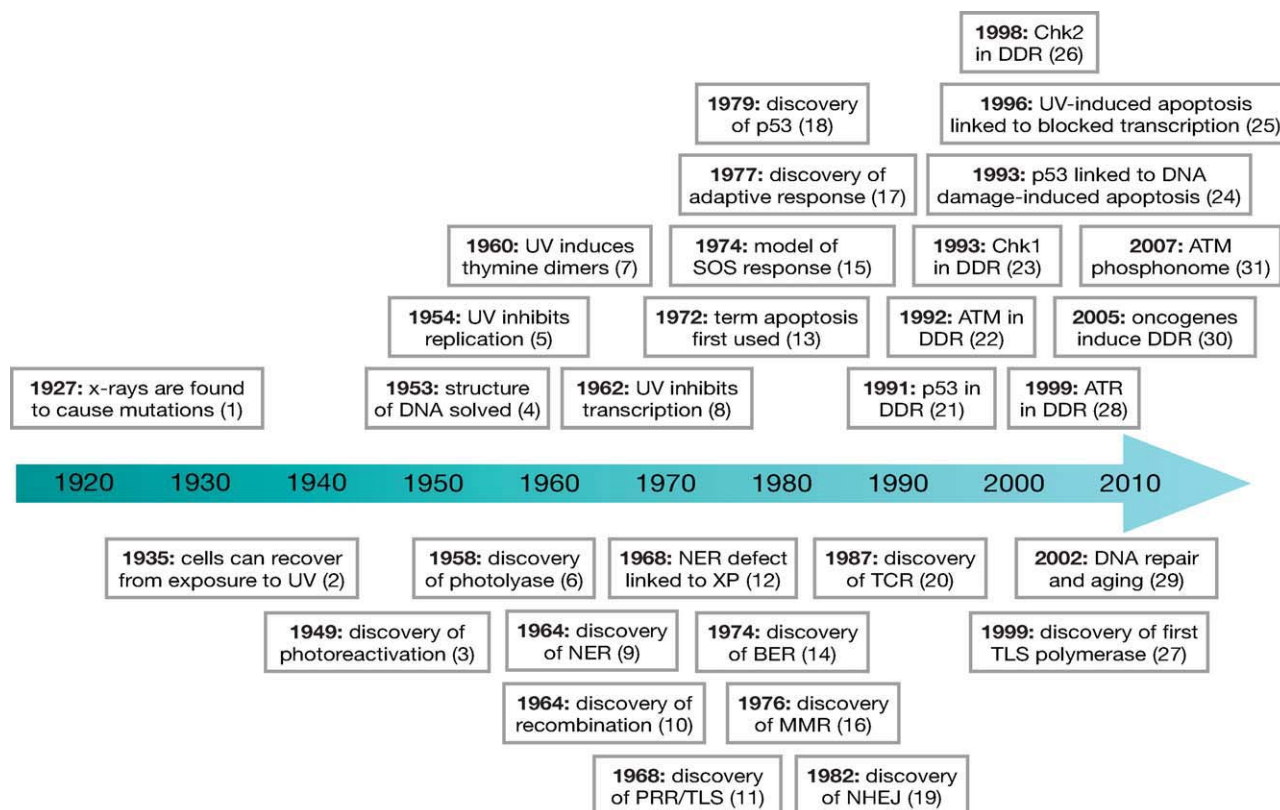Environmental and Molecular Mutagenesis. DOI 10.1002/em

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Fig. 1. Timeline of major discoveries in the DDR field. The discoveries listed on top are related to various effects of DNA damage on cellular functions and DNA damage signaling while the discoveries listed on the bottom are related to DNA repair. The numbers in parentheses denote the discovery's corresponding publications, which are listed below: (1) [Muller, 1927]; (2) [Hollaender and Curtis, 1935]; (3) [Dulbecco, 1949; Kelner, 1949]; (4) [Watson and Crick, 1953]; (5) [Kanazir and Errera, 1954]; (6) [Rupert et al., 1958]; (7) [Beukers and Berends, 1960]; (8) [Kameyama and Novelli, 1962; Masters and Pardee, 1962]; (9) [Boyce and Howard-Flanders, 1964; Pettijohn and Hanawalt, 1964; Rasmussen and Painter, 1964; Setlow and Carrier, 1964]; (10) [Holliday, 1964]; (11) [Rupp and Howard-Flanders, 1968]; (12) [Cleaver, 1968]; (13) [Kerr et al., 1972]; (14) [Lindahl, 1974]; (15) [George et al., 1974; Witkin, 1974]; (16) [Wagner and Meselson, 1976]; (17) [Jeggo et al., 1977; Samson and Cairns, 1977]; (18) [Lane and Crawford, 1979; Linzer and Levine, 1979]; (19) [Wilson et al., 1982]; (20) [Mellon et al., 1987]; (21) [Kastan et al., 1991]; (22) [Kastan et al., 1992]; (23) [Walworth et al., 1993]; (24) [Lowe et al., 1993]; (25) [Ljungman and Zhang, 1996]; (26) [Matsuoka et al., 1998]; (27) [Johnson et al., 1999; Masutani et al., 1999a, b]; (28) [Tibbetts et al., 1999]; (29) [de Boer et al., 2002]; (30) [Bartkova et al., 2005; Gorgoulis et al., 2005]; (31) [Matsuoka et al., 2007]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

repair enzymes is enormous considering that the DNA molecule is not unusually stable and is further damaged by endogenous reactive oxygen species and by the wear and tear of replication and transcription. It has been estimated that more than 20,000 lesions are induced on a daily basis in each cell by endogenous forces [Friedberg et al., 2006]. Adding all these events up in one individual, one would get a number that is larger than there are grains of sand on all the beaches of this planet [Derheimer, 2007]. The great majority of the endogenously induced lesions are repaired by the base excision repair (BER) machinery and loss of any of the BER enzymes APE1 [Xanthoudakis et al., 1996], DNA polymerase β [Gu et al., 1994] or DNA ligase III [Puebla-Osorio et al., 2006] is not compatible with life.

DNA repair enzymes are constantly engaged in probing the integrity of the DNA molecule. In general, there does not seem to be a need for additional DNA damage sensors, such as ataxia telangiectasia mutated (ATM) and ATM and RAD3-related (ATR), to directly activate the DNA repair pathways. However, some examples exist in which ATM or ATR-mediated phosphorylation stimulates repair. For example, it has been shown that ATR promotes repair after UV-irradiation by phosphorylating the nucleotide excision repair (NER) protein XPA [Wu et al., 2006; Shell et al., 2009] or stimulating global genomic repair in S-phase [Auclair et al., 2008]. Furthermore, ATM promotes repair in heterochromatin following exposure to ionizing radiation [Beucher et al., 2009; Goodarzi et al., 2008; Noon et al., 2010]. Finally, by phosphorylating and activating p53, ATM and ATR promote global genomic nucleotide excision repair [Ford and Hanawalt, 1995, 1997] by the induced expression of DNA repair genes such as p48 (DDB2) [Hwang et al., 1999] and XPC [Adimoolam and Ford, 2002] (Fig. 2). Conversely, DNA repair intermediates formed by NER trigger the activation

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Fig. 2. The DNA damage response. The ATM kinase responds to alterations in DNA/chromatin topology while the ATR kinase monitors interruptions in transcription and replication as well as responds to DNA repair intermediates induced during NER. The induced ''phosphonome'' involves more than 700 substrates including p53 that collectively promote cell cycle arrest, DNA repair and/or apoptosis depending on the efficiency of DNA repair and on cell type. DNA repair can work in ATM/ATR-dependent and -independent ways to restore DNA integrity and to reverse DDR signaling. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Fig. 3. Formation of a DDR complex at site of DNA DSB. A DSB induces a topological alteration in the DNA/chromatin that leads to the activation of ATM and the C-terminal tail of H2AX becomes available for phosphorylation by ATM. Phosphorylation of H2AX then trigger the assembly of a large DDR complex consiting of MDC1, RNF8 BRCA1, 53BP1, and DNMT (see text for details). It has been recently shown that a similar DDR complex containing MDC1 and RNF8 assemble on chromatin following UV-irradiation and that DNA repair intermediates may trigger such assembly. The precise function of these DDR complexes is not well established but they may give cells the option to utilize recombination for repair, induce cell cycle checkpoints and may play a role in the restoration of chromatin structure following repair. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of ATR leading to the phosphorylation of the histone variant H2AX (γH2AX) [Hanasoge and Ljungman, 2007].

Cell Cycle Checkpoints

Proliferating cells are in general much more susceptible than resting cells to the toxic and mutagenic effects of DNA-damaging agents. This is due to the formidable tasks of DNA replication and chromosome segregation, processes easily derailed by DNA damage. To prevent cells with damaged DNA to enter S-phase or mitosis, ATM and ATR phosphorylate specific substrates, such as p53, CHEK1, and CHEK2, which promote cell cycle arrest [Reinhardt and Yaffe, 2009]. This arrest will ''buy'' time for the repair enzymes to clean up the DNA before DNA synthesis or chromosome segregation begin.

In addition to setting up cell cycle checkpoints, ATM regulates the recruitment of a number of DDR factors to sites of some types of DNA damage, e.g., DNA double strand breaks (DSB), by the phosphorylation of H2AX (Fig. 3). This phosphorylation nucleates the formation of a large complex consisting of MDC1, which binds to phosphorylated H2AX and in turn allows ubiquitin ligase RNF8 to bind. RNF8 then ubiquitylates histones in the chromatin surrounding the damage, thereby recruiting BRCA1 via the RAP80 protein and 53BP1 via chromatin structure alterations [Huyen et al., 2004]. When assembled, this complex enhances DNA double strand break repair, partakes in activating cell cycle arrest and

increases resistance to radiation [Yan and Jetten, 2008]. It has been recently shown that following UV-irradiation, DNA repair intermediates may trigger formation of γH2AX [Hanasoge and Ljungman, 2007] and the assembly of a DDR complex containing MDC1 and RNF8 [Marteijn et al., 2009]. While inactivation of some of the components in this complex, such as RNF8, resulted in increased sensitivity of cells to UV light, DNA repair was not affected. It is possible that the DDR complexes forming after the induction of DNA repair intermediates play a role in restoring the chromatin structure following repair to allow for resumption of transcription and/or replication.

Another protein that is recruited to the multiprotein DDR complex is the DNA methyltransferase DNMT (Fig. 3). This enzyme methylates nearby CpG islands to inactivate any ongoing transcription that may interfere with repair [O'Hagan et al., 2008]. Interestingly, the removal of the DNA methylation following repair was found not to be fully complete which may result in the epigenetic silencing of genes, including tumor suppressor genes. Thus, genotoxic damage may contribute to carcinogenesis by altering either the genome or the epiginome.

As repair continues, the pool of activated ATM or ATR will diminish to a level that finally will allow the traffic light to turn green and the cells can resume progression of the cell cycle. Although the mechanisms regu-

lating the activation and deactivation of cell cycle checkpoints are fairly well understood [Reinhardt and Yaffe, 2009; Toettcher et al., 2009], it is not fully clear whether checkpoints act as binary on/off switches determined by some critical level of DNA damage or whether cell progression continues but is slowed down in a DNA damage dose-dependent manner.

Apoptosis

Apoptosis is the last resort mode for cells if repair of DNA damage is slow or incomplete. Cells appear to possess an internal ''apoptotic clock'' or ''timer'' which is set to activate caspases leading to the induction of apoptosis when the remaining time on the timer runs out. Cells can evidently reset the timer before it runs out if sufficient DNA repair has occurred and essential processes such as transcription or replication have resumed.

In 1993, Scott Lowe et al. showed that radiation-induced apoptosis was strongly dependent on p53 in thymocytes [Lowe et al., 1993]. This led to the hypothesis that p53 is the master regulator of apoptosis and therefore, tumors with mutant p53 may respond poorly to radiation or chemotherapy. It is now clear that the role of p53 in regulating apoptosis is very cell type specific [Gudkov and Komarova, 2003] and that p53 may actually protect certain types of cells against UV light and cisplatin [McKay and Ljungman, 1999; McKay et al., 2000, 2001].

While apoptosis induced by certain agents such as UV light, cisplatin, or photoactivated psoralen, is strongly correlated to blockage of transcription [Derheimer et al., 2009; Ljungman and Zhang, 1996; Ljungman et al., 1999], cells appear to preferentially undergo apoptosis when attempting to traverse the S-phase of the cell cycle [McKay et al. 2001, 2002; Derheimer et al., 2009]. How can apoptosis correlate to both blocked transcription and to the traversing of S-phase? It is likely that during normal replication, a mechanism is in place to clear replicons to be replicated from ongoing transcription to avoid conflicts between transcription and replication [Tuduri et al., 2009] (Fig. 4). In fact, it has been shown that active transcription factories do not generally occupy the same chromatin regions as active replication factories [Wei et al., 1998]. The retinoblastoma protein (Rb) [Ahlander et al., 2008], the minichromosome maintenance (MCM) proteins [Bailis and Forsburg, 2004], and the ATR kinase [Cha and Kleckner 2002; Brown and Baltimore, 2003] may play roles during S-phase ensuring that replication origins only fire when transcription has been moved out of the path. In addition, cells lacking DNA topoisomerase I induce high levels of γH2AX in active genes during S-phase, suggesting that DNA topoisomerase I normally plays a role in suppressing any interference occurring by transcription during replication [Tuduri et al., 2009]. It is tempting to speculate that the ''replication stress'' that
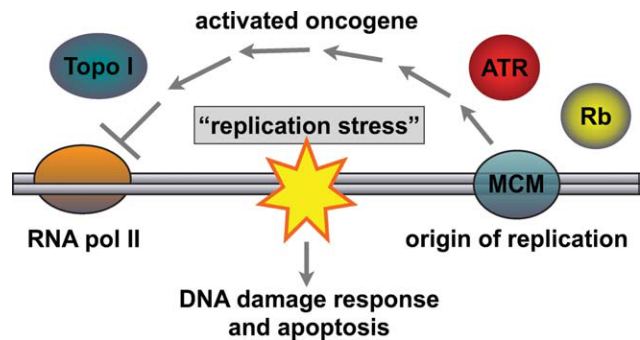
Fig. 4. Tug-A-War between replication and transcription. It is likely that initiating replication factories must negotiate with nearby transcription units to ''finish up'' so that replication origins can be fired. Many proteins regulate the timing of replication firing such as Rb, MCM, ATR and Topo I (see text for details). If transcription units are stalled at DNA lesions and replication initiates or if activated oncogenes drive cells into S-phase prematurely, cells will experience ''replication stress'' activating DDR. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

activated oncogenes induce in cells is due to a lax communication between replication and transcription prior to origin firing resulting in a tug-a-war between transcription and replication factories.

A similar situation may occur when the transcription elongation machinery encounters blocking DNA lesions as cells commit to entering S-phase [Ljungman and Lane, 2004; Tuduri et al., 2009].

DDR AND GENE EXPRESSION

An important outlet of DDR is to modify gene expression so that cells can better counteract the deleterious effects of genotoxic exposures and to adapt to potential future insults [Fornace et al., 1988; Herrlich et al., 1992]. The expression of many genes is upregulated at the level of transcription by p53-mediated transactivation [El-Deiry et al., 1993; Zhao et al., 2000], but gene expression may also be regulated post-transcriptionally through alternative splicing [Munoz et al., 2009], stabilization of specific mRNAs [Jackman et al., 1994; Blattner et al., 2000; Wang et al., 2000], and by preferential translation of certain mRNAs [Lu et al., 2006b; Kumaraswamy et al., 2008; Braunstein et al., 2009] (Fig. 5). Some of these mRNAs are regulated by microRNAs, which can be induced or repressed after exposure to DNA damage [Pothof et al., 2009; Shin et al., 2009; Simone et al., 2009].

What regulates gene expression at all these levels? The ATM kinase is one of the first responders following exposure to ionizing radiation and it has been shown that ATM can phosphorylate over 700 substrates after becoming activated [Matsuoka et al., 2007]. The largest group of proteins phosphorylated by ATM is a group of proteins
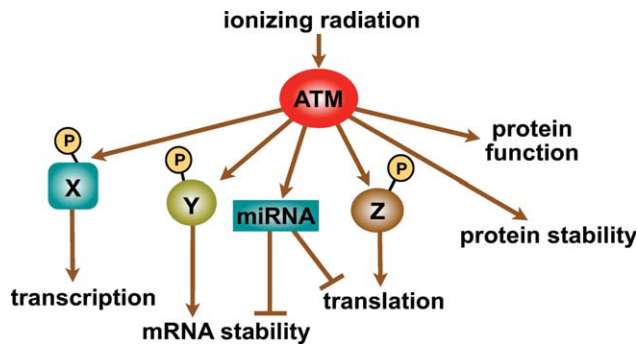
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Fig. 5. Radiation induces alterations in gene expression at multiple levels. ATM is activated by ionizing radiation leading to the phosphorylation of many downstream substrates resulting in the regulation of gene expression and protein function on many different levels (see text for details). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

involved in regulating ''RNA metabolism'' such as transcription, splicing, mRNA stability and translation. Only for a few of these proteins, such as p53, has the effect of ATM phosphoylation on gene expression been clarified (Fig. 4). There are many proteins known to regulate mRNA stability by binding to 3′UTR sequences. Some of these RNA-binding proteins are regulated by DNA damage including HuR [Abdelmohsen et al., 2007; Lafarga et al., 2009], nucleolin [Zhang et al., 2006], RNPC1 [Shu et al., 2006], and AUF [Lal et al., 2006]. It is not known whether ATM is involved in the regulation of mRNA stability or translation via these RNA-binding proteins.

EVOLUTION OF DDR

During evolution, genomic instability promoted diversity, thereby ensuring a greater probability of survival for at least some offspring if the environment suddenly changed. As organisms evolved more complex genomes, however, genomic instability became mostly detrimental and systems safeguarding the integrity of DNA became in demand. While most DNA repair systems found in higher organisms also exist in lower eukaryotes and bacteria [Friedberg et al., 2006], higher eukaryotes are equipped with additional layers of defense systems suppressing mutagenesis and tumorigenesis. Cell cycle checkpoints, regulated by an intricate network of sensors, transducers and mediators, allow cells more time for repair before entering replication or mitosis [Reinhardt and Yaffe, 2009; Toettcher et al., 2009]. Furthermore, the expression of many DDR genes is induced following DNA damage leading to enhanced DNA damage surveillance, repair and apoptosis.

As natural selection rewards improvements in biochemical processes that increase survival, such as DNA repair and cell cycle checkpoints, how could a genetic program carrying instructions for cellular suicide be allowed to develop during evolution? The process of apoptosis has an important role during development and normal tissue homeostasis for the elimination of altered cells or cells that are not longer needed [Cotter, 2009]. During the course of evolution, this programmed cell death pathway became incorporated into DDR to eliminate cells that had sustained damage exceeding their repair capacity [Norbury and Zhivotovsky, 2004]. To counterbalance apoptosis, cell cycle checkpoint mechanisms evolved that allowed cells more time to repair before entering critical cell cycle stages in which DNA damage may lead to cell death. The balance between pathways promoting survival and pathways promoting death must be finely regulated as to suppress cancer but promote longevity [Ljungman and Lane, 2004].

CELLULAR DNA DAMAGE DOSIMETERS

How do cells determine the severity of an insult acquired by a genotoxin to formulate a decision on whether to induce cell cycle checkpoints and DNA repair or induce apoptosis? The assessment of the level of DNA damage is not trivial for a cell since the genome is vast in molecular terms and it is decorated with histones and numerous chromatin-binding proteins limiting accessibility of the lesions to be detected. Cells may assess the severity of the acquired DNA damage in multiple ways.

First, cells may monitor the activity of DNA repair factors operating on DNA lesions or the level of DDR factors assembled in nuclear foci. If a certain critical level of DNA repair activity or DDR occupancy at nuclear foci is achieved, a strong enough signal surpassing a particular threshold may be generated triggering apoptosis. How the cell might monitor repair activity or foci occupancy is unknown.

Second, dedicated DNA damage sensors activated at sites of DNA damage may transmit stress signals as long as some minimal level of DNA lesions persists. This signal may be amplified in a ''feed-forward'' cascade putting time pressure on cells to expeditiously repair the DNA lesions before a critical level of signal is reached. If the damage is severe, cells may not be able to keep up with a progressively increasing damage signal with cell death to ensue.

Third, as discussed above, cells may monitor processes that normally operate on DNA, such as transcription and replication, to gather information on the status of the DNA template. If lesions in the DNA template interrupt transcription and/or replication, DNA damage signals may be generated in proportion to the overall severity of the interruption. We and others have shown that blockage of transcription is linked to the accumulation of p53 [Yamaizumi and Sugano, 1994; Ljungman et al., 1999] and that

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blockage of transcription elongation in particular generates an ATR-dependent stress response resulting in p53 phosphorylation [Derheimer et al., 2007; Ljungman et al., 2001]. Induction of p53 may then trigger apoptosis dependent on the cell type [Gudkov and Komarova, 2003].

Fourth, extended transcription blockage leads to the induction of apoptosis [Ljungman and Zhang, 1996] perhaps by the loss of particular RNAs coding for critical survival-promoting proteins. One example is the loss of the apoptosis antagonist Mcl-1 as a result of UV-mediated transcription blockage leading to apoptosis [Nijhawan et al., 2003]. Alternatively, inhibition of transcription may lead to the loss of a protein(s) involved in the processes of transcription or translation itself. This scenario would paint the cell into a corner where it would run out of any possibility of regenerating the missing essential protein(s) or any other protein for that matter. This would represent a point-of-no-return in which the cell is doomed and ultimately would be eliminated.

DDR, CANCER, AND AGING

DDR has been proposed to act as a major barrier for tumorigenesis by activating cell cycle checkpoints, apoptosis or senescence as a result of oncogene-induced ''replication stress'' [Bartkova et al., 2005, 2006; Gorgoulis et al., 2005]. This puts selective pressure on precancerous lesions and favors clones that have obtained mutations in DDR genes for continued proliferation. In addition to allowing these cells to proliferate, such mutations are also critical for creating a ''mutator phenotype'' that will accelerate the process of carcinogenesis by promoting genetic instability [Loeb et al., 2003]. Oncogene-mediated replication stress and defects in DDR in cancer cells are promising new areas for cancer therapeutic exploitation [Helleday et al., 2008; Ljungman, 2009].

While individuals with inactivating mutations in DDR pathways are profoundly susceptible for cancer, individuals with certain genetic polymorphisms, epigenetic silencing or heterozygosity in DDR genes also have elevated risks for contracting cancer. For example, about 1% of the population is estimated to be heterozygotic for the ATM allele, leading to haploinsufficiency and an elevated risk for cancer [Spring et al., 2002; Thompson et al., 2005; Lu et al., 2006a]. Furthermore, ATM alleles are found to be frequently mutated or epigenetically inactivated in cancer, suggesting that selective pressure favors inactivation or haploinsufficiency of ATM and the DDR pathway [Vo et al., 2005]. Similarly, heterozygosity of p53 [Srivastava et al., 1990], BRCA1/2 [Venkitaraman, 2002] or mismatch repair genes [Jiricny and Nystrom-Lahti, 2000] leads to familial predisposition to cancer.

The aging process has been linked to the accumulation of endogenous lesions in the genome [Hoeijmakers,

2009]. In particular, lesions interfering with transcription appear to be the major type of lesion associated with the aging process and ''premature aging'' syndromes caused by certain DNA repair defects [Ljungman and Zhang, 1996; de Boer et al., 2002; Garinis et al., 2009]. The gene expression profiles from cells of old individuals differ from those of younger individuals and interestingly, when cells from young mice are UV-irradiated, their expression profile changes so that it resemble the profile of cells from older mice [Garinis et al., 2009]. Since UV light randomly introduces transcription-blocking lesions in the genome, large genes are predicted to be inactivated more easily while small genes are less effected by UV light [McKay et al., 2004; Sauerbier and Hercules, 1978]. Thus, it is possible that genes not needed later in life, such as the insulin growth factor 1 receptor (IGF-1R), may have been selected to reside in large transcription units that have a high likelihood of becoming inactivated over time by random transcription-blocking lesions. Correspondingly, genes needed throughout life may be more compact so as to avoid being inactivated. It is also possible that repair mechanisms may be programmed to selectively remove lesions from genes important for longevity but evidence for such selective repair is lacking.

FUTURE STUDIES OF DDR

Studies of DNA repair dominated the DDR field during the 1960s, 1970s, and 1980s, while explorations of DNA damage signaling, cell cycle checkpoints, and apoptosis have blossomed during the last 20 years. We are in a very exciting period where technological advances in DNA sequencing, mass spectrometry, crystallography, and microscopy make it possible to comprehensively interrogate the genome, the epiginome, the RNAome, the proteome, the phosphonome and many other ''omes'' and how protein complexes are assembled and where in cells they operate.

What are the biggest questions in the field that may be answered in the next 5–10 years? Here is my personal top ten list:

10. *How is DNA topology and chromatin structure restored following DNA repair?* DNA of eukaryotic cells contains localized domains of unconstrained supercoiling that is assumed to be lost following induction of DNA strand breaks [Ljungman and Hanawalt, 1992, 1995]. Following the repair of a DNA strand break, the cells need to restore DNA topology and chromatin structure to completely restore the intact genomic region and for transcription or replication to resume [Ljungman, 2005]. How these events occur are not well understood but studies have suggested that p53 [McKay and Ljungman, 1999; McKay et al., 2000] and the DDR complex consisting

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of MDC1 and RNF8 [Marteijn et al., 2009] may play important roles in restoring the chromatin structure and to allow resumption of transcription following repair.

9. *What is a DDR nuclear focus and why are so many proteins aggregating in one place?* Following induction of a double strand break, long stretches of H2AX on either side of the break are phosphorylated, providing a ''landing strip'' for DDR proteins. Although only a few of the recruited DDR proteins will eventually be directly involved in the repair of the lesions, why is there such an excess of these proteins aggregating near the break site? Is the formation of this landing strip merely a mechanism to increase the local concentration of proteins that may be needed in the repair? Does this local accumulation act as a dosimeter of the severity of the damage and an indicator of the kinetics of recovery or is it needed for the restoration of chromatin structure following repair?

8. *How do translesion DNA polymerases kick out processive polymerases during translesion DNA synthesis?* When DNA polymerases encounter certain DNA lesions in the DNA template, translesion DNA polymerases replace the processive DNA polymerases [Friedberg et al., 2005]. This process depends on the coordination of many factors to induce ubiquitylation of PCNA at stalled replication forks. Does the exchange take place on either the leading or lagging strand? What happens to the released processive polymerase? To what extent is this process altered in cancer cells?

7. *How do replication and transcription communicate with each other to avoid ''tug-a-war'' conflicts during S-phase?* Little is known about how replication negotiates with transcription to clear transcription factories from the path of DNA to be replicated. Many proteins are involved in regulating the initiation and elongation of replication but it is not known whether they communicate with transcription (Fig. 4). Do DNA methyl transferases (DNMTs) methylate CpG islands of genes to be inactivated when they are in the path of replication? Does the presence of transcription factories stalled at sites of UV-lesions introduce a particularly difficult challenge when located on the same stretch of DNA as the replication machinery [McKay et al., 2002; Derheimer et al., 2009]?

6. *Have the sizes of genes been selected to allow for differential expression after UV light and as part of an aging program?* As discussed above, transcription-blocking lesions accumulate in DNA over time leading to the inactivation of genes [Garinis et al., 2009]. The degree of inhibition would be influenced by the size of the gene with large genes expected to have a higher probability of attracting blocking lesions [McKay et al., 2004]. Therefore, do genes that are not needed later in life reside in larger transcription units than genes that are in demand at old age?

5. *How does the apoptotic timer work and what do cells use as dosimeters for DNA damage?* As shown in Figure 2, DDR signaling can lead to multiple outcomes, such as enhanced repair, activation of cell cycle checkpoints or apoptosis. It is not well understood what regulates the choice of these very different outcomes but cells must rely on molecular dosimeters and timers that have different settings in different cell types.

4. *What are the mechanisms leading to oncogene-induced ''replication stress''?* Overexpression or activation of oncogenes forces cells to prematurely enter S-phase resulting in ''replication stress'' [Bartkova et al., 2005; Gorgoulis et al., 2005]. This stress activates many DDR proteins suggesting that DNA damage is induced when oncogenes are activated. Does expression of oncogenes force cells to fire replication origins before the path is cleared of transcription and may this be the cause of replication stress [Tuduri et al., 2009]? If we knew the nature of this damage and the factors the cell utilizes to overcome this stress, we may identify new therapeutic targets that tumor cells uniquely rely upon.

3. *How do DNA-damaging agents affect transcription, mRNA stability and translation?* As described in Figure 5, ATM and ATR phosphorylate numerous proteins involved in RNA metabolism and thus may regulate DNA damage-induced/repressed transcription, RNA splicing, mRNA stability and translation via these proteins. Next generation sequencing technologies will make it possible to comprehensively assess the RNAome, RNA splicosome, transcriptome, RNA ''stabilome'' and ''translatome'' and the effect DNA damage may have on these ''omes.''

2. *How is DNA damage sensed and how are ATM and ATR activated?* We have learned that ATM respond to topological alterations in DNA/chromatin [Bakkenist and Kastan, 2003] and that ATR monitors replication [Guo et al., 2000; Hekmat-Nejad et al., 2000], transcription [Derheimer et al., 2007] and DNA repair intermediates [Hanasoge and Ljungman, 2007; Marteijn et al., 2009]. However, the molecular mechanisms by which such sensing and monitoring are accomplished are not fully understood.

1. *How can we best translate the knowledge in the DDR field into new therapies?* There is a growing knowledge of how to explore targets in the DDR pathway for novel cancer therapies [Helleday et al., 2008; Ljungman, 2009]. Some exciting new therapies include the targeting of PARP1 in HR-defective cancers [Bryant et al., 2005; Farmer et al., 2005] and the targeting CHEK1 in p53-mutated cancers [Chen et al., 2006; Blasina et al., 2008]. The simultaneous targeting of DDR pathways to which cancer cells are ''addicted'' would be effective, especially in combina-

tion with radiation or chemotherapy. Furthermore, the reestablishment of DDR growth barriers in tumors that have lost them may prove to be a powerful and selective way to target tumor cells for elimination.

The DDR field has made some extraordinary advances over the last 50 years. We are now in a good position to translate some of this vast knowledge into clinical therapies to prevent and treat human diseases such as cancer and perhaps aging. However, our knowledge is still limited and many breakthroughs in the DDR field that will impact public health are waiting to be made. Thus, it is of great importance to strengthen the support of basic research to attract and sustain bright scientists working in this exciting and important field.

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