

# The ATM-mediated DNA-damage response: taking shape

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**Cellular responses to DNA damage are crucial for maintaining homeostasis and preventing the development of cancer. Our understanding of the DNA-damage response has evolved: whereas previously the focus was on DNA repair, we now appreciate that the response to DNA lesions involves a complex, highly branched signaling network. Defects in this response lead to severely debilitating, cancer-predisposing 'genomic instability syndromes'. Double strand breaks (DSBs) in DNA are potent triggers of the DNA-damage response, which is why they are used to study this pathway. The chief transducer of the DSB signal is the nuclear protein kinase ataxia-telangiectasia mutated (ATM). Genetic, biochemical and structural studies have recently provided insights into the ATM-mediated DSB response, reshaping our view of this signaling pathway while raising new questions.**

## The DNA-damage response: repair and signaling

DNA damage is a serious threat to cellular homeostasis because it compromises one of its cornerstones – namely, the stability and integrity of the cellular genome. Sequence alterations in DNA arise from normal genomic transactions, spontaneous chemical changes in DNA constituents, replication errors, and endogenous and exogenous agents that inflict damage on the DNA. The greatest challenge to genome stability comes from these last agents, which induce various types of DNA lesion [1]. If not repaired, some of these lesions are extremely cytotoxic, whereas others are mutagenic with consequences ranging from malfunction of the cell to its malignant transformation [1,2].

DNA damage initiates various repair mechanisms that recognize and repair specific DNA lesions. It has recently become clear, however, that there is more to the DNA-damage response than simply DNA repair; indeed, it is actually a complex signaling network [3–6] that encompasses many additional processes. Genetic defects in crucial parts of this network lead to a group of human genetic disorders that are collectively called 'genomic instability syndromes' [7–10]. These diseases are characterized by degeneration of specific tissues, sensitivity to particular DNA damaging agents, chromosomal instability and a marked predisposition to cancer. Attempts to understand the molecular basis of these diseases have led

to the identification of various components of the DNA-damage response [9,11–18]. Of the many kinds of DNA lesion, double-strand breaks (DSBs) are particularly effective in triggering the DNA-damage response, and the study of genomic instability syndromes that are caused by defective response to DSBs [9] has been very rewarding in terms of understanding this pathway.

The extremely cytotoxic DSB is induced by ionizing radiation, radiomimetic chemicals and oxygen radicals formed in the course of normal metabolism, and can also follow replication fork stalling. DSBs are also part of normal genomic transactions, such as meiotic recombination and the maturation of the immune system genes via V(D)J recombination [19]. Importantly, it has been recently shown that uncapped telomeres in senescent cells attract the same damage-response proteins that are recruited to DSBs (see later) and evoke an unregulated DNA-damage response [20,21]. In healthy cells, functional telomeres are recognized as DSBs in the G2 phase of the cell cycle and consequently partially activate the DNA-damage response [22]. An ongoing DSB response has been also observed in precancerous cells [23] and tumor tissues [24].

Eukaryotic cells use two main mechanisms to repair DSBs: nonhomologous end-joining (NHEJ), an error-prone ligation mechanism that acts throughout the cell cycle [25]; and a high-fidelity process based on homologous recombination between sister chromatids, which is functional in the late S and G2 phases of the cell cycle [26]. The overall cellular response to DSBs, however, goes far beyond repair. This complex signaling network works swiftly to affect numerous cellular systems [3–5]. One of its hallmarks is the activation of cell-cycle checkpoints, which temporarily halt the cell cycle while the damage is assessed and repaired [27]. The sudden arrest of the cell cycle involves marked alterations in numerous physiological processes. Indeed, DSBs lead to profound changes in basic cellular processes such as gene expression [28,29], and protein synthesis, degradation and trafficking. These changes mean that the DNA-damage signal must be conveyed swiftly and precisely to numerous pathways across the cell. Here, I summarize recent work that has provided new insights into the different tiers of this process.

## The DSB response: a three-tiered signaling cascade

### *From sensors to transducers*

Mounting evidence indicates that dissemination of the DNA-damage alarm is based on a signal transduction

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mechanism that begins with ‘sensor’ proteins that sense the damage and/or chromatin alterations that occur after damage induction. These sensors transmit a signal to ‘transducers’, which in turn convey the signal to numerous downstream ‘effectors’ involved in specific pathways [3–5].

A well-studied sensor in mammalian cells is the Mre11–Rad50–Nbs1 (MRN) complex. This complex, comprising the nuclease Mre11, the structural maintenance of chromosomes protein Rad50 and the protein Nbs1, is rapidly recruited to DSB sites, where it tethers and processes the broken ends [30,31]. It has become apparent that, in addition to its DNA processing activities, the MRN complex controls the early steps in transduction of the DNA-damage signal (see later). Additional factors in the sensor tier of the DSB response are recruited to the damaged sites, where they create rapidly expanding nuclear foci and take part in signaling damage to the transducers. These factors include the multifunctional tumor suppressor protein BRCA1 [32], the p53-binding protein 53BP1 [33] and mediator of DNA-damage checkpoint protein 1 (MDC1) [34], all of which typically contain a phosphoprotein-binding BRCA1-terminal (BRCT) domain [35,36].

The primary transducer of the DSB alarm is the nuclear protein kinase ataxia-telangiectasia mutated (ATM) [3,17]. In response to DSB induction, ATM is rapidly activated and phosphorylates various substrates, each of which is a key factor in a damage-response pathway. The clinical phenotype associated with loss of ATM activity is that of a prominent genomic instability syndrome – ataxia telangiectasia (A-T). This syndrome is characterized by neuronal degeneration, which primarily affects the cerebellum and leads to severe neuromotor dysfunction, immunodeficiency, chromosomal fragility, a marked predisposition to lymphoreticular malignancies, and extreme sensitivity to ionizing radiation and other DSB-inducing agents [37,38]. Cultured cells from individuals affected with A-T show a broad defect in responding to DSBs that spans almost all of the known branches of this response network.

ATM belongs to a conserved family of proteins termed the ‘PI3K-like protein kinases’ (PIKKs), most of which possess a serine/threonine kinase activity and all of which, as their name indicates, contain a domain with motifs that are typical of the lipid kinase phosphatidylinositol 3-kinase (PI3K) [39]. At present, the mammalian members of this family include five protein kinases: ATM, ataxia-telangiectasia- and Rad3-related (ATR), hSMG-1, mTOR (also known as FRAP) and the catalytic subunit of the DNA-dependent protein kinase (DNA-PK). Another member of the PIKK family is TRRAP, a protein component of histone acetyltransferase complexes that does not possess protein kinase activity [3,39]. The PIKK protein kinases, which are conserved from yeast to mammals, respond to various stresses by phosphorylating substrates in the appropriate pathways.

Four mammalian PIKKs are involved in the DNA-damage response: DNA-PK, which has a role in the NHEJ repair pathway [40]; ATM; ATR; and hSMG-1. Whereas ATM and DNA-PK primarily respond to DSBs, ATR mainly transduces the signal emanating from UV damage

and stalled replication forks [39,41], but it also responds to DSBs, albeit later and with slower kinetics. The hSMG-1 kinase responds to both UV damage and DSBs [42]. Whereas ATM and ATR share substrates in the DSB response, they show selective substrate specificities in response to different genotoxic stresses and DSB inducers [43].

#### *ATM and ATR: distinct and cooperative roles*

ATR has a role in damage surveillance at the DNA replication fork and might be directly involved in regulating replication progression [41,44]. It is probably this essential function that leads to an embryonic lethal phenotype in *Atr* knockout mice [45] and possibly in humans. Hypomorphic mutations in human *ATR* give rise to a severe disease, termed ATR–Seckel syndrome, that is characterized by intrauterine growth retardation, dwarfism, microcephaly, mental retardation and marked defects in various cellular DNA-damage responses [46,47]. Being a hypomorphic phenotype, ATR–Seckel syndrome demonstrates the crucial role of ATR and shows that it has no functional redundancy.

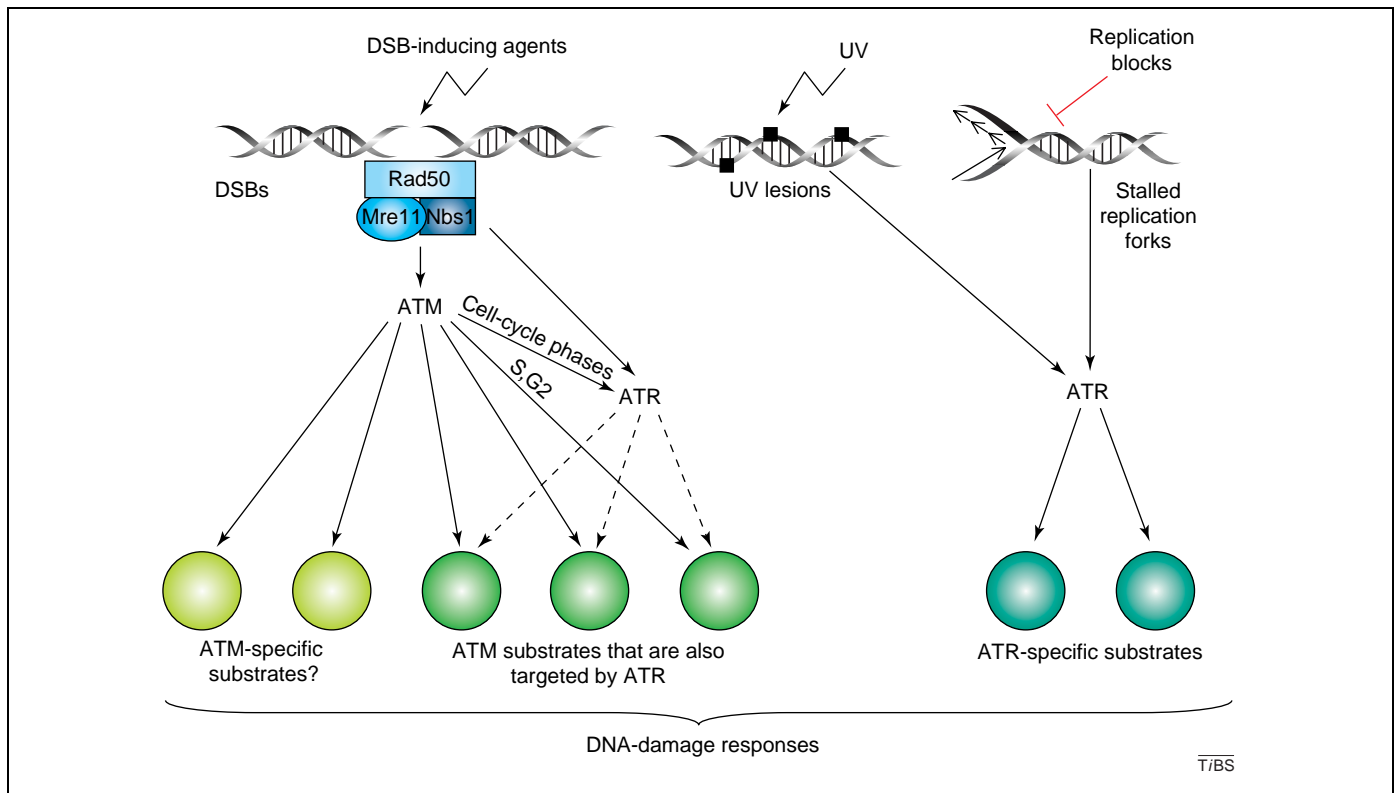
The functional relationships between ATM and ATR in the DSB response have turned out to be more complex than was previously thought. It is generally assumed that after the rapid activation of ATM in response to DSB induction and the subsequent phosphorylation of its numerous substrates, ATR is independently triggered and maintains phosphorylation of some of these substrates. This functional redundancy is observed in A-T cells, which are devoid of ATM activity but can mount a belated, moderate DSB response that could be ascribed to ATR. Thus, ATM and ATR have been thought to act in parallel, independently of each other.

Recent observations indicate, however, that during the S and G2 phases of the cell cycle, the response of ATR to DSBs that are not part of the replication process is dependent on ATM: the latter protein is required for ATR to bind to the single-stranded ends formed at DSB sites, probably by Mre11-mediated resection [48–51]. Binding of ATR to these sites is mediated by an interaction between the ATR accessory protein ATRIP and replication protein A, which coats the single-stranded ends. ATR binding to DSBs is evident as nuclear foci and is important for efficient phosphorylation of its substrates [52]. By contrast, the response of ATR to UV lesions and stalled replication forks occurs independently of ATM. These observations place ATM upstream of ATR in some phases of the cell cycle, in addition to their functional redundancy (Figure 1).

#### **ATM activation and early steps in the DNA-damage response**

##### *Dormant kinase becomes active*

It has long been known that the kinase activity of ATM is enhanced by DSBs [53,54]. In a seminal study, Bakkenist and Kastan [55] showed that in unprovoked cells ATM is present as inert dimers or multimers that, after DNA damage, release highly active ATM monomers. During this process, ATM undergoes intermolecular autophosphorylation on Ser1981 [55]. Activated ATM has been



**Figure 1.** Separate and overlapping roles of ATM and ATR in responses to genotoxic stress. The protein kinases ATM and ATR activate response networks after genotoxic stress by phosphorylating key proteins in various signaling pathways. The two protein kinases share substrates in the DSB response, but ATM functions upstream of ATR in some phases of the cell cycle during this response.

recently shown to undergo additional phosphorylation events (M. Lavin, the 2005 Ataxia Telangiectasia Workshop, Belgirate, Italy) and acetylation mediated by the Tip60 acetylase [56,57]. Two protein phosphatases have been reported to be involved in damage-induced modification of ATM: dynamic interaction of ATM with the phosphatase PP2A suggests that PP2A-mediated dephosphorylation inhibits ATM activation [58]; by contrast, the serine/threonine phosphatase PP5 has been reported to contribute to activation of ATM [59]. The current repertoire of activating modifications of ATM is probably far from complete, and the activation process might involve interactions of ATM with even more proteins.

Another important facet of ATM dynamics after DNA damage is the recruitment of ATM to the damaged sites. The first study of this phenomenon demonstrated damage-induced, tight binding of part of the ATM present in the cell to DSB sites, which receded with kinetics similar to that of DSB repair [60]. Chromatin-bound ATM was found to be autophosphorylated and thereby activated [61]. Importantly, many substrates of ATM are phosphorylated at the DSB sites by the chromatin-bound ATM [17,62]. An important substrate of ATM and probably other PIKKs in the vicinity of the damaged site is histone H2AX, a member of a subfamily of histone H2A [63]. Phosphorylated H2AX ( $\gamma$ H2AX) has an important role in anchoring damage-response proteins to the damaged sites (see later).

A central issue in the DNA-damage response is to understand the early sequence of events that take place between DSB formation and transducer activation. It turns out that the key factors at this stage are sensor

proteins such as the MRN complex, 53BP1 and MDC1, which are the first recruits to the sites of DNA damage.

#### Sensors are also activators

The early stage of the DSB response is characterized by the rapid formation of nuclear foci at the DSB sites that represent huge conglomerates of recruited damage-response proteins. Elegant imaging technology in live mammalian and yeast cells has elucidated the temporal order in which the early damage-response proteins are recruited to the damaged sites [64]: the MRN complex is the first to bind to these sites [65–67]; MDC1, whose binding to chromatin requires phosphorylation of histone H2AX [65], follows; and next comes 53BP1 [68]. Although not the first to be recruited, MDC1 is required for sustained binding of MRN and 53BP1 to the damaged chromatin [65,68]. These observations document one of the hallmarks of this process: namely, stabilization and augmentation of protein conglomerates at the damaged sites through repeated protein–protein interactions and recruitment of additional molecules.

By applying meticulous imaging analysis, Bekker-Jensen *et al.* [69] have demonstrated internal spatial organization in the nuclear foci at DSB sites and have shown that specific subcompartments are occupied by different classes of proteins. Thus, ATM is present in DSB-flanking chromatin together with MDC1, MRN, 53BP1 and BRCA1. Single-stranded DNA ends delineate a different subcompartment, in which ATR and ATRIP are present together with replication protein A, other portions of MRN and BRCA1, and additional damage-response

proteins. This fine spatial organization of what previously seemed to be uniform nuclear foci reflects the specific functions of the building blocks of these foci.

How, then, do these proteins mediate the next step in the DNA-damage signaling cascade – namely, activation of the transducers? Early evidence that transducer activation is dependent on the sensor proteins was provided by Uziel *et al.* [61], who showed that activation of ATM and its recruitment to damaged sites requires a functional MRN complex. Their study was based on another genomic instability syndrome, ‘A-T-like disease’, which is similar to A-T, but has a later onset and slower progression [70]. The defective protein in A-T-like disease is the MRN component Mre11 [71].

The similarity of the two phenotypes might be explained by the fact that Mre11 is required for damage-induced ATM activation. The study of Uziel *et al.* [61] confirmed this hypothesis and showed that full activation of ATM, particularly after treatment of the cells with low doses of DSB-inducing agents, requires the nuclease activity of Mre11 [61]. A whole series of studies have since documented the dependence of ATM activation on MRN and the physical interaction between these two proteins in yeast, mouse, *Xenopus* and human cells [50,61,72–86]. Genetic analysis in mice has shown the importance of MRN for activation of an ATM-mediated apoptotic pathway [87]. Other studies have reported that activation of ATM also requires functional 53BP1 [88,89], and that ATM recruitment and sustained interaction with damaged DNA requires MDC1 [67,88,89].

These results initially created a conceptual difficulty, because Nbs1 (an MRN component), MDC1 and 53BP1 were known to be phosphorylated in an ATM-dependent manner and thus had been placed downstream of ATM in the DNA-damage response cascade [17]. The new data placed these proteins upstream of ATM and made them activators in addition to sensors. Thus, rather than being a simple hierarchy of ‘sensors upstream of transducers’, the initial phase of the DNA-damage response is now viewed as a cyclic process that amplifies the damage signal by repeated interactions among these proteins [6,67,90]. Evidence in favor of this model is accumulating. The signal amplification process depends on interaction of the sensors and activators with damaged chromatin on the one hand and with ATM on the other.

How are the sensors and activators anchored at the chromatin? Structure analysis has shown that 53BP1 binds to methylated Lys79 of histone H3, which becomes exposed at the break sites [91]. MDC1 has been found to bind through its BRCT domain to the phosphorylated tail of  $\gamma$ H2AX [92,93]. How, then, do these proteins interact with ATM?

A protein complex containing activated ATM, MRN and damaged DNA has been isolated from *Xenopus* egg extracts [75]. Furthermore, Lee and Paull [76,77] have shown that MRN is essential for activation of ATM *in vitro* and have demonstrated that MRN binds tightly to both DNA and ATM, further implicating MRN in the recruitment of ATM to damaged DNA. The MRN component that binds ATM has been recently identified as a C-terminal domain of the Nbs1 protein [82,83]. The physical

interaction among ATM, MRN and the damaged DNA is thus required for activation of ATM.

Recent work by Dupré *et al.* [86] has demonstrated that in *Xenopus* egg extracts ATM is activated in two steps, both of which require MRN. The first involves the recruitment of dimeric ATM to DSBs and the formation of monomers that are not yet autophosphorylated and active; the second leads to the actual conversion of these monomers to active, autophosphorylated kinase molecules. Of note, this model implies that activation of ATM requires physical contact of the ATM–MRN complexes with DSB ends. Bakkenist and Kastan [55], working with mammalian cells, have suggested that activation of ATM could be triggered by changes in chromatin conformation that accompany DSB formation or even chromatin alterations that are not associated with DSBs at all. The latter possibility could explain the swift activation of the whole cellular content of ATM by very few breaks in the DNA. Whether DSBs are a prerequisite for activation of ATM in mammalian cells, and the role of chromatin alterations and other signals in ATM activation are likely to remain key issues in ATM research.

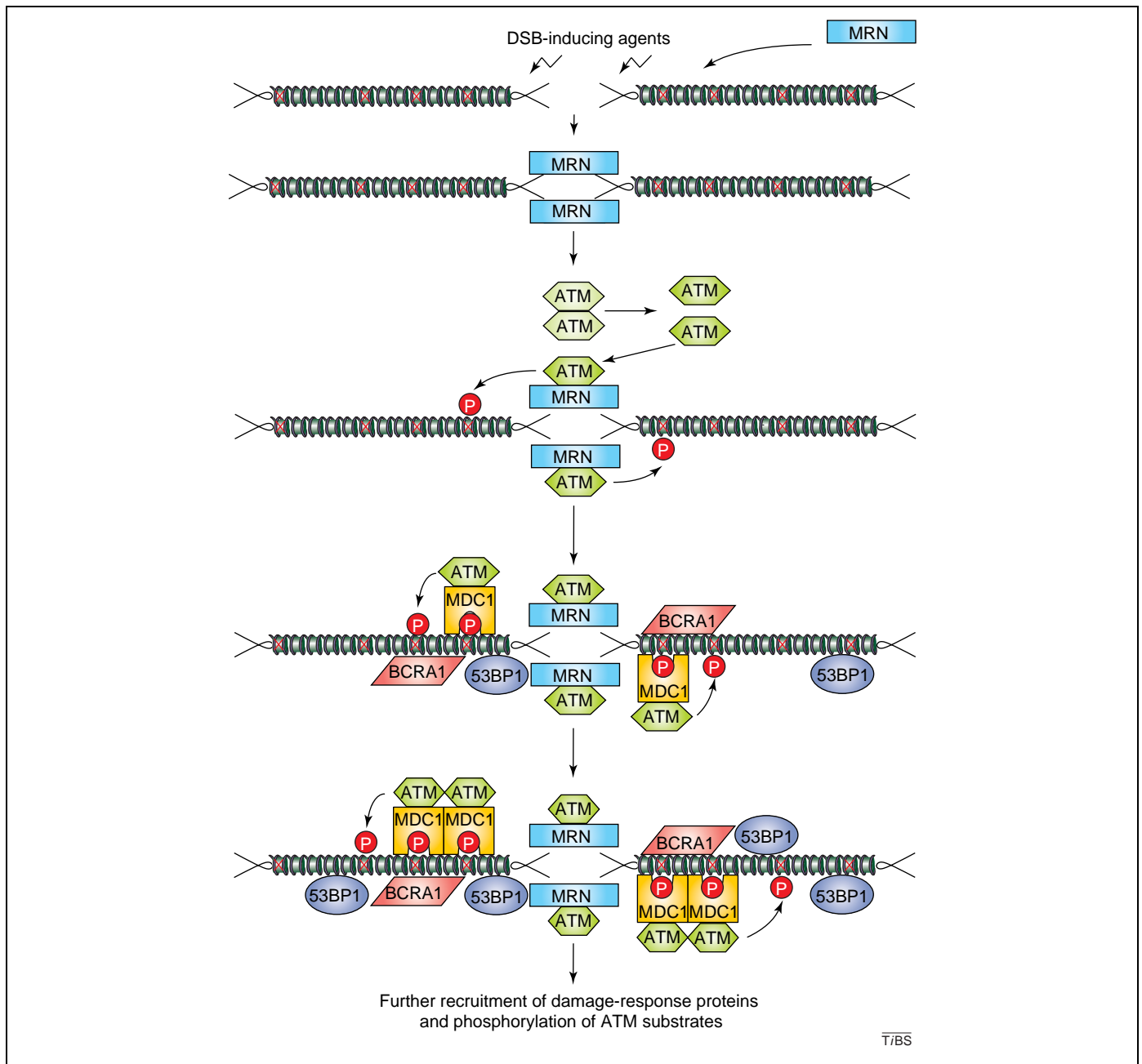
MRN is not the only factor involved in attracting ATM to the damaged sites. Recent work by J. Chen and colleagues [67] has shown that while the BRCT domain of MDC1 holds on to  $\gamma$ H2AX [93], another protein–protein interaction domain of MDC1 (the FHA domain) binds to ATM. Given that ATM phosphorylates H2AX, J. Chen and colleagues have proposed a cycle in which an MDC1–ATM pair is recruited, and H2AX is phosphorylated and subsequently binds MDC1, thereby stabilizing the attachment of MDC1–ATM to the DSB site. ATM then phosphorylates additional H2AX molecules in the vicinity, setting the scene to bind more MDC1 molecules. This cyclic process could be the driving force behind the observed expansion of H2AX phosphorylation over megabases of DNA flanking DSBs [69] and the consequent formation of an expanding platform for the recruitment of additional damage-response proteins (Figure 2).

One of the remaining questions is whether the recruitment of ATM to DSB sites must indeed precede its activation and is absolutely required for it. Conflicting data obtained in mammalian and *Xenopus* egg extract systems [77,82,83,86] preclude a definite answer at this point. The rapid pace at which the whole cellular content of ATM in mammalian cells becomes activated after the induction of low levels of DSBs [55] seems to argue against the ‘recruitment first’ model [86]. Further studies of the physical nature of the signal that actually activates the dormant ATM dimers should clarify this point.

### Downstream of ATM: an ever-expanding network

The list of reported ATM substrates is far from complete, and many ATM-dependent responses are likely to involve ATM targets that are currently unknown. Nevertheless, the study of these pathways has revealed the diversity of the ATM-dependent response to DSBs (Figure 3).

In addition to the versatility of ATM as a protein kinase with numerous substrates, the ATM signaling network contains protein kinases that are themselves capable of targeting several downstream effectors simultaneously

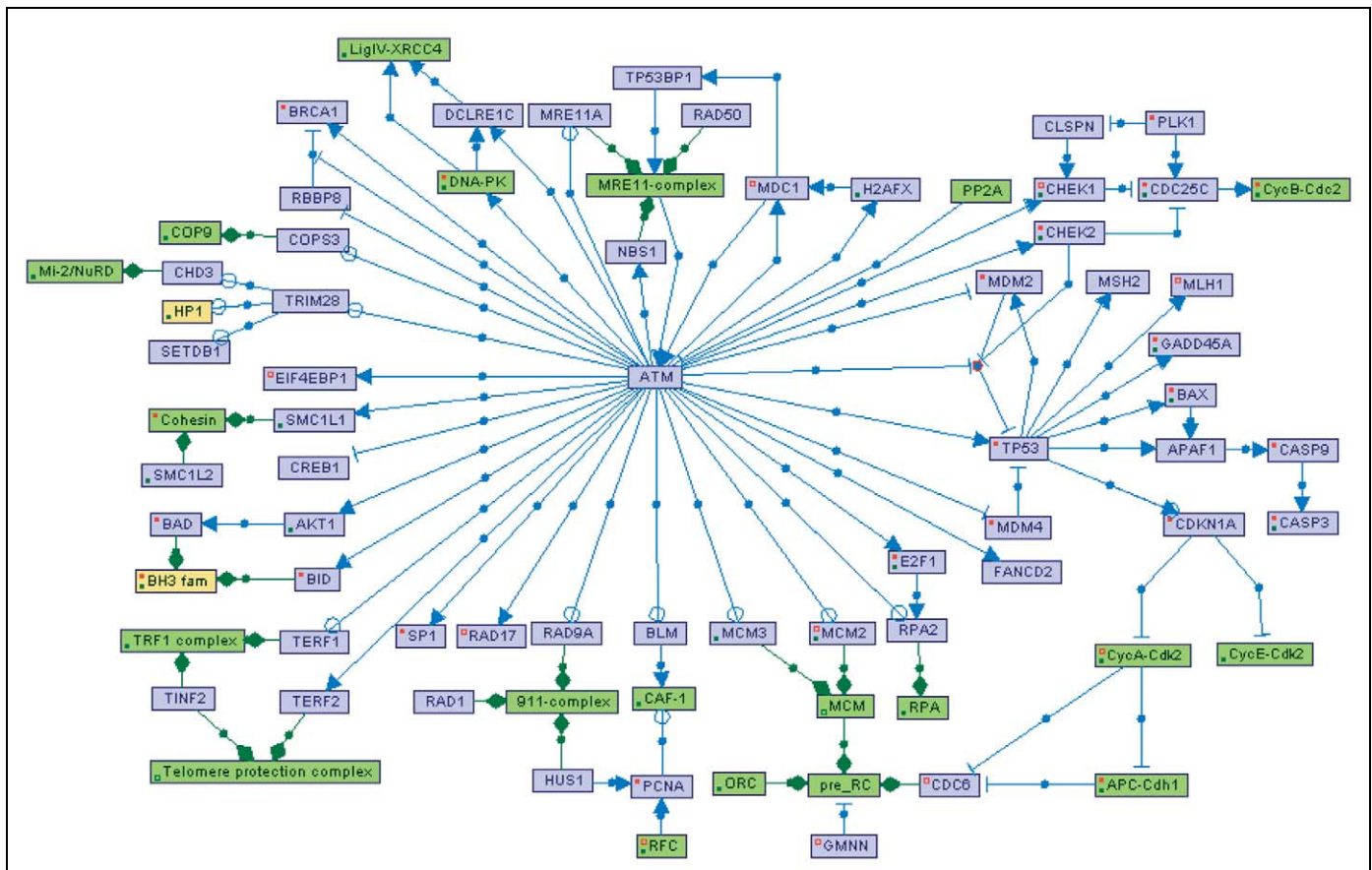


**Figure 2.** Early events in the DSB response. X denotes the location of histone H2AX molecules in chromatin. The MRN complex is the first recruit to the damaged site (i) and is a prerequisite both for activation of ATM – that is, conversion of the inert ATM homodimer (light green) to active monomers (green) bearing various posttranslational modifications (ii) – and for initial recruitment of ATM to the damaged site (iii). MDC1, which binds to phosphorylated H2AX, is required for sustained interaction of ATM with chromatin and the further recruitment of ATM. Additional damage-response proteins are recruited to the damaged site at this point, creating a growing protein conglomerate (iv). Subsequent events at the site include MRN-mediated processing of DSB ends and DSB repair, and phosphorylation of many ATM substrates (v). Figure is based on data in Refs [6,55,60,61,67].

and, as such, controlling subsets of pathways (e.g. Chk1 and Chk2). An important aspect of ATM-mediated signaling is its ability as a transducer to target the same endpoint by using different pathways; for example, the cell-cycle checkpoints are each governed by several ATM-mediated pathways. Furthermore, the same ATM effector can be approached by several different ATM-dependent mechanisms. A prominent example is ATM-mediated activation and stabilization of the p53 protein, an important mediator of cell-cycle checkpoints on the one hand and damage-induced apoptosis on the other. ATM mediates this process by controlling various

posttranslational modifications of p53 itself [94] and at the same time phosphorylating Mdm2, a ubiquitin ligase of p53, thereby targeting p53 for degradation [3,17,95]. A new ATM target – the p53 inhibitor Mdmx – has been recently identified in the p53 control loop; this inhibitor also undergoes several ATM- and Chk2-dependent phosphorylation events that enhance its degradation [96–99]. The existence of additional ATM targets in this pathway is not unlikely.

A major response to DNA damage is represented by marked alterations in gene expression patterns [28], which are largely dependent on ATM when the underlying



**Figure 3.** Current view of the ATM-regulated network. This signaling map was created with a computerized tool established in our laboratory called 'Showcase for ATM Related Pathways (SHARP), a curated knowledge base for signaling pathways induced by DNA damage (<http://www.cs.tau.ac.il/~sharp>). SHARP has been designed to assist researchers in integrating, visualizing and interpreting pre-existing and new information on the ever-growing ATM-mediated network. The map contains three types of node: purple nodes represent single proteins (denoted by the official HUGO-approved symbol of their encoding gene); yellow nodes represent protein families (e.g. the BH3 family); green nodes represent protein complexes (e.g. the MCM complex). The map also contains two types of edge: blue edges denote regulatory relations (arrow indicates activation, 'T' indicates inhibition, and an open circle indicates interactions whose regulatory effect is unclear); green edges denote containment relations among protein families or protein complexes and their members (e.g. MCM2 is contained in the MCM complex). Red and green dots within a node indicate that not all of the regulations and associations stored in the SHARP database for this particular node are shown in the map, but can be viewed by clicking on this node when it is presented on a computer screen. This map contains all information published on ATM as of 15 April 2006, and data presented at the recent International Workshop on Ataxia-Telangiectasia, ATM and the DNA-damage Response (Belgirate, Italy, 2005) [111]. Modified, with permission, from Ref. [111].

lesion is a DSB [29,100,101]. The relays that mediate the signal downstream from ATM to gene promoters are specific transcription factors. A combination of microarray and computational analysis has recently supported earlier evidence that two of the key molecules involved are the p53 protein and the transcription factor NF- $\kappa$ B [29,100,101]. Interestingly, in lymphoid cells p53 mediates the apoptotic response to DNA damage, whereas NF- $\kappa$ B activates genes associated with cellular survival [100,101]. The functional link between ATM and NF- $\kappa$ B has been found by Wu *et al.* [102] to be the NF- $\kappa$ B essential modulator (NEMO). NF- $\kappa$ B is held inactive in the cytoplasm by its inhibitor, I $\kappa$ B, until an appropriate stimulus leads to activation of the I $\kappa$ B kinase (IKK) complex, which phosphorylates I $\kappa$ B and thus marks it for proteasome-mediated degradation. NF- $\kappa$ B is subsequently liberated to enter the nucleus and to act on its downstream target genes. NEMO (also called IKK $\gamma$ ) is the regulatory subunit of the IKK complex. Wu *et al.* [102] have shown that, after DNA damage, ATM-mediated phosphorylation of NEMO in the nucleus leads to its ubiquitin-dependent nuclear export and subsequent activation of cytoplasmic IKK, eventually leading to stimulation of NF- $\kappa$ B.

Another transcription factor that has been recently found to be a direct target of ATM is the Ca<sup>2+</sup>/cAMP response element binding protein (CREB), which is involved in various cellular growth pathways [103]. In this study, genotoxic-stress-induced phosphorylation of CREB by ATM was found to lead to inactivation of CREB, and a mutant protein that could not be phosphorylated by ATM showed enhanced activity that was resistant to DNA damage [103]. Interestingly, ATM is involved in phosphorylation of CREB not only after DSB induction but also, together with ATR, in response to UV damage [104].

One of the long-standing obstacles to understanding the A-T phenotype has been to explain the extreme radiosensitivity of A-T cells. Such sensitivity to the cytotoxic effect of ionizing radiation and radiomimetic chemicals is expected to result from defective DSB repair, but attempts to show such a defect in A-T cells have traditionally led to ambiguous results. Thus, it is commonly thought that such a defect might affect only a few DSBs, which would be difficult to identify in A-T cells with routine methods. Jeggo, Lobrich and co-workers [105,106] have provided evidence for this hypothesis, attributing the repair of these few DSBs to a new pathway

mediated by ATM and a novel substrate – the nuclease Artemis. They suggest that Artemis, previously known to resolve hairpin end intermediates in V(D)J recombination, is involved in the NHEJ repair pathway. After its phosphorylation by ATM, it engages in processing the ends of some DSBs in preparation for ligation, and its lack of phosphorylation might interfere with this process. During (VD)J recombination, the activity of Artemis is regulated by DNA-PK rather than by ATM. These observations have established a long-awaited direct link between ATM and a DSB repair pathway.

One of the main lines of research attempts to understand how cells make the crucial choice between activation of the survival response and apoptosis in the face of heavy DNA damage. A recently identified ATM target – the BID protein, a member of the ‘BH3-only’ pro-apoptotic member of the BCL-2 family – has thrown light on this choice. ATM-mediated phosphorylation of BID has an anti-apoptotic role in the damage response and is also involved in the S-phase checkpoint [107,108]. This change in role after ATM-mediated phosphorylation might be typical of other ATM effectors. Thus, activating transcription factor 2 (ATF2), which is known to be involved in the JNK/p38 stress response, has been found to be phosphorylated by ATM at DSB sites and is required to mount an efficient DNA-damage response. The role of ATF2 in the DNA-damage response is completely uncoupled from its well-documented function as a transcription factor [109].

### Concluding remarks and future directions

Investigations into the cellular response to DSBs continue to provide the most comprehensive view of how cells respond to DNA damage, in addition to detailed mechanistic insights into this elaborate response. Each of the three tiers of this signaling system provides many research directions. In particular, several questions remain open about the nature of the initial signal sent from DSBs to the MRN complex, which seems to be the first molecule to rush to the damaged sites and to start the response signaling cascade.

How does MRN recognize the damage, and how does it initially bind to it? What is the nature of the signal that activates ATM and breaks up the inert ATM dimers? Where in the nucleus does this process take place? What role does the recruitment of ATM to damaged sites play in the activation of this protein? Similarly, what mechanistic roles do the posttranslational modifications of ATM play in its activation?

Elucidating the many targets of ATM and its downstream signaling pathways is a continuous endeavor. But understanding the DNA-damage response will certainly have a big impact on the search for new treatments for A-T and other genomic instability syndromes [110]. It might have even more far-ranging ramifications on our understanding of key biological processes such as coping with environmental hazards, aging, cancer formation and tumor responses to therapy. Thus, research in this field is expected to maintain its position at the forefront of biomedical research in the foreseeable future.

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