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INVITED EDITORIAL
Multiple ATM-Dependent Pathways: An Explanation for Pleiotropy

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Ataxia-telangiectasia (AT), a rare autosomal recessive human genetic disorder, is a syndrome remarkable for its pleiotropic phenotype. The hallmark features of AT are degenerative neurological function, particularly in the cerebellum, and oculocutaneous telangiectasias. These features are often accompanied by immunodeficiency, infertility, small size, sensitivity to the effects of ionizing radiation (IR), and increased predisposition to cancer. Cell lines derived from patients with AT also display characteristic abnormalities, including poor growth, premature senescence, sensitivity to IR, and failure to establish effective cell-cycle arrest after genotoxic insult. Mice deficient in *Atm*—the mouse homologue (Pecker et al. 1996) of ATM, the gene mutated in AT (Savitsky et al. 1995)—display a similar pleiotropic phenotype in vivo and in vitro (Barlow et al. 1996; Elson et al. 1996; Xu et al. 1996; Herzog et al. 1998). Thus, ATM is crucial for the maintenance of normal function in a wide variety of mitotic, postmitotic, and meiotic cell types and tissues.

Determining how the loss of function of a single gene product can lead to such diverse phenotypes in humans and mice is central to understanding the molecular nature of the AT disorder. An understanding of proteins that directly interact with ATM, the pathways that these interacting proteins modulate, and the functional consequences attributable to ATM's absence will likely clarify the pathophysiological disturbances that result in the AT phenotype. In particular, what biological functions are disrupted in various tissues when ATM is missing?

Several recent studies have identified direct downstream targets of ATM, and in some cases, clues about the biological function of these interactions are beginning to emerge. In this review we have placed these findings in the context of the pleiotropic phenotype displayed by patients with AT and by *Atm*-deficient mice. For more comprehensive reviews on AT, see Shiloh (1997) and Rotman and Shiloh (1998).

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ATM and Its Product

Disease-causing mutations in *ATM* are found scattered throughout its coding sequence and include both point mutations and intragenic deletions (Gilad et al. 1996). As is common when mutations introduce premature termination codons, no truncated *ATM* gene product is detected in AT cells that harbor frameshift mutations within *ATM* (Brown et al. 1997; Watters et al. 1997); such mutations constitute the clear majority of *ATM* mutations mapped to date (Gilad et al. 1996). Thus, AT is likely caused by complete loss of *ATM* functions, and this view is supported by rescue experiments using full-length recombinant *ATM* in AT cell lines (Zhang et al. 1997; Ziv et al. 1997) and by depletion of endogenous ATM in normal cells determined by means of antisense approaches (Zhang et al. 1998).

The ATM protein possesses a carboxy-terminal kinase domain with distinct homology to the lipid kinase phosphotidylionsitol-3 kinase (PI-3K) and the serine/threonine protein kinase DNA-dependent protein kinase (DNA-PK). ATM is one of a growing family of high–molecular-weight kinases that are conserved throughout eukaryotic evolution from yeast to mammals (Zakian 1995). Analyses of many of the ATM family members and related lipid kinases show that they regulate diverse processes such as mitotic checkpoints, meiosis, the monitoring of telomere length, V(D)J recombination, apoptosis, proliferation, and membrane trafficking. On the basis of the AT phenotype, we may assume that the diverse functions of these family members overlap substantially with the inferred function of ATM.

Despite the homology to PI-3K, ATM does not appear to possess lipid kinase activity, but ATM and several of its relatives are known from both in vitro and in vivo studies to act as serine-specific protein kinases (Hartley et al. 1995; Keegan et al. 1996; Baskaran et al. 1997; Banin et al. 1998; Canman et al. 1998; Gately et al. 1998). Given that every member of the ATM kinase family investigated so far exhibits a strict requirement

for an intact kinase domain (see Cliby et al. 1998 and references therein), it seems likely that ATM-associated catalytic activity is critical to its function.

ATM is a single high–molecular-weight phosphoprotein (~350 kD; Chen and Lee 1996; Lakin et al. 1996; Brown et al. 1997; Watters et al. 1997) that is ubiquitously expressed, although at heterogeneous levels in different cell and tissue types. ATM is present predominantly within the nucleus of cultured human cells (Chen and Lee 1996; Lakin et al. 1996; Brown et al. 1997; Watters et al. 1997), consistent with the proposed role of ATM in cellular response to DNA damage, but a fraction of ATM is also present in the cytoplasm. Several nuclear substrates have been identified for ATM (fig. 1), and it is likely that defects in the ATM-dependent phosphorylation of these molecules may explain some of the effects of ATM deficiency in individuals with AT and in mouse models of the disease.

c-Abl

The first direct target identified for ATM catalytic activity was the nuclear tyrosine kinase c-Abl. IR-induced c-Abl activation is defective in AT cells lines (Shafman et al. 1997) and in cells or tissues from *Atm*-deficient mice (Baskaran et al. 1997). Both genetic and biochemical approaches determined that an SH3 motif of c-Abl binds directly to a proline-rich region of the ATM molecule (Shafman et al. 1997), consistent with a direct stimulation of c-Abl activity by ATM. In addition, the minimal kinase domain of ATM can phosphorylate c-Abl Ser465 directly, and such modification activates c-Abl in vitro (Baskaran et al. 1997). The biological consequences of this interaction and activation are not fully understood. Still, the identification of RNA polymerase II as a c-Abl substrate suggests that an ATM/c-Abl–dependent pathway could modulate cellular patterns of gene expression in response to genome damage. ATM-dependent activation of c-Abl is unlikely to result in cell-cycle arrest, since Abl-deficient cells activate IR-induced cell-cycle checkpoints normally (Baskaran et al. 1997).

Replication Protein A

Another candidate target for regulation by nuclear ATM is the p34 subunit of replication protein A (RPA). RPA, a known substrate for ATM-mediated phosphorylation in vitro (Gately et al. 1998), is a heterotrimeric single-stranded DNA binding complex involved in DNA replication, repair, and recombination. Several groups have shown that, after IR exposure, p34 fails to become phosphorylated in AT cells (Liu and Weaver 1993; Morgan and Kastan 1997). These observations led to speculation that this deficiency is responsible for the failure of AT cells to arrest in S-phase in response to IR (Liu and Weaver 1993). However, Morgan and Kastan (1997) found that p34 phosphorylation was not required for S-phase arrest, leaving unclear the physiological outcome of this modification.

p53

Perhaps the most compelling potential target for nuclear ATM is the tumor suppressor protein p53, since it has long been known that AT cells show irradiation-induced cell-cycle checkpoint defects and, after IR, fail to upregulate p53 to levels observed in normal cells (Kastan et al. 1992). p53, acting as a transcription factor, plays a crucial role in several cellular reponses triggered by IR exposure. Among the proteins whose expression p53 is known to induce are $p21^{WAF1/CIP1}$—a potent inhibitor of cyclin-dependent kinase activity—and Bax, which activates apoptosis. Recently, we demonstrated in vivo that ATM is required in the thymus for cell-cycle arrest after IR exposure, although, as we and others found, IR-induced apoptosis in thymocytes is independent of ATM (Barlow et al. 1997*a;* Herzog et al. 1997). At the molecular level, thymocytes from irradiated *Atm*-deficient mice show markedly reduced p53-dependent

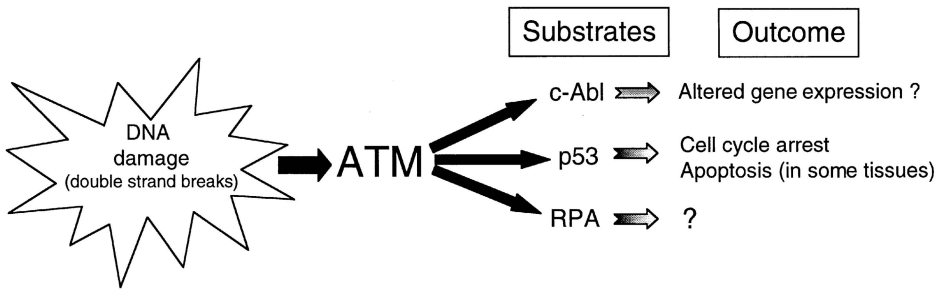

Figure 1 Function of nuclear ATM after DNA damage. The catalytic activity of ATM has been shown to be upregulated after the introduction of double-strand breaks into the genome. Currently, there are three recognized downstream targets for ATM phosphorylation within the nucleus: c-Abl, p53, and the p34 subunit of RPA. Phosphorylation of these proteins results in the indicated cellular responses. Note: ATM interacts with the cytoplasmic protein β-adaptin; however, it is unclear whether this molecule is a target for ATM phosphorylation.

induction of $p21^{WAF1/CIP1}$, consistent with the lack of appropriate cell-cycle arrest after irradiation. Conversely, both the rate of apoptosis and the p53-dependent induction of Bax in thymocytes from irradiated mice are normal (Barlow et al. 1997*a;*). IR-induced apoptosis is reduced, however, in the brains of newborn *Atm*-deficient mice (Herzog et al. 1998), indicating that the functional requirement for ATM in apoptotic pathways may differ among cell types. Taken together, these findings suggest that ATM exerts a differential effect on p53 to specify timely transcriptional induction of $p21^{WAF1/CIP1}$ and normal cell-cycle arrest in response to DNA damage, but that the role of ATM in apoptosis is dispensable in some cell types.

Consistent with these functional studies, Watters et al. (1997) report that ATM interacts with p53, and the ATM-related protein DNA-PK is also known to bind and phosphorylate p53 directly (Hartley et al. 1995). After DNA damage, p53 is phosphorylated on Ser15 (Shieh et al. 1997; Siciliano et al. 1997). The rate of this modification is lower in AT cell lines than in wild-type cell lines (Siciliano et al. 1997), indicating that some of the relevant kinase activity requires ATM expression and that modification of p53 may be a common feature of the ATM kinase family. Support for the hypothesis that p53 is a direct catalytic substrate of ATM comes from recent reports demonstrating that endogenous or recombinant ATM phosphorylates p53 on Ser15 and that ATM displays increased kinase activity in response to IR or to the radiomimetic compound neocarzinostatin (Banin et al. 1998; Canman et al. 1998), but not to UV irradiation. This finding is consistent with the longstanding observation that AT cells are defective in their response to double-strand breaks (DSBs) within their genome, the type of damage caused by IR, but that they possess normal responses to base damage caused by agents such as UV irradiation or alkylating agents. Another ATM family member, ATR/FRP-1 (Keegan et al. 1996; Cliby et al. 1998), also phosphorylates p53 on Ser15, but at only ~5% of the activity, when assayed under the same conditions (Canman et al. 1998).

This body of evidence makes a convincing case that ATM directly phosphorylates p53 Ser15 in response to genotoxic insults that induce DSBs. Intriguingly, this modification of p53 may activate cell-cycle checkpoints, since mutation of Ser15 leads to checkpoint defects (Fiscella et al. 1993). This possibility is consistent with observations that p53-dependent cell-cycle checkpoint responses to IR are defective in the thymus of *Atm*-deficient mice (Barlow et al. 1997*a*).

β-Adaptin

Neither the involvement of ATM in post-IR activation of cell-cycle checkpoints nor any of the other known intranuclear events mediated by ATM suggest any obvious explanation for the neuronal dysfunction and degeneration seen in individuals with AT. However, along with the finding that ATM is a nuclear protein, this molecule was also found to cofractionate (Lakin et al. 1996; Brown et al. 1997; Watters et al. 1997) and colocalize (Watters et al. 1997) with cytoplasmic vesicles. The cytoplasmic pool of ATM does not appear to change in quantity or localization in response to IR (Brown et al. 1997; Watters et al. 1997), so it is unlikely that ATM is recruited to the nucleus after DNA damage; hence any role of cytoplasmic ATM is likely to be independent of its function in genome surveillance.

Lending a biochemical basis to the observation that cells contain microsomal ATM is the recent finding that ATM binds to the vesicle-associated protein β-adaptin (Lim et al. 1998). β-Adaptin is a component of the AP-2 adaptor complex, which interacts with several proteins that are important in membrane trafficking and cell signaling and in endocytosis of clathrin-coated vesicles. Several ATM-related lipid kinases, in particular Vps34 and PI-3K, also play a critical role in stimulating vesicle and protein transport (DeCamilli et al. 1996).

The consequences of the interaction between ATM and β-adaptin are unknown, but it is tempting to speculate that the cytoplasmic fraction of ATM may act in vesicle transport and that this function is disrupted in patients with AT. Thus, the neurological dysfunction seen in both AT patients and *Atm*-deficient mice, as well as the neurodegeneration displayed by AT patients, could result from abnormal axonal or synaptic transport. Indeed, ATM is present primarily in the cytoplasm of human (Oka and Takashima 1998) and mouse neurons, and neurons in *Atm*-deficient mice have an increased number of lysosomes, as judged by electron microscopy (C. Barlow, unpublished data). These observations are consistent with the hypothesis that ATM is important in vesicle transport, perhaps via interaction with β-adaptin or related proteins such as β-NAP, and that disruption of this function may contribute to the neurological phenotype of patients with AT and of *Atm*-deficient mice.

Forming a Basis for the Pleiotropic Nature of the AT Disorder

The recent studies described in this review indicate that ATM interacts with multiple downstream targets. Since these targets are located both in the nucleus and in the cytoplasm, this protein is most likely involved in several distinct signaling pathways. Now that some of these targets have been identified, it is hoped that they will provide an entry point to the pathways regulated by ATM in various tissues in vivo. For example, in the thymus, p53 is phosphorylated directly by ATM after IR, probably in the nucleus, leading to transcriptional activation of $p21^{Waf1/Cip1}$ and consequential cell-cycle ar-

rest. In the absence of ATM, this pathway is disrupted, and this defect perhaps results in the immunodeficiency and abnormal cellular responses to IR seen in patients with AT. Furthermore, the infertility noted in both AT patients and Atm-deficient mice is due to abnormal meiotic progression and subsequent germ-cell degeneration, a phenotype that is partially corrected by concomitant loss of p53 and p21 function (Barlow et al. 1997*b*). ATM interactions with β-adaptin in the cytoplasm might mediate axonal transport and vesicle trafficking in the central nervous system and so account for the neuronal dysfunction and eventual neurodegeneration seen in AT.

In this view, the phenotypic pleiotropy of AT results from the fact that different tissues express different ATM targets and perhaps also express a different complement of ATM family members whose functions may overlap with those of ATM. This model is attractive and testable, given the existence of mouse models of AT that permit various tissues, including brain, to be isolated and studied. In addition, genetic manipulations will allow the breeding of mice harboring mutations in multiple genes for putative targets of ATM. Such analysis should clarify the role of the various genes in specific AT phenotypes. As more targets of ATM (and ATM-related kinases) are defined and scrutinized, we should develop a deeper understanding of the molecular basis of the pleiotropic AT phenotype. Our hope is that this understanding will provide a framework for developing therapies for this devastating disorder.

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References

Banin S, Moyal L, Shieh S, Taya Y, Anderson CW, Chessa L, Smorodinsky NI, et al (1998) Enhanced phosphorylation of p53 by ATM in response to DNA damage. Science 281: 1674–1677

Barlow C, Brown KD, Deng C-X, Tagle DA, Wynshaw-Boris A (1997*a*) Atm selectively regulates p53-dependent cell cycle checkpoints and apoptotic pathways. Nat Genet 17: 453–456

Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, et al (1996) *Atm*-deficient mice: a paradigm of ataxia telangiectasia. Cell 86:159–171

Barlow C, Liyanage M, Moens PB, Deng CX, Ried T, Wynshaw-Boris A (1997*b*) Partial rescue of the prophase I defects of *Atm*-deficient mice by p53 and p21 null alleles. Nat Genet 17:462–466

Baskaran R, Wood LD, Whitaker LL, Canman CE, Morgan SE, Barlow C, Baltimore D, et al (1997) Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. Nature 387:516–519

Brown KD, Ziv Y, Sadandanan SN, Chessa L, Collins FS, Shiloh Y, Tagle DA (1997) The ataxia-telangiectasia gene product, a constitutively expressed nuclear protein that is not upregulated following genome damage. Proc Natl Acad Sci USA 94:1840–1845

Canman CE, Lim D-S, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, Appella E, et al (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science 281:1677–1679

Chen G, Lee EY-HP (1996) The product of the *ATM* gene is a 370 kDa nuclear phosphoprotein. J Biol Chem 271: 33693–33697

Cliby WA, Roberts CJ, Cimprich KA, Stringer CM, Lamb JR, Schreiber SL, Friend SH (1998) Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA-damaging agents and defects in cell cycle checkpoints. EMBO J 17: 159–169

DeCamilli P, Emr SD, McPherson PS, Novick P (1996) Phosphoinositides as regulators in membrane traffic. Science 271: 1533–1539

Elson A, Wang Y, Daugherty CJ, Morton CC, Zhou F, Campos-Torres J, Leder P (1996) Pleiotropic defects in ataxia-telangiectasia protein–deficient mice. Proc Natl Acad Sci USA 93:13084–13089

Fiscella M, Ullrich SJ, Zambrano N, Shields MT, Lin D, Lees-Miller SP, Anderson CW, et al (1993) Mutation of the serine 15 phosphorylation site of human p53 reduces the ability of p53 to inhibit cell cycle progression. Oncogene 8: 1519–1528

Gately DP, Hittle JC, Chan GKT, Yen TJ (1998) Characterization of ATM expression, localization, and associated DNA-dependent protein kinase activity. Mol Biol Cell 9: 2361–2374

Gilad S, Khosravi R, Shkedy D, Uziel T, Ziv Y, Savitsky K, Rotman G, et al (1996) Predominance of null mutation in ataxia-telangiectasia. Hum Mol Genet 5:433–439

Hartley KO, Gell D, Smaith GCM, Zhang H, Divecha N, Connelly MA, Admon A, et al (1995) DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. Cell 82:849–856

Herzog K-H, Chong MJ, Kapsetaki M, Morgan JI, McKinnon PJ (1998) Requirement for Atm in ionizing radiation–induced cell death in the developing central nervous system. Science 280:1089–1091

Kastan MB, Zhan Q, El-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, et al (1992) A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71:587–597

Keegan KS, Holtzman DA, Plug AW, Christenson ER, Brainerd EE, Flaggs G, Bentley NJ, et al (1996) The Atr and Atm protein kinases associate with different sites along meiotically pairing chromosomes. Genes Dev 10:2423–2437

Lakin ND, Weber P, Stankovic T, Rottinghaus ST, Taylor AM, Jackson SP (1996) Analysis of the ATM protein in wild-type and ataxia telangiectasia cells. Oncogene 13:2707–2716

Lim D-S, Kirsch DG, Canman CE, Ahn J-H, Ziv Y, Newman LS, Darnell RB, et al (1998) ATM binds to beta-adaptin in

cytoplasmic vesicles. Proc Natl Acad Sci USA 95: 10146–10151

Liu VF, Weaver DT (1993) The ionizing radiation–induced replication protein A phosphorylation response differs between ataxia telangiectasia and normal human cells. Mol Cell Biol 13:7222–7231

Morgan SE, Kastan MB (1997) Dissociation of radiation-induced phosphorylation of replication protein A from the S-phase checkpoint. Cancer Res 57:3386–3389

Oka A, Takashima S (1998) Expression of the ataxia-telangiectasia gene (ATM) product in human cerebellar neurons during development. Neurosci Lett 252:195–198

Pecker I, Avraham KB, Gilbert DJ, Savitsky K, Rotman G, Harnik R, Fukao T, et al (1996) Identification and chromosomal localization of Atm, the mouse homolog of the ataxia-telangiectasia gene. Genomics 35:39–45

Rotman G, Shiloh Y (1998) ATM: from gene to function. Hum Mol Genet 7:1555–1563

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, et al (1995) A single ataxia telangiectasia gene with a prduct similar to PI-3 kinase. Science 268:1749–1753

Shafman T, Khanna KK, Kedar P, Spring K, Kozlov S, Yen T, Hobson K, et al (1997) Interaction between ATM protein and c-Abl in response to DNA damage. Nature 387: 520–523

Shieh SY, Ikeda M, Taya Y, Prives C (1997) DNA damage–induced phosphorylation of p53 alleviates inhibition by MDM2. Cell 91:325–334

Shiloh Y (1997) Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. Annu Rev Genet 31:635–662

Siciliano JD, Canman CE, Taya Y, Sakaguchi K, Appella E, Kastan MB (1997) DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev 11:3471–3481

Watters D, Khanna KK, Beamish H, Birrell G, Spring K, Kedar P, Gatei M, et al (1997) Cellular localisation of the ataxia-telangiectasia (ATM) gene product and discrimination between mutated and normal forms. Oncogene 14:1911–1921

Xu Y, Ashley T, Brainerd E, Bronson RT, Meyn MS, Baltimore D (1996) Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev 10: 2411–2422

Zakian VA (1995) ATM-related genes: what do they tell us about functions of the human gene? Cell 82:685–687

Zhang N, Chen P, Gatei M, Scott S, Khanna KK, Lavin MF (1998) An anti-sense construct of full length ATM cDNA imposes a radiosensitive phenotype on normal cells. Oncogene 17:811–818

Zhang N, Chen P, Khanna KK, Scott S, Gatei M, Kozlov S, Watters D, et al (1997) Isolation of full-length ATM cDNA and correction of the ataxia-telangiectasia cellular phenotype. Proc Natl Acad Sci USA 94: 8021–8026

Ziv Y, Bar-Shira A, Pecker I, Russell P, Jorgensen TJ, Tsarfati I, Shiloh Y (1997) Recombinant ATM protein complements the cellular A-T phenotype. Oncogene 15:159–167