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# ATM to the Rescue: Repairing DNA Damage

Human genetic disorders present a logical starting point for the identification and functional analysis of numerous proteins. In addition to understanding a disease and searching for a cure, the identification of a “culprit” gene and the corresponding protein may shed light on fundamental biological processes. The human autosomal recessive disorder ataxia-telangiectasia (A-T) is a prime example of a genetic disease that is leading to major discoveries in cell biology—in plants as well as in animals.

## A-T AND THE ATM GENE

A-T is a rare disease characterized by a loss of motor control (ataxia), dilated blood vessels in the eyes and facial area (telangiectasia), and an assortment of other problems, including immunodeficiency leading to recurrent pulmonary and sinus infections and a predisposition to cancer. A-T patients are not mentally impaired and can lead productive lives, although they often require assistance and usually become wheelchair bound at an early age. Although some have survived into their 40s and 50s, most A-T patients die at an earlier age from respiratory failure or cancer. In addition, heterozygous carriers of an A-T mutation (~1% of the general population) are three to five times more susceptible to cancer than are noncarriers (Swift et al., 1991).

The gene responsible for A-T, called *Ataxia Telangiectasia Mutated (ATM)*, encodes a large protein with a phosphatidylinositol 3-kinase (PI3K)-like domain at the C terminus (reviewed by Rotman and Shiloh, 1998). PI3K-related proteins make up a large family of Ser-Thr protein kinases, numerous members of which are involved in the regulation of cell cycle progression, responses to DNA damage, and the maintenance of genomic stability

(Hoekstra, 1997). Mammalian ATM has been found to play critical roles in cellular responses to ionizing radiation (IR) and in normal cell cycle progression and meiosis (Xu and Baltimore, 1996; Rotman and Shiloh, 1998). More specifically, it is believed to function in the response to DNA double-strand breaks that occur as a result of IR and also during normal cellular processes (reviewed by Rotman and Shiloh, 1998).

For example, immunodeficiency in A-T patients is believed to be related to an essential role of ATM in V(D)J recombination that is essential for normal lymphocyte development. (Genes that encode antigen receptor variable regions are not inherited intact but are assembled from different germline DNA segments via V(D)J recombination during the early stages of lymphocyte differentiation.) ATM deficiency in mammals also results in severe meiotic disruption and sterility (Barlow et al., 1998) and is believed to be involved in the repair of DNA breaks that occur normally during meiotic recombination (Rotman and Shiloh, 1998). ATM also is active in the response to DNA damage induced by IR. In mammalian cells, ATM-mediated responses to DNA damage induced by IR include the activation of cell cycle checkpoints, DNA repair, and apoptosis (Rotman and Shiloh, 1998).

Garcia et al. (2000) identified a homolog of *ATM* in *Arabidopsis*, *AtATM*, which is present in a single copy on the short arm of chromosome 3. The gene contains 79 exons, which is the largest number of exons in the *Arabidopsis* genome. The *AtATM* protein has 3856 amino acid residues and is more similar to mammalian ATM than to various other PI3K-related proteins. In this issue of *The Plant Cell*, **Garcia et al. (pages 119–132)** describe the isolation and characterization of two T-DNA insertion mutants of *AtATM* in *Arabidopsis* and show that *AtATM* plays an essential role in meiosis and in the somatic

response to DNA damage in plants, similar to the function of ATM in mammals and other eukaryotes.

Garcia et al. (2003) analyzed two independent T-DNA insertion mutants of *AtATM*. The *atm-1* mutant, in the Wassilewskija background, contains a T-DNA insertion in exon 78. A second mutant, *atm-2*, was isolated in the Columbia background and contains a complex insertion in intron 64 that includes the insertion of filler DNA from other regions of the genome on both sides of the T-DNA and a truncated right border. Both mutants exhibit similar phenotypic characteristics that are consistent with a dual role for the ATM protein in the DNA damage response pathway and in the regulation of meiosis.

## PLANT RESPONSE TO IR

Both *atm-1* and *atm-2* mutants were found to be hypersensitive to IR and to treatment with the radiomimetic alkylating agent methyl methanesulfonate but not to treatment with UV-B light. This result is consistent with observations from mammalian wild-type and *atm* mutant cells and suggests that *AtATM*, like its mammalian counterpart, responds specifically to DNA double-strand breaks. However, IR and alkylating agents cause various other types of lesions, including single-strand breaks, nucleotide deletions and modifications, and the generation of free radicals (e.g., hydroxyl radical). It also has been suggested that ATM may respond to free radical byproducts of DNA damage (Rotman and Shiloh, 1997).

Garcia et al. (2003) characterized the response to IR in wild-type and *atm* mutant plants with regard to the expression of four genes reported previously to be induced by IR treatment. These were *AtRAD51*, *AtPARP1*, *AtGR1*, and *AtLIG4*, all of which

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have been shown or hypothesized to be involved in DNA damage repair and/or cell cycle regulation. Garcia et al. (2003) found that the expression of *AtRAD51*, *AtGR1*, and *AtPARP1*, which was very low to non-existent in wild-type plants before treatment with IR, was induced strongly and rapidly to a maximum at 30 to 60 min after IR treatment. *AtLIG4* expression showed a more moderate twofold induction that peaked at 4 h after IR treatment. Induction of transcript accumulation for all four genes was reduced greatly in the *atm-1* mutant. This represents an important contribution to the literature with regard to the characterization of the plant response to DNA damage induced by IR and indicates that ATM function includes the upregulation of genes involved in DNA repair.

*AtATM* itself appears to be expressed constitutively in Arabidopsis and is not induced by IR (Garcia et al., 2000). Furthermore, no evidence of alternative splicing of *AtATM* was detected, although it could not be excluded completely. Savitsky et al. (1997) found that exons within the 5' untranslated region of the human *ATM* gene undergo extensive alternative splicing, suggesting that *ATM* gene expression might be subject to complex post-transcriptional regulation. However, the mammalian gene also is expressed constitutively in numerous tissues and does not appear to be expressed differentially during normal cell cycle progression or upregulated after treatment of cells with IR (Brown et al., 1997). Thus, the nature of any post-transcriptional regulation of *ATM* remains largely unknown.

### ROLE OF ATM IN MEIOSIS

Both *atm-1* and *atm-2* homozygous mutants were found to be partially sterile. Examination of meiotic progression in pollen mother cells of wild-type and *atm* mutant plants showed that meiosis is disrupted severely in the *atm* mutants. Frequent chromosome fragmentation was observed, particularly during anaphase I, and extrane-

ous chromosome bridges were observed during anaphase II, suggesting further fragmentation.

Cells from *atm*-deficient mice also show severe meiotic disruption and complete arrest of meiosis in prophase I (Barlow et al., 1998). The meiotic arrest observed in *atm* mice is likely the result of a p53-mediated apoptotic response to double-strand breaks that are not repaired, a notion that is supported by the observation that meiosis progresses farther in *atm p53* double mutants (Barlow et al., 1997). In Arabidopsis *atm* mutant plants, meiosis was disrupted severely but was not arrested, and it progressed through the formation of abnormal tetrads, leading Garcia et al. (2003) to surmise that the high lethality of gametophytes likely was attributable to aberrant chromosomal content. Although this observation alone suggests the presence of an ATM-dependent meiotic checkpoint in plants, a similar lack of meiotic arrest in numerous different Arabidopsis mutants that have disruptions in meiosis suggests that Arabidopsis simply lacks a strong meiotic checkpoint altogether. The difference in cell cycle arrest characteristics between plants and animals may reflect a difference in downstream targets of ATM. For example, Arabidopsis does not appear to have a homolog of the p53 protein, which in mammals is involved in the control of cell cycle arrest and apoptosis and has been shown to be a target of ATM (Xu and Baltimore, 1996).

Mammalian ATM also has been shown to have an important function in the promotion of normal mitotic cell cycle progression in fibroblasts, because *atm* mutant fibroblasts show severely compromised ability to progress from G<sub>1</sub>- to S-phase (Xu and Baltimore, 1996). What might be the principal role(s) of ATM in promoting or facilitating normal meiosis and normal progression of the mitotic cell cycle? ATM is believed to lie at or near the top of the signal transduction pathway activated in response to double-strand breaks (and/or other signals resulting from DNA damage) and to transduce the signal via activation of its protein kinase activity and phosphorylation of numerous downstream targets.

(It is important to note that the putative protein kinase activity of *AtATM* has not yet been demonstrated.)

Double-strand breaks occur during the normal cell cycle [e.g., at stalled replication forks and, notably, during V(D)J recombination, which is unique to lymphocyte development] and during meiosis, which helps to explain how ATM could play critical roles in normal cell cycle progression and meiosis as well as in the response to xenobiotic DNA damage-inducing agents. Meiotic recombination in yeast is initiated by double-strand breaks produced by the activity of the DNA topoisomerase/transesterase SPO11, and this pathway is believed to be conserved among all eukaryotes. Grelon et al. (2001) showed that SPO11 homologs in Arabidopsis are required for meiotic recombination in Arabidopsis. Interestingly, Garcia et al. (2003) found no significant differences in the expression of three genes associated with meiotic recombination in Arabidopsis (*AtRAD51*, *AtSPO11*, and *AtDMC1*) in young inflorescence tissue from wild-type relative to *atm-1* mutant plants. In addition, meiotic recombination frequencies appeared to be normal in *atm-1* mutants, as assessed by crossing the wild type and *atm-1* mutants with lines expressing visible phenotypic markers linked to known recessive mutations. These observations do not exclude a critical role for *AtATM* in meiotic recombination, but we have few clues to the function of *AtATM* in meiosis, and its function in meiotic recombination remains an open question.

In yeast and mammalian cells, ATM interacts with and phosphorylates a component of the MRE11 complex, a multisubunit nuclease that is believed to be a primary sensor of DNA double-strand breaks and to be associated intimately with the DNA damage response and checkpoint signaling in mitosis and meiosis (reviewed by D'Amours and Jackson, 2002). These authors present a model wherein the MRE11 complex perceives and binds to double-strand break regions, which causes the activation of MRE11 nuclease activity that produces regions of

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single strandedness, which in turn are potent activators of kinases, including ATM, that induce checkpoint responses and DNA repair. In this model, subsequent phosphorylation of the MRE11 complex by ATM serves to amplify the signal and/or further regulate MRE11 complex activity. Bundock and Hooykaas (2002) recently showed that Arabidopsis T-DNA insertion mutants of a homolog of *MRE11* (*AtMRE11*) are hypersensitive to DNA-damaging treatments and exhibit lengthened telomeres. It also has been shown that disruption of *AtRAD50*, a component of the MRE11 complex, leads to hypersensitivity to DNA damage and disruptions in meiosis (Gallego et al., 2001) as well as lengthened telomeres (Gallego and White, 2001). Mutations in several other genes, such as *AtKu70* (Riha et al., 2002) and telomerase (McKnight et al., 2002), also are associated with abnormal telomere lengths. Further analysis of ATM function in Arabidopsis in relation to the MRE11 complex and related proteins (e.g., the creation of double mutants) may reveal critical features of meiosis, the DNA damage response, and the control of telomere length in plants.

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