Viral Defense and Counterdefense: A Role for Adenosine Kinase in Innate Defense and RNA Silencing

Geminiviruses are single-stranded DNA viruses that cause severe disease (Figure 1) and considerable damage to crops worldwide, including tomato, cotton, maize, bean, and cassava. They have small circular genomes that lack genes for DNA and RNA polymerases, and viral replication and transcription are dependent on host plant enzymes. Many geminiviruses encode proteins, such as the AL2 protein from *Tomato golden mosaic virus* (TGMV) and L2 from *Beet curly top virus* (BCTV), that confer enhanced susceptibility to viral disease in host plants (Sunter et al., 2001). TGMV AL2 is a transcription factor necessary for the expression of late viral genes, but its ability to confer enhanced susceptibility apparently is not related to its transactivation function in viral gene expression. BCTV L2 is not required for viral gene expression and shares only limited identity with AL2 in a small region that might form a zinc binding domain. A number of studies have shown that AL2 and L2 function both to disable an innate metabolic defense pathway mediated by SNF1 kinase and to inhibit the adaptive RNA-silencing defense pathway in plants. In this issue of *The Plant Cell*, Wang et al. (pages 3020–3032) show that AL2 and L2 interact with and inactivate host plant adenosine kinase (ADK), a nucleoside kinase that may function in both the SNF1-mediated and RNA-silencing defense pathways. The authors present evidence that AL2 and L2 inactivate ADK in infected plant cells and present hypotheses for the role of ADK in plant defense.

**INTERACTION OF VIRAL AL2 AND L2 WITH PLANT ADK**

Wang et al. first used the yeast two-hybrid assay to identify putative interacting factors of AL2 and L2 among Arabidopsis proteins, which led to the identification of Arabidopsis ADK as capable of specific interaction with AL2 and L2. Arabidopsis has two ADK genes, ADK1 and ADK2, and both were found to interact specifically with AL2 and L2. Subsequent experiments were performed with recombinant Arabidopsis ADK2 expressed in *Escherichia coli* and yeast, or involved measuring ADK activity in extracts of *Nicotiana benthamiana*. A series of experiments showed that the viral AL2 and L2 proteins inhibit ADK activity in *E. coli* cells and in vitro, in eukaryotic yeast cells, and in *N. benthamiana* plants.

Because AL2 is known to function as a transcriptional activator of late viral genes and to be localized in the nucleus of host plant cells, it was important to determine if it also is found in the cytoplasm, where it would have the opportunity to interact with cytosolic ADK. Wang et al. used AL2 antiserum to examine AL2 localization and showed that AL2 was present in both the cytoplasm and nucleus of cells in transgenic *N. benthamiana* plants expressing a truncated AL2 protein and of nontransgenic TGMV-infected plants.

**ADK AND SNF1-MEDIATED STRESS RESPONSES**

SNF1 kinase is a homolog of mammalian AMP-activated protein kinase, which plays a role in stress responses that deplete cellular ATP, and SNF1-mediated defense responses appear to be related to the maintenance of cellular energy homeostasis. Nutritional deprivation and other environmental stresses often result in the depletion of ATP, resulting in the activation of SNF1 kinase, which in turn functions to inhibit numerous ATP-dependent anabolic...
pathways and enhance ATP-generating pathways. For example, SNF1 has been shown to phosphorylate and inactivate key enzymes that control steroid and isoprenoid synthesis, nitrogen assimilation for amino acid and nucleotide synthesis, and sucrose synthesis (Sugden et al., 1999b). In yeast, SNF1 is required for the derepression of glucose-repressed genes (i.e., genes required for growth on alternative fermentable carbon sources such as sucrose, galactose, and maltose, as well as genes required for oxidative metabolism and hence for growth on nonfermentable carbon sources such as glycerol and ethanol). Plant SNF1 kinases can complement yeast snf1 mutants, suggesting that their functions are highly conserved, and a number of studies have shown that SNF1 is an important regulator of carbon metabolism in both plants and yeast (Halford and Hardie, 1998; Rolland et al., 2002).

Hao et al. (2003) demonstrated that SNF1-mediated responses can be viewed as an innate metabolic defense system in plants, because transgenic N. benthamiana plants with reduced SNF1 activity showed enhanced susceptibility to viral disease, whereas plants that overexpress SNF1 showed enhanced resistance. They also showed that AL2 and L2 inhibit SNF1 activity in vitro and in vivo, providing support for the idea that SNF1 induces a viral defense pathway that is counteracted by the geminivirus proteins.

The maintenance of AMP homeostasis represents a possible link between ADK activity and SNF1 activity, because yeast SNF1 and its mammalian counterpart AMP-activated kinase (AMPK) are activated by increased AMP:ATP ratios. Although yeast and plant SNF1 enzymes are not activated allosterically by AMP, as is AMPK, Sugden et al. (1999a) provided evidence that plant SNF1 is regulated by AMP via other mechanisms that are similar to the regulation of the mammalian enzyme. These authors showed that AMP inhibits the dephosphorylation and inactivation of spinach SNF1 enzymes and suggested that increased AMP in plant cells in response to nutritional or environmental stress might activate SNF1 kinases (Sugden et al., 1999a). ADK is responsible for recycling adenosine and maintaining intracellular AMP levels, and Wang et al. hypothesize that AMP generated by ADK might function indirectly as an early activator of SNF1 after infection with a virus that lacks ADK-inactivating protein. Consistent with this model, ADK activity was increased by 15 to 30%, relative to mock-infected controls, in extracts of plants infected with BCTV L2 mutant virus (which does not produce L2 protein) or with unrelated RNA-containing Potato virus X and Cucumber mosaic virus. These results suggest that enhanced ADK activity might be a general signaling mechanism in plants that activates SNF1-mediated defense pathways and that geminivirus AL2 and L2 proteins constitute an effective counterdefense mechanism against this pathway. Whatever the mechanism, the observations that AL2 and L2 interact with and inactivate both ADK (Wang et al., 2003) and SNF1 (Hao et al., 2003) provide support for the notion that both proteins are involved in plant viral defense responses.

**ADK AND RNA SILENCING**

RNA silencing also is recognized as a viral defense mechanism. Essentially, it acts to destroy transcripts from foreign genomic material, thereby inhibiting the replication and spread of viral particles and the development of viral disease (Vance and Vaucheret, 2001; Waterhouse et al., 2001). A key feature of RNA silencing is that it is triggered by the formation of double-stranded RNA (dsRNA), which is cleaved into small interfering RNAs (siRNAs) 21 to 25 nucleotides in length that subsequently direct the degradation of homologous sequences (i.e., RNA sequences encoded by the viral genome, or in the case of transgene silencing, encoded by the transgene and/or endogenous genomic DNA).

It is clear that DNA viruses as well as RNA viruses are capable of generating dsRNA or siRNA, which can trigger the development of RNA silencing within a host plant (Voinnet et al., 1999). Although the presence of foreign dsRNA is a powerful trigger for RNA silencing, plant cells also appear to have the ability to recognize “aberrant” single-stranded RNA molecules from different sources and then to direct the synthesis of homologous dsRNA or siRNA, which serves as a trigger for RNA silencing. For example, geminiviruses replicate to high copy number and generate abundant viral RNAs in the cytoplasm; these viral RNAs might be recognized as aberrant RNA and subsequently processed by host enzymes into dsRNA or siRNA that could trigger RNA silencing directed against the viral genome. Alternatively, dsRNA might be produced by readthrough transcription of viral genes in the small circular genome. Numerous DNA viruses also undergo illegitimate integration into the host genome, which could trigger the production of dsRNA or aberrant RNA in a manner similar to transgene-induced gene silencing (although geminiviruses are not known to integrate, this might be a factor with other DNA viruses).

Although RNA silencing acts at the post-transcriptional level, it is associated with changes in the chromatin structure of DNA sequences identical to the dsRNA silencing trigger. In particular, dsRNA and/or siRNAs guide the DNA methylation of identical sequences in a process called RNA-dependent DNA methylation. When RNA-directed DNA methylation occurs in the transcribed region of a gene, it typically does not impede transcription; instead, it might contribute to the production of aberrant RNA precursors for dsRNA (Bender, 2001). In support of this view, plants disrupted in DNA methylation also are impaired in their ability to trigger or to sustain RNA silencing from a transgene that produces aberrant RNAs (Morel et al., 2000). Furthermore, if RNA-directed DNA methylation occurs on sequences necessary for transcription initiation, it can cause transcriptional silencing. In addition to DNA methylation, the methylation of particular residues on histone tails is associated with transcriptionally silent chromatin (Gendrel et al., 2002; Volpe et al., 2002).

Although the mechanism(s) of dsRNA or siRNA production and the induction of RNA
silencing by DNA viruses is unclear, it is apparent that geminiviruses and other DNA viruses (as well as a number of RNA viruses) have the ability to suppress RNA silencing in host plants. This ability has been traced to AL2-like proteins from the geminiviruses *African cassava mosaic virus* and *Tomato yellow leaf curl virus* (Voinnet et al., 1999; van Wezel et al., 2002), and the TGMV AL2 and BCTV L2 proteins also may have silencing suppressor activity (H. Wang and D.M. Bisaro, unpublished data). Wang et al. hypothesize a possible connection between the viral suppression of ADK activity and RNA silencing in that the maintenance of RNA silencing is associated with the methylation of target DNA sequences and/or histone proteins, and ADK activity plays a role in transmethylation.

The S-adenosyl-L-Met (SAM)–dependent methylation cycle performs transmethylation of a number of substrates and is a key source of adenosine in plants. It has been shown in yeast (Lecoq et al., 2001) and in plants (Weretilnyk et al., 2001; Moffatt et al., 2002) that ADK activity controls flux through the SAM-dependent methyl cycle by recycling adenosine. Moffatt et al. (2002) showed that ADK-deficient Arabidopsis plants have reduced SAM-dependent transmethylation activity, and Weretilnyk et al. (2001) found that ADK activity increases in plants in response to increased methyl demand. Some plant species, such as spinach and sugar beet, synthesize the osmolyte glycin betaine in response to salt stress, and this requires SAM-dependent methylation of precursors. Therefore, salt stress increases methyl demand in these species. Weretilnyk et al. (2001) showed that ADK activity increases in spinach and sugar beet after salt stress treatment but not in two other species (tobacco and canola) that are nonaccumulators of glycin betaine. Because methylation appears to be important to maintain silencing in plants, Wang et al. propose that by inhibiting ADK, AL2 and L2 might indirectly suppress silencing by interfering with methylation. By contrast, increases in ADK activity in plants after infection by a virus that lacks AL2-like or L2-like proteins might reflect an increase in methyl demand concomitant with the induction and maintenance of silencing.

Methylation of viral DNA also may have an effect on viral replication independent of RNA silencing. Geminivirus genomic DNA is largely unmethylated, and Brough et al. (1992) showed that methylation interferes with viral replication. Therefore, viral inhibition of ADK activity might be important to ensure efficient geminivirus replication instead of or in addition to an effect on the suppression of RNA silencing.

Additional experiments to investigate the role of ADK in response to viral infection have been hampered by the inability to generate multiple transgenic lines in Arabidopsis or *N. benthamiana* that either overexpress ADK (via the expression of a sense ADK construct) or underexpress ADK (via the expression of an antisense ADK construct or an ADK RNA interference construct). To date, the authors have generated only one ADK-deficient line in Arabidopsis (via RNA interference), which contains ~25% of wild-type ADK activity and produces extremely stunted plants. Interestingly, Moffatt et al. (2002) produced only ADK-deficient lines from the expression of sense or antisense ADK constructs in Arabidopsis and also reported a high frequency of reversion to the wild type. These authors suggested that reversion of silencing might be the result of reduced transmethylation activity caused by reduced ADK activity. Therefore, Wang et al. hypothesize that ADK activity is tightly regulated and that attempts to alter activity artificially often are overcome by post-translational processes, epigenetic events, or some combination of these mechanisms.

Clearly, many questions remain concerning the relevance of ADK to viral infection and plant defense mechanisms. Nonetheless, the work of Wang et al. opens new avenues of investigation in the realm of viral defense and counterdefense.

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**REFERENCES**


Plants expressing tomato golden mosaic virus AL2 or beet curly top virus L2 transgenes show enhanced susceptibility to infection by DNA and RNA viruses. Virology 285, 59–70.


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