transgenic plants, for some species at least, and it often seems that there are few limits to the phenotypic diversity that can be created by molecular breeding. Among the most dramatic and potentially useful phenotypes is viral resistance conferred by transformation with viral genes, which is known as pathogen-derived resistance (PDR). It has long been known that plants transformed with viral sequences may show a heightened resistance to infection by the same virus, and sometimes many other viruses as well. Although many such plants have been created and studied, little has been learned about the molecular mechanisms that allow plants expressing viral transgenes to fend off infection, information that is essential if we are to predict the long-term stability of PDR and the possible ecological and biological effects of growing crops engineered to resist viruses in this way.

In all likelihood, no single mechanism will account for all instances of PDR. Indeed, in some cases the presence of the transgenic viral protein is required for the expression of resistance, whereas in other cases it is the transgene transcript itself that mediates resistance (for reviews, see Lindbo et al., 1993a; Reimann-Philipp and Beachy, 1993). On pages 1441–1453 of this issue, Smith et al. describe a possible mechanism for RNA-mediated resistance that emerges from their analysis of tobacco expressing an untranslatable version of the potato virus Y (PVY) coat protein (CP) gene. What is especially exciting is that the implications of this mechanism extend beyond PDR to the oft-observed but little-understood phenomenon of sense suppression, in which attempts to overexpress transgenes often result in a paradoxical reduction in expression of both the transgene and a chromosomal homolog, when one exists (for reviews, see Finnegan and McElroy, 1994; Flavell, 1994). The same cellular mechanism that results in a reduction of viral transcript levels in plants expressing an untranslatable version of the PVY CP may account for at least some instances of sense suppression.

The work of Lindbo and Dougherty (1992a, 1992b) provided one of the first hints that virus-derived transgenes altered to contain in-frame stop codons after the initiating ATG might confer virus resistance. These workers observed that some tobacco plants transformed with such untranslatable versions of the tobacco etch virus (TEV) CP were resistant to TEV infection. (These “RC” plants were originally meant to serve as RNA controls for plants expressing truncated CPs; Lindbo and Dougherty, 1992a.) Plants of some RC transgenic lines showed striking TEV resistance in which inoculated plants remained symptomless. In fact, protoplasts of such plants, when electroporated with TEV, produced no detectable viral protein or infectious particles. By contrast, resistance mediated by the translatable CP gene (Lindbo and Dougherty, 1992a) or by an antisense CP gene (Lindbo and Dougherty, 1992b) was weaker: resistant CP plants developed symptoms more slowly than susceptible plants upon TEV inoculation, and protoplasts from resistant CP and antisense CP plants could support some virus replication. Moreover, whereas CP expression often confers resistance to related viruses, RC expression conferred resistance only to TEV.

One possible explanation for the ability of the TEV-RC transgenic lines to block TEV infection was that the untranslatable sense strand RNA, which would be free of ribosomes, would be available to hybridize to minus sense genomes, thus interfering with replication (Lindbo and Dougherty, 1992b). Pang et al. (1993) offered a similar explanation to account for the observation that transgenic plants expressing an untranslatable nucleocapsid protein gene from tomato spotted wilt virus (TSWV) are able to fend off TSWV infection. However, both groups noted that the degree of resistance did not correlate with the steady state levels of transgene transcript. In fact, Pang et al. (1993) pointed out that the plants that showed resistance were the ones with the lowest steady state transcript levels.

A clue to the origin of this inverse correlation came from Lindbo et al.’s (1993b) finding that transgenic plants expressing a translatable TEV CP gene (either full length or a truncated form), although initially susceptible to TEV infection, “recovered” within several weeks and then displayed a high TEV resistance comparable to that in the TEV-RC lines. This high resistance was elicited only in response to viral challenge. The resistance did not involve systemic signaling; instead, it appeared to result from the combination of transgene expression and the presence of viral RNA. Resistant (i.e., “recovered”) plants had low steady state CP transcript levels, at least 10-fold less than those of susceptible (i.e., not pretreated with TEV) plants. What was most intriguing, however, was that the transcription rates, as assayed by nuclear run-off assays, were similar in both unchallenged and recovered plants.

These results suggested that infection of the TEV CP plants with TEV results in the degradation of the transgene transcript rather than a reduction in transgene transcription. Although it is possible that the reduction in transcript levels and the establishment of virus resistance are independent events, Lindbo et al. (1993b) proposed that the two are linked by a process in which the presence of the infecting TEV stimulates the cell to reduce the lev-
els of both the TEV transgene transcript and the viral transcript in a sequence-specific manner.

In light of these results, it was possible that the TEV RC plants (Lindbo et al., 1992b) were showing a "recovered" phenotype in the absence of viral challenge—that is, that the expression of the untranslatable CP transcript alone stimulates the cellular process that reduces the level of both transgene transcript and that of the homologous virus (i.e., TEV) should it happen to be present. Smith and coworkers now show that this is exactly what seems to happen in tobacco plants transformed with an untranslatable CP gene from a different potyvirus, PVY. Expression of this gene can lead to a highly resistant state that is accompanied by low steady state levels of transgene transcript, high rates of transgene transcription, and, in some cases, transgene methylation.

To simplify the genetic analysis of resistant plants, Smith and coworkers took advantage of a relatively simple technique for generating haploid tobacco plants. Leaf disc tissue from haploid plants transformed with an untranslatable CP gene from a different potyvirus, PVY. Expression of this gene can lead to a highly resistant state that is accompanied by low steady state levels of transgene transcript, high rates of transgene transcription, and, in some cases, transgene methylation.

What this cellular response system might comprise is, at this point, anybody's guess. Lindbo et al. (1993b) have proposed that sequence-specific RNA binding proteins might bind to overexpressed or aberrant RNA and thereby target it for degradation or that an RNA-dependent RNA polymerase (which plant cells are known to contain; Schiebel et al., 1993) produces short antisense transcripts to overexpressed transcripts that hybridize to these transcripts, targeting them for degradation. The advantage of the latter mechanism is that it doesn't depend on a large stable of sequence-specific RNA binding proteins.

Whatever the mechanism, the authors point out that it might well be responsible for some instances of sense suppression. Although sense suppression sometimes involves a transgene-induced reduction in transcription, other times it seems to involve a post-transcriptional reduction in transcript levels that could result from effects on pre-mRNA processing, export of mature mRNA from the nucleus, or transcript stability in the cytoplasm (for reviews, see Jorgensen, 1992; Flavell, 1994). Assuming that the same cellular process is involved in both RNA-mediated virus resistance and post-transcriptional sense suppression, this process likely occurs in the cytoplasm, which is where viral transcripts accumulate. Therefore, it is likely to involve the modulation of transcript stability.

An important feature of sense suppression is that it is frequently epigenetically reversible. This reversibility is thought to reflect the mechanism of suppression: that is, suppression probably involves modifications such as methylation and chromatin restructuring, both of which can change over development or from generation to generation. RC1-mediated resistance to PVY appears to share this aspect of sense suppression, because genetically identical plants (either individual plants from an HR fertile line or individual lines from a single DH family) vary in their susceptibility to PVY.

What kind of epigenetic regulation could control the degradation of PVY-RC1 transcripts? Presumably, it is the initial transcription rate that determines whether or not the transgene transcript will reach the critical threshold; if so, the transgene must be transcribed at different rates in these genetically identical plants. One simple possibility is that in HR fertile lines, the transcript accumulates to levels that are close to the threshold. Alternatively, the threshold itself might be particularly variable in these lines. In either case, transcript levels might be sufficient to activate the system in some plants but not in others. However, were such a stochastic mechanism to operate, one would not always expect the majority of plants in HR fertile lines to be resistant. Moreover, it is inconsistent with the threefold difference in transgene transcription rates seen between resistant and susceptible plants from different lines of a single DH family.
Different trapsgene transcription rates could also be achieved if the transgene were subject to some form of epigenetic regulation, such as methylation. Smith and colleagues did find variations in the extent of transgene methylation, but, interestingly enough, methylation correlated with low steady state RNA levels and high transcription rates. Methylation is generally credited with reducing, not increasing, transcriptional activity, and the authors propose that methylation of the more rapidly transcribed transgenes might in fact represent the plant's attempt to reduce transcription of the transgene. Indeed, Wassenegger et al. (1994) recently reported that viroid RNA directs methylation of a homologous transgene in the plant genome and suggested that this sequence-specific methylation might serve to silence an overexpressed gene.

A genetic approach may help identify the components that participate in the process by which transgene transcript levels are assessed and, ultimately, targeted for degradation. Dehio and Schell (1994) have initiated a genetic dissection of transgene silencing in Arabidopsis by selecting for mutants in which silencing of a particular transgene (the Agrobacteri-um rolB gene) occurs during the regeneration of transgenic plants. Suppression of rolB expression is regulated at least in part at a post-transcriptional level, and the genetic loci identified by Dehio and Schell (1994) may eventually provide information that will help in understanding how the cell detects excess transcripts, whether of viral or plant origin, and targets them for destruction.

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Making sense (suppression) of viral RNA-mediated resistance.
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