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Interfering with Viral Infection: Plants Do It Too

Viruses can be classified into taxa according to any number of criteria: whether the viral genome consists of DNA or RNA, and whether it is single- or double-stranded; the existence and nature of RNA intermediates that are required for viral replication; the variety of proteins that house or otherwise associate with the virion genome and whether the protein coat is in turn surrounded by membrane; virion size; and host range. Clearly, these characteristics can be interrelated. Indeed, they all reflect the basic viral strategy of invading the host cell so as to exploit the macromolecular and enzymatic machinery that is unique to the intracellular milieu of the living organism.

Because viral infections are generally deleterious to invaded cells, host organisms have evolved a variety of mechanisms that combat viruses. The vertebrate immune system generates, in addition to specialized cells that directly attack viruses and infected cells, a battery of secreted immunoglobulin molecules that is sufficiently diverse to neutralize a broad spectrum of viral antigens. In contrast to animal systems, a general barrier to viral infection is afforded by the cell walls of plants and bacteria. Correspondingly, viruses have evolved a variety of elegant strategies to exploit, for their own purposes, the very molecular defenses that the host may use to ward off viral entry into cells. (The human immunodeficiency virus, utilizing the CD4 membrane protein to specifically bind to and infect leukocytes, exemplifies viral abilities to directly usurp host proteins.) And once it has circumvented obstacles to entering the host cell, the virus pursues an intracellular agenda that involves uncoating, the synthesis of proteins and nucleic acids necessary for genome replication and intercellular movement, and assembly into mature virion particles.

Given that the intracellular stages of viral infection depend so intimately upon the biochemistry that is essential to cellular life itself, it makes evolutionary sense that host organisms would place a premium on preventing viral entry. After all, processes of cellular growth and homeostasis, dependent as they are on the constant turnover of proteins and nucleic acids, impose considerable limits upon mechanisms whereby viral nucleic acids or proteins might be recognized within the infected cell as foreign and thereby targeted for destruction. Nevertheless, various cellular defense strategies appear to be marshaled to combat viruses once they have reached the cytoplasm. Most intriguingly, these intracellular defenses can indeed act against an impressively broad range of viral invaders while allowing normal processes of cellular metabolism to proceed.

The ability of bacteria to restrict the intracellular replication of phage is an example of an antiviral strategy that has been recognized for decades, although the origin of the term “restriction enzyme” has perhaps been obfuscated in the past twenty years through the routine use of such endonucleases as laboratory reagents. Viral restriction, however, was not the first documented phenomenon whereby viral replication is hampered intracellularly. It was, rather, in the late 1950s that the multiplication of a given virus in animal cells had been observed to “interfere” with subsequent infection by the same or different viral species, and the cellular protein that was responsible for such interference, present in minute quantities, was named interferon. In the early 1980s, interferon became one of the first gene products to be produced, through the availability of purified restriction enzymes, by means of the new recombinant gene technology.

The antiviral mechanisms mediated by interferon have since been elucidated in some detail. To begin with, the primary signal that induces the expression of interferon is provided by the molecules of double-stranded RNA (dsRNA) that represent either the genome of the invading virus or an intermediate in the replication of DNA and single-stranded RNA viruses. The means by which the inducing dsRNA causes the nucleus to turn on the expression of interferon continue to be elaborated (see, e.g., Kumar and Carmichael, 1998), but it has long been evident that the interferon thereby produced must be released into the extracellular environment in order to establish the “antiviral state” in neighboring cells that bear specific cell surface interferon receptors. Upon binding to such neighboring cells, interferon primes them for antiviral defense through a signal transduction cascade that includes the induction of two enzymes, the activities of which depend upon the binding of dsRNA supplied by a subsequently invading virus. The first of these two enzymes, 2′,5′-oligoA synthetase, synthesizes oligoadenylate, an activator of a cellular endoribonuclease (RNase L) that proceeds to degrade mRNA. The second dsRNA-dependent activity to be induced by interferon is a protein kinase that participates in a cascade of reactions, including the inhibitory phosphorylation of eIF-2. Although both wings of the interferon pathway inhibit cellular as well as viral processes, host dynamics are generally better able to compensate and withstand the inhibitory mechanisms (see, e.g., Nilsen et al., 1983).

Plants also manifest antiviral activities

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that resemble, at least at the macroscopic level, viral interference. "Recovery," also discussed in terms of "cross-protection," is the phenomenon whereby plants that have undergone an initial round of viral infection nevertheless develop new, healthy, virus-free growth that is resistant to subsequent infection by the same or a related virus. Viral recovery in plants has been the focus of intensive research in recent years as a result of increased interest in the ways that plants respond to transgenic manipulation, which, in many ways, can be likened to an experimental form of the intracellular genetic exploitation practiced by viruses. Thus, many experimentalists, frustrated by the finding that transgene expression often declines with time and successive generation—not to mention that homologous endogenes are similarly "cosuppressed"—look to viral recovery as a model for the thwarted expression of foreign (viral) genes. Transgenic technology has thus brought renewed urgency to investigations of the molecular mechanisms that underlie viral recovery in plants. Many questions remain to be answered regarding the commonality between recovery and cosuppression and the extent that these phenomena represent broader, intracellular antiviral strategies in plants.

On pages 1207–1215 of this issue, Ratcliff et al. continue their exploration of the molecular bases upon which *Nicotiana benthamiana* recovers to viral infections. The authors' previous work (Ratcliff et al., 1997), along with that of others (Covey et al., 1997), has established that the recovery of plants to both RNA and DNA viruses, as represented by a nepovirus and caulimovirus, respectively, is dependent on the transcription of viral genes into mRNA. This was an especially important finding in that the transgene-induced cosuppressive silencing of genes had similarly been found to occur via a post-transcriptional mechanism (reviewed in Matzke and Matzke, 1995). The linking of post-transcriptional gene silencing (PTGS) to recovery to nepovirus and caulimovirus furthermore strengthened the suspicion that cosuppression in transgenic studies could be the manifestation of an antiviral strategy to an artificial manipulation.

In their present report, Ratcliff et al. extend our understanding of PTGS in viral recovery by studying the molecular outcomes of infecting *N. benthamiana* with two viruses (i.e., a tobravirus and a potexvirus) that are not related to the viral taxa previously analyzed. Significantly, the two additional viruses examined in this study manifest the properties of PTGS, thereby demonstrating that the phenomenon is characteristic of a broad spectrum of plant viruses. And in agreement with earlier results that initially seemed daunting to practitioners of transgenic methodologies, both the tobraviral and potexviral infections prevented the subsequent expression of homologous genes introduced via viral or T-DNA vectors. In this way, the authors account for the observation of cross-protection, whereby an initial round of viral infection can prevent the successful infection of a second viral species.

The authors show, however, that it would be shortsighted to equate PTGS with "recovery" from viral infection per se. Indeed, despite the fact that their potexviral construct is clearly capable of preventing the accumulation of mRNA from subsequently administered tobraviral constructs (i.e., capable of promoting cross-protection by PTGS), the infected plants do not recover from the initial potexviral infection. In this way, the authors elegantly show that PTGS is not sufficient for viral recovery.

So what additional elements are involved in recovery, and what have we learned about antiviral strategies in plants? First, as in animal systems, plants respond to intracellular viral invaders by mobilizing a defense that can interfere with invasion by a second viral type. (And similar to animal viruses, it is now becoming evident that plant viruses have evolved means to defy antiviral defense mechanisms such as PTGS [see Brigneti et al., 1998; Kasschau and Carrington, 1998].) We have, furthermore, learned that PTGS, as an element of antiviral intracellular initiatives, is elicited by viruses—as observed by early transgenic experimentalists—in order to target viruses. To the extent that viral defense is PTGS-mediated, one could go so far as to liken the plant response to animal interferon systems by asserting that the antiviral state is promoted by an (m)RNA intermediate.

Does this place us in a position to look for an interferon-like antiviral response in plants? To date, specific molecular elements analogous to those of the mammalian interferon system (e.g., oligoA synthetase and RNase L) have not been found in plants, and forthcoming experiments to elucidate the links between PTGS and plant systemic recovery will likely not reveal an interferon system (see, e.g., Mitra et al., 1996). Indeed, the "antiviral state" that is established in plants is not as robust as that characterized by the interferon system; cross-protection is limited to viral strains that are significantly related (Mueller et al., 1995).

It is intriguing, however, that the RNA signal within the cascade of molecular events along the pathway from viral invasion to recovery via PTGS in plants, like the dsRNA effector in mammalian systems, appears to involve more than the immediate transcription of viral genes. Various studies have in fact suggested that both sense and antisense RNA molecules—perhaps together in the form of dsRNA—may act as antiviral mediators (reviewed in Grant, 1999), and RNA-dependent RNA polymerases that might participate therein have been cloned from plant systems (Schiebel et al., 1998). The elaboration of systemic RNA movement in plants, promoted by specific proteins, moreover, offers a conceptually attractive notion of an RNA-mediated signaling

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mechanism in recovery (see Lazarowitz and Beachy, 1999). In any event, it is clear that antiviral mechanisms in plants are currently proving to be as evolutionarily incisive as those that are already recognized in animal and bacterial systems.

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