Geminiviruses: A Twin Approach to Replication

Viruses play a dual role in biology research. Their ability to cause disease certainly makes them important subjects of study in their own right, and their interactions with their hosts have made them invaluable sources of potent tools that have immeasurably enhanced our understanding of biological processes. For example, M13 phage has been useful for gene cloning, the promoter of the cauliflower mosaic virus (CaMV) 35S gene is useful for driving high-level gene expression in plants, and retroviruses have been used to transfected foreign genes into mammalian cells. Viruses have also provided direct insights into processes that would otherwise have proven very difficult to study. Simian virus 40 (SV40) replication, for example, depends heavily on host replication factors, and because replication of the viral genome is so much easier to follow than that of the host genome, the study of SV40 replication has illuminated replication processes in remarkable detail.

Analogous studies would also be valuable for learning about plant replication, but most plant viruses—even some DNA viruses (e.g., the caulimoviruses)—replicate via RNA intermediates. One group of plant viruses whose members do appear to replicate via DNA intermediates is the geminiviruses, so-called because of the "twinned" nature of their particles. The geminiviruses, which are single-stranded DNA viruses, are subdivided into three subgroups based on the structures of their genomes (bipartite or monopartite), on their hosts (monocots or dicots), and on their insect vectors (whitefly or leafhopper) (for review, see Timmermans et al., 1994). If geminiviruses are to be useful for exploring the mechanisms of plant DNA replication, it is necessary to ascertain the extent to which they interact with the host replication apparatus. On pages 705–719 of this issue, Nagar and co-workers show that the bipartite geminivirus tomato golden mosaic virus (TGMV) induces the accumulation of proliferating cell nuclear antigen (PCNA), a protein associated with DNA replication, in differentiated cells ofNicotiana benthamiana plants. Their results suggest that TGMV not only uses host replication proteins but also influences their expression, raising the possibility that geminivirus replication may have much to reveal about both the components and control of plant DNA replication.

All geminiviruses encode just one highly conserved protein that is essential for viral replication (Elmer et al., 1988) (in the case of the bipartite geminiviruses this protein is called AL1, because it is the product of the first leftward open reading frame on the A genome component), although in the bipartite geminiviruses a second protein, AL3, is necessary for high-level replication (Sunter et al., 1990). Geminiviruses, like the bacteriophages M13 and φX174, appear to replicate via a rolling circle mechanism. The single-stranded viral strand is first converted to a double-stranded replicative form (RF) with the synthesis of a complementary strand (the mechanism of whose priming is not known). The viral strand of the RF is nicked at a site within the origin of replication, presumably as a result of the activity of AL1, which recently has been found to have site-specific endonuclease activity (Laufs et al., 1995); according to the rolling circle model, a new viral strand would then be synthesized, displacing the original viral strand. The displaced viral strands could then serve again as templates for complementary strand synthesis or be packaged into a virion, depending on the stage of the infection.

The finding that only one viral protein is essential for geminivirus replication (albeit a multifunctional protein—in addition to being an endonuclease, AL1 is an origin recognition protein [Fontes et al., 1992] and it negatively regulates its own transcription [Eagle et al., 1994]) raises the possibility that, like bacteriophages such as φX174 and papovaviruses such as SV40, geminiviruses must rely on host proteins for their replication. Consistent with this hypothesis is the fact that geminiviruses appear to replicate in the nucleus (rather than, say, in the cytoplasm). In addition, geminivirus replication in cultured cells has been observed to be correlated with cell division, although in some cases it has been observed to precede proplast division. A flow cytometry study of infected plants also indicated that virus replication is associated with host cell replication; those cells in S phase (i.e., with a DNA content of between 2C and 4C) contained the most double-stranded viral DNA (Accotto et al., 1993).

Assuming that geminivirus replication does rely to a great extent on host replication factors, an important question is whether in infected plants, geminiviruses can replicate only in cells that are already actively dividing or whether they can, like some animal viruses, induce quiescent cells to produce replication proteins and even, perhaps, to enter S phase. Accotto et al. (1993) noted that Digitaria streak geminivirus-infected Digitaria plants may have more S-phase and fewer G2-phase nuclei than uninfected plants, raising the possibility that geminivirus infection somehow influences the plant cell cycle. Evidence that geminivirus replication in plants does not take place in actively dividing cells comes from the work of Horns and Jeske (1991) on the phloem-limited geminivirus Abutilon mosaic virus (AbMV). Their in situ hybridization studies of the leaves of infected Abutilon plants indicated that AbMV DNA does not accumulate in meristematic tissues; instead, the viral DNA was found in the nuclei of differentiated phloem cells (and was rarely
seen in two adjacent cells, as would have been expected if dividing cells were infected.

Nagar and coworkers have now found that for TGMV as well, which unlike AbMV infects a large number of cell types, not just phloem cells, the virus appears to replicate in differentiated rather than dividing cells. To study replication, they assayed for expression of the AL1 and AL3 proteins, under the assumption that detectable amounts of these proteins will be present only where the virus is abundant and, presumably, replicating. Immunolocalization of both proteins in infected N. benthamiana plants showed that they are present in the nuclei of terminally differentiated leaf cells, including phloem, epidermal, and mesophyll cells, as well as in the nuclei of differentiated cells in the stem. The authors point out that the absence of AL1 and AL3 expression in dividing cells of the leaf and stem makes it unlikely that TGMV replicates in dividing cells and then moves into differentiated cells.

The finding that AL1 and AL3 are expressed—and therefore that TGMV is replicating—in differentiated cells implies that these cells are expressing replication proteins that are not normally expressed in nondividing cells. Nagar and colleagues show that this is indeed the case, at least for one protein, PCNA. This protein, which interacts with DNA polymerase δ and endows it with processivity (i.e., the ability to replicate thousands of nucleotides without “falling off” the DNA), is thought to fold into a clamp-like structure that encircles the replicating DNA and that can slide along it as replication proceeds (for review, see Wyman and Botchan, 1995). PCNA is normally present in dividing plant cells only, but Nagar and coworkers show that it is present in the nuclei of differentiated leaf cells of TGMV-infected plants; indeed, AL1 and PCNA colocalize to the nuclei of the same differentiated cells. In addition to expressing PCNA, and presumably other replication proteins as well, the cells show some morphological evidence of dedifferentiation: the nuclei are round and have moved toward the center of the cell. The accumulation of PCNA appears to be due to the AL1 protein alone, because transgenic plants expressing AL1 (Hanley-Bowdoin et al., 1990) also express PCNA in differentiated cells.

An important question for future work concerns the mechanism by which TGMV influences PCNA expression. That is, does the virus impinge on cell cycle regulation? Or does it have a less global effect, specifically inducing the expression of only those genes whose products it needs for its replication? Many viral replication proteins, such as SV40 large T antigen and the adenovirus E1A protein, both regulate the expression of specific genes and perturb progress through the cell cycle by interacting with cell cycle control proteins such as the retinoblastoma protein, which represses the expression of genes whose products are involved in DNA replication (Nevins, 1992). The authors note that AL1 expression appears to lengthen the cell cycle of transgenic tobacco cells, so it is possible that this protein acts to promote or maintain S phase in host cells.

The data in this paper thus provide strong evidence that TGMV replication takes place in differentiated cells and that it can induce these cells to synthesize host proteins that are presumably required for viral replication. (Indeed, at least some geminiviruses, including TGMV and AbMV, appear not to replicate in actively dividing cells in plants, most likely because, for reasons that are as yet unknown, they cannot access those cells.) Defining the requirements for geminivirus replication should thus provide a potent means of defining the requirements for host DNA replication. An exciting prospect is the development of an in vitro geminivirus replication system analogous to those developed for SV40 and some bacteriophages.

Nagar and coworkers’ results also illustrate how geminiviruses may help in deciphering the details of how the plant cell cycle is controlled. That is, finding out how AL1 induces PCNA accumulation, and possibly that of other DNA synthesis proteins as well, may reveal how PCNA accumulation is normally induced in cells that have become activated to divide. Other geminiviruses may also provide complementary information about regulation of the plant cell cycle. Whereas TGMV infection seems not to induce cell proliferation, certain other geminiviruses, such as beet curly top virus (BCTV) may stimulate cell division. In the case of BCTV, which is a monopartite geminivirus, vein swelling, which may involve the induction of cell division, appears to be due to an open reading frame (C4) that encodes a protein of unknown function (and that is not produced by TGMV) (Stanley and Latham, 1992). Examining how C4 influences putative cell cycle regulators may give insights into additional levels of plant cell cycle control.

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REFERENCES


Disputed Ancestry: Comments on a Model for the Origin of Incompatibility in Flowering Plants

In a recent review published in THE PLANT CELL, Bell (1995) argues that self-incompatibility (SI) in flowering plants is derived from isolating mechanisms, typically involving thickened callosic cell walls, that develop between the gameto- phyte and sporophyte generations of lower plants. He writes that "an interfacial reaction leading to the separation of the two generations at the time of reproduction is a fundamental property of land plants" and suggests that the recognition loci governing this isolation evolved to produce the multiallelic SI loci in angiosperms. This model requires that the first angiosperms had a genetically determined incompatibility and that all SI systems of modern plants derive from this ancestral feature. We restate here the view, based on current molecular data, that SI arose separately in different lineages of angiosperms. We also present alternative views to those of Bell concerning the function of callose in the SI response and the role of carbohydrates in mediating SI recognition.

Origins of SI

The phylogenetic origin of SI has been debated for many years, and arguments exist both for and against the early origin of SI in angiosperms (Whitehouse, 1950; Bateman, 1952). The wide distribution of SI among the 320-odd angiosperm families has been used as evidence of its ancestral nature. However, the distribution is rather sporadic, and SI is uncommon in "primitive" woody angiosperms and entirely absent from several very large families (Charlesworth, 1985).

Molecular information is now available through the cloning of SI genes from the Solanaceae (Anderson et al., 1989), Brassicaceae (Nasrallah et al., 1987), Papaveraceae (Foote et al., 1994), and Poaceae (Li et al., 1994). There is substantial evidence that these genes encode the molecules identified as S gene products, and their sequences give considerable insight into the evolution of SI. In three cases, the likely function of the S gene products can be inferred from the corresponding sequence: the S genes of Brassica encode receptor-protein kinases (SRKs) and glycoproteins (SLGs), those of the Solanaceae encode RNases, and those of Phalaris encode thioredoxins. Some, such as the SLGs and SRKs, are encoded by part of a multigene family present in the genome of Brassica and other plants, whereas others, such as the S-RNases and thioredoxins, are of more ancient lineage and are found in both eukaryotic and prokaryotic organisms (Nasrallah and Nasrallah, 1993; Green, 1994; Li et al., 1994).

The genes currently known to be present at the S loci in these four families have no sequence similarity—indeed, they have independent evolutionary histories. This indicates that these SI systems are unrelated at the molecular level and leads to the conclusion that SI arose independently in each family. No traces of residual similarity between SI genes have been reported, as would be required to support the idea of divergence from a single, ancestral SI system (Bell, 1995). The lack of sequence similarity between the S genes in these different families, coupled with the existence of related genes with other functions, indicate that the various S genes were independently recruited to their current functions in reproduction from previously existing genes in the genome. The molecular data therefore point to several origins of SI.

Callose: Cause or Effect of SI?

Bell argues that the expression of SI involves deposition of the polysaccharide callose as a barrier between the angio- sperm gametophyte and sporophyte, with