RNA-dependent RNA polymerases of plants

(RNA replication in eukaryotes/cucumber mosaic virus/template preference)

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ABSTRACT The existence of RNA-dependent RNA polymerases (EC 2.7.7.48) in plants has been definitely proven by their isolation in pure form from cucumber and tobacco in our laboratory and from cowpea at Wageningen. These enzymes are single-chain proteins of 100–130 kilodaltons. They show clear physical and biochemical differences characteristic for a given plant species, even when their amounts in the plants were greatly increased prior to isolation by infection with the same virus. The role of these enzymes in plant physiology remains unknown.

The existence of RNA-dependent RNA polymerase activity (EC 2.7.7.48) in eukaryotic cells was shown by Astier-Manifacier and Cornuet (1) in 1971 in Chinese cabbage plants. Two years later the same was found true for tobacco plants (2). The central dogma of molecular biology, however, does not call for RNA being made other than by transcription from DNA. Only for RNA viruses was a need for RNA replicating enzymes acknowledged. Because the two groups finding such enzymes in healthy plants were composed of virologists, the consensus of the scientific community, including other virologists, was that these observations were probably due to RNA virus contamination or cryptic viruses in these plants. Actually, no consensus was needed—disregarding such findings by each individual reader of these papers sufficient to make them almost nonexistent, and the authors soon abandoned this line of research or at least refrained from publishing any more on this topic.

A few years later several laboratories took up the study of the RNA-dependent RNA polymerase in tobacco, and they showed that the properties of still very crude enzyme preparations were indistinguishable from those of the enzyme present in greater amounts in plants infected with tobacco necrosis virus or alfalfa mosaic virus (3–6). Biochemists and molecular biologists, however, have shown scant interest in the existence of this class of enzymes of eukaryotic cells. Thus we continue to see books, chapters, and papers entitled “RNA polymerases” with the tacit assumption that these are all DNA transcripases.

Because the amounts of these enzymes in plants are usually low, but increased by RNA virus infection, interest in them has remained mostly restricted to plant virologists. Among these there were several camps holding at different times different opinions. One small group for a while retained doubts that such enzymes really existed in their uninfected control plants. Actually in one plant, cucumber, no definite evidence for this enzyme has been reported. Another group claimed that the virus-specific enzymes (the viral RNA replicases) differed from the plant RNA-dependent RNA polymerases. As stated, virus infection is often used to increase the amounts and thus facilitate the purification and biochemical study of these enzymes. The fact that in association with viral RNA they are largely membrane bound, whereas in healthy plants they are mostly soluble or cytoplasmic, has represented an obstacle in the realization that these were nevertheless the same enzymes. Our conviction (3, 5) that they differed only in amount and localization has, however, gained ground in recent years. Data published since 1978, as well as data presented at recent international congresses, have illustrated this evolution of increased harmony (7–12, 13).

The progress in this research and the consensus arrived at in the past few years will now be briefly reviewed, followed by a consideration of the important questions: how do viruses increase the amounts of these enzymes, and what is their physiological role in the plants?

The successful isolation of a pure plant RNA-dependent RNA polymerase from healthy cauliflower was reported in the second and very brief paper by Astier-Manifacier and Cornuet (11). It, like their 1971 paper, met with some skepticism and has not been confirmed or followed up. On the other hand, successful purification has been reported for three other plant RNA polymerases in 1982 (12, 13). "Pure" in this context is defined as preparations that show one or two specifically located very predominant peptide chains on sodium dodecyl sulfate/polyacrylamide gels. The purified RNA-dependent RNA polymerases reported in 1982 were obtained from virus-infected cucumber, tobacco, and cowpea. In these plants very great increases in enzyme were obtained by particular virus infections, cucumber mosaic for the first two (13, 14) and cowpea mosaic for the third (12). Such virus-infected plants were utilized for the isolation of pure enzymes, whereas from the corresponding healthy plants no pure enzyme preparations have as yet been obtained. This makes the French workers' (11) results, obtained with healthy cauliflower, so unexpected. On the other hand, the sedimentation rates of all plant RNA-dependent RNA polymerases indicate molecular weights of 100,000–150,000, and as their purification has advanced it became evident that the molecular weights of the characteristic peptide chains on denaturing gels were similar. The unexpected and tentative conclusion that we are dealing with single-chain enzymes of this size agrees with the findings of Astier-Manifacier and Cornuet (11) regarding the enzyme from healthy cauliflower. The cucumber and tobacco enzymes show specific activities that are similar to that of the phage Qb RNA polymerase.

The enzymes of different plants are clearly different in many respects. Early comparative studies of different plants, healthy or infected with the same or different viruses (15–18), showed that various properties of the enzyme from tobacco and cowpea differed, even when the plants were infected by the same virus. This proved that the enzyme upon virus infection was still completely host-specific, thus not virus-coded, a conclusion that is confirmed by the recent isolation of pure cucumber and tobacco enzymes, both greatly increased in amount by infection with the same virus, cucumber mosaic virus. The RNA polymerases

of the two plants show clear differences in protein molecular weights, the tobacco enzyme forming a doublet band near 125,000 (13) and the cucumber enzyme a strong band at 100,000 and a lesser one at 110,000 (8, 13). Further evidence for the nonviral nature of the cucumber enzyme has since been reported by Symons and co-workers, namely that neither the 100,000-dalton enzyme polyadenylc acid nor the other minor proteins regarded as enzyme components (110,000 and 35,000 daltons) showed similarities in peptide patterns with those of corresponding viral RNA translation products (*). Differences in the enzymological properties of active preparations from different plants have also frequently been reported. Among these properties are template preferences. By definition, RNA-dependent RNA polymerases require an RNA or polynucleotide template, although it was recently shown that the cucumber enzyme can utilize poly(dC), though with lower efficiency than poly(rC) (13). To the extent that these activities in plants or at least their sometimes very great increase was attributed to a virus, they were expected to be more or less specific in requiring that viral RNA as template. This is the case for the bacterial and animal RNA virus replicases (19, 20). However, the plant virologists were uniformly disappointed, in that their solubilized enzymes would accept any RNA and many polynucleotides as templates. Now that we know that these are host-specific and not virus-specific enzymes this is not surprising. A report of specific binding of cowpea mosaic viral RNA to the enzyme from cowpeas infected with this virus could not be confirmed and has been withdrawn (12, 21).

One special case, however, must be noted, that may in the future prove to have important mechanistic implications. A polymerase-active membrane-bound fraction from brome mosaic virus-infected barley, which must contain that viral RNA because it incorporates triphosphates without added template, is greatly increased in activity by the addition of the RNA of brome mosaic virus or closely related viruses but not by other RNAs (22). One can hypothesize that in vivo and in situ the enzyme becomes modified as a consequence of infection and membrane association, which renders it template specific under these conditions. Solubilization apparently reverses this effect and releases the enzymes in their customary promiscuous state. However, lack of template specificity does not preclude lack of template preference. In quantitative terms, each plant enzyme responds differently to different templates. Poly(U,C), poly(U,G), and turnip yellow mosaic virus RNA are, among those compared, the best templates for the tobacco enzyme, whereas poly(C) and tobacco mosaic virus RNA are the best for the cucumber enzyme, and tobacco mosaic virus RNA is also the best template for the cowpea enzyme (13, 15–17). All these quite reproducible differences hold, regardless of the nature of the infecting virus, if any.

The finding that cowpea mosaic virus stimulates the production of cowpea RNA polymerase so much that this enzyme could be isolated in pure form (12) is particularly surprising. This virus resembles the animal picornaviruses in many regards and has even more RNA than these viruses, which definitely code for their specific RNA polymerase (20, 23). Without doubt viral genes are involved in eliciting the increased production of plant enzymes, and in two divided-genome viruses we know that these genes are on the larger RNA [more than 2 × 10^6 daltons in cowpea mosaic virus (24) and tobacco rattle virus (25)]. The mechanism of this effect is, however, not understood. It must be very complex, because none of the three genome compo-

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1 Kuśmierek, J. T. & Singer, B., Twelfth International Congress of Biochemistry, Perth, Western Australia, Aug. 15–21, 1982, p. 162 (abstr.).

nents of the cucumber mosaic virus (1.1, 1.0, and 0.7 × 10^6 daltons) singly or in pairs was able to elicit the great increase in enzyme activity that resulted from inoculation of all three components, which is also required for infection (26). Similar conclusions can be derived from data with alfalfa mosaic virus components (27). That we are really observing increased production, and not activation, is shown by the fact that considerably more enzyme in terms of weight can be isolated from the virus-infected than from the healthy plants. However, this increase can range for different viruses and hosts from negligible to possibly 100-fold.

We know nothing concerning the physiological role of these enzymes in healthy plants. A small amount of double-stranded RNA, seemingly rather homodisperse, has been detected in tobacco leaves, and it has been suggested that such an RNA might have a regulatory function and that it might be produced by the RNA polymerases that we have been studying (28), but this is no more than a guess.

The question whether in virus-infected plants these enzymes actually replicate the viral RNA remains controversial. I believe the answer is yes, for the following reasons: the enzymes of the infected plants are indistinguishable in all known properties, including their molecular weights, from the enzyme of the healthy plant; they are greatly increased in amount by the virus infection that specifically calls for high RNA polymerase activity; no other RNA replicates are found in infected plants; no change in enzymological properties, as is observed with poliovirus RNA replicase (23), is observed in the course of purification of the plant virus RNA replicases.

One important feature of a replicase system is its capability of making not only the complementary strand, but ultimately the replicate of the original template. Only the first stage, a largely double-stranded complex of template and complement, is usually detected in tests in vitro with the plant RNA polymerases, as is also the case when poliovirus RNA is copied by its specific polymerase in vitro (29). However, it has been reported that upon use of a limiting amount of template, one-third of the product was not hybridizable to the template and thus presumably was identical to it (9). If this could be confirmed it would represent important support for the belief that these enzymes can act as viral RNA replicases. However, the plant enzymes may well become reversibly modified when they become membrane bound together with the template RNA. The requirement for virus-specific cofactors at that stage cannot be ruled out. This would be an interesting variation of the mode of bacterial and animal virus RNA replication. For, in these instances, the activity resides in a viral gene, while host protein cofactors are necessary for proper viral RNA replication (23, 30, 31).

While the presence of RNA-dependent RNA polymerases in plants is now beyond doubt, there exists, to my knowledge, no valid evidence that such enzymes occur in animal cells. However, because in a particular plant, cucumber, the enzyme level is too low for its incontrovertible demonstration, the failure to detect such enzymes in animal cells cannot be regarded as proof of their absence. On the hypothetical grounds that very little of such an enzyme may be needed for a specific biological function in eukaryotes, a renewed search for their existence in animal cells might be justified.

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