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THE SELF-ASSEMBLY OF TOBACCO MOSAIC VIRUS
Influence of the Viral RNA and Protein Components upon the Assembly Process

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The initial stage in the self-assembly of tobacco mosaic virus (TMV) RNA and coat protein into virions involves insertion of a loop of TMV RNA between layers of a double-layered disk of TMV protein (containing 17 coat protein subunits per layer) via the central channel of the disk, followed by conversion of the disk-RNA complex into a two-turn protohelix and addition of more protein (elongation). This mechanism leads to assembling particles in which one of the uncoated RNA tails, that containing the 5′-terminus, runs back along the central channel of the growing rod (1, 2).

Bidirectional Elongation

The nucleotide sequence recognized during initiation is located 950 residues (~15% total chain length) from the 3′-end of the RNA (3). Thus, the looped-back 5′-tail of short incompletely assembled rods is much longer than the 5′-tail. It has been suggested that the proximity of the looped-back 5′-tail to the growing point of the particle in the 3′-direction may hinder encapsidation of the 3′-tail (1, 4). Study of the reconstitution of nearly complete particles shows that, for such particles at least, encapsidation can proceed simultaneously on both tails although growth is on the average much faster in the 5′ than in the 3′-sense if we consider the whole of the assembly process. Surprisingly, the ultimate step in assembly appears to be encapsidation of the last several hundred nucleotides of the 5′-tail, as incomplete particles having about nine-tenths the full length (but with no visible RNA tail) may persist for several hours after the beginning of reconstitution.

Heterologous Reconstitution and the Specificity of Initiation

The mechanism for assuring the specificity of self-assembly is thought to reside in the initiation stage of the reaction. The portion of the RNA chain which first binds to coat protein during initiation has been characterized and shown to possess a hairpin loop structure

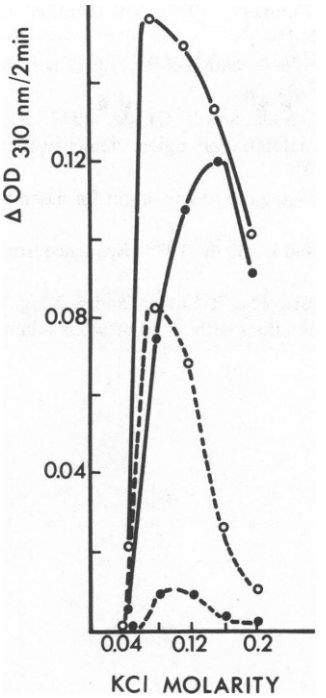

Figure 1 Initial rate of assembly of TMV particles as measured turbidometrically for heterologous and homologous reconstitutions between the RNA and protein components of vulgare and U2 strain TMV. Assembly was performed at 20°C in 10 mM MES, pH 7.0, plus indicated KCl concentration. Protein concentration: 4 mg/ml; RNA concentration: 50 μg/ml. (—o—) TMV RnA plus U2 protein; (—·—) U2 RNA plus U2 protein; (– – – o – – –) TMV RNA plus TMV protein; (– – – · – – –) U2 RNA plus TMV protein.

with the semirepetitive triplet sequence GAA.GAA.GUU.GUU.GAU at its summit (5, 6). Presumably, TMV protein has a heightened affinity for such a sequence although other features of sequence and secondary structure probably reinforce the binding. Heterologous reconstitution experiments have confirmed, however, that the specificity of assembly is not complete. Coat protein from TMV, vulgare strain, can distinguish between its own RNA and that of TMV strain U2, encapsidating the one but not the other. U2 coat protein, on the other hand, accepts RNA from either strain and, indeed, reassembles about twice as rapidly with vulgare strain RNA as with its own RNA (Fig. 1). U2 coat protein differs from vulgare coat protein at 38 positions. Two of these substitutions, Gly (85) →Asn and Ala (86)→Ser, occur in the proximal end of the right radial helix, a region of the coat protein subunit close to the RNA binding site (7). Conceivably, these changes may be responsible for the diminished specificity of U2 coat protein in reconstitution. Alternatively, the diminished specificity may be a consequence of changes in distant parts of the subunit which work their effect by slightly altering the configuration of the portion of the polypeptide chain which interacts with the RNA.

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