THE OCCURRENCE OF THE COAT PROTEIN MESSENGER RNA IN THE MINOR COMPONENTS OF TURNIP YELLOW MOSAIC VIRUS

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1. Introduction

Previous studies from this laboratory [1] showed that the infectivity of turnip yellow mosaic virus (TYMV) is confined to RNA molecules with a M_r of 1.9×10^6. These molecules resist heat treatment at 65°C [1] and solving in 98% formamide (unpublished results). This strongly suggests that the genome of TYMV consists of an uninterrupted RNA chain with a M_r of 1.9×10^6. In vitro translation of this RNA in a cell-free extract of wheat germ yields none or very little coat protein, which means that the coat protein cistron of the genome RNA is closed under our in vitro conditions. Very efficient synthesis of coat protein occurs, however, with a small RNA (M_r 240 000) which can be isolated from a virus preparation. A similar coat protein messenger was observed and described by Klein et al. [2]. Since this active messenger comprises about 1% of the unfractionated RNA, not all of the virus particles will contain the coat protein messenger.

TYMV preparations contain in addition to the main infectious component B_1 a number of minor nucleoprotein components (B_0, B_{00} and B_{000}), the origin and function of which are as yet unknown [3–7]. When fractionated in a CsCl density gradient B_1 can be partly converted to a B_2 particle with a higher buoyant density. A more thorough analysis by Mathews [8] revealed the presence of double bands for each of the five nucleoprotein components described above.

Considering this heterogeneity of TYMV the question arises in which of these components the coat protein messenger is present.

Isolation of the various components of TYMV with CsCl density gradient centrifugation and in vitro translation of the extracted RNAs in a cell-free extract of wheat germ revealed that none or very little coat protein is synthesized with the RNAs from the main components B_{1a} and B_{2a} (compare fig.1). Coat protein synthesis is found, however with RNA from particles with densities between that of B_{1a} and B_{2a} and higher than that of B_{2a}. The minor components B_{00} and B_{000} also contain the messenger RNA for the coat protein.

2. Materials and methods

TYMV (type strain) was grown on Chinese cabbage (Brassica pekinensis, var. Witkrop) and isolated according to the method of Dunn and Hitchborn [9]. The virus was stored at 4°C in 0.01 M Na acetate buffer, pH 6.0, in the presence of 10^{-3} M NaN_3. Isolation of TYMV-RNA and determination of the RNA concentration were as described elsewhere [10].

CsCl density gradient centrifugation was essentially as described by Matthews [8]. An appropriate amount of CsCl (Merck; reinst) was dissolved in 0.01 M Na acetate buffer, pH 6.0, and mixed with 0.5–1.0 ml of a virus solution in the same buffer, so that the desired density (usually 1.43 g/cc) was reached. Centrifugation was carried out at 30 000 rev./min for 48 h in the Spinco SW-41 rotor. No equilibrium for the CsCl is reached under these conditions (see figures).

After centrifugation the tubes were viewed and/or photographed in a darkened room under a beam of light and the gradients were pumped through a flow cell of the Gilford 2000 spectrophotometer and monitored continuously for absorbance at 260 nm.

Fractions of 5–10 drops were collected and the pooled fractions were dialyzed against 0.01 M Na acetate buffer, pH 6.0. The density of the CsCl solution was determined by refractometry.

In vitro translation of TYMV-RNA in the cell-free extract of wheat germ and the subsequent analysis of the biosynthetic products with SDS slab gel electrophoresis was performed as described previously [1].

3. Results

Figure 1a shows the distribution of the various components of TYMV after equilibrium centrifugation in a CsCl density gradient. Three main bands are seen representing the infectious component B_{1a}, its conversion product B_{2a} and the empty protein shell (NTC) floating at the top. All the components are designated according to the nomenclature of Matthews [8]. The nucleoprotein particles B_{000a}, B_{000b}, B_{1b} and B_{2b} can be observed either when the tubes are viewed in a darkened room under a beam of light or when the gradient is pumped through a flow cell of a spectrophotometer and the absorbance is continuously recorded (fig.1b). Fractions of ten drops were collected, pooled from six gradient tubes and dialyzed against low salt buffer. The nucleoprotein components were purified by a further 3 cycles of CsCl density centrifugation and the result of the last cycle is shown for 9 components in fig.2. We could not purify B_{1b} as yet, probably due to the presence of large amounts of B_{1a} and B_{2a} on both sides of this minor component. B_{2a} remains present in the B_{1a} preparation because of the continuous conversion of B_{1a} into B_{2a} (see also ref.[8]). B_{2b} is slightly contaminated with the less dense particle B_{2a} while all other minor components remained contaminated with more dense particles. B_{000a}

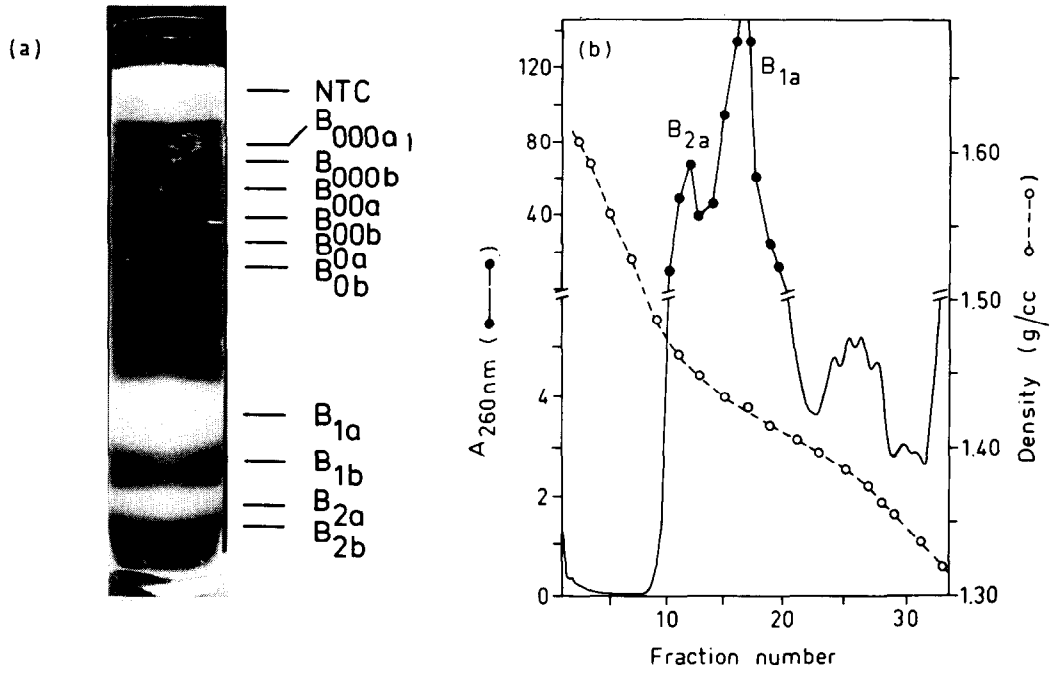

Fig.1. Cesium chloride density gradient analysis of a TYMV preparation. TYMV (25 mg) was solved in a CsCl solution (initial density 1.43 g/cc) buffered with 0.01 M Na acetate, pH 6.0. Centrifugation was carried out at 30 000 rev./min for 48 h at 5°C in the Spinco SW-41 rotor. (a) Photograph of a gradient tube viewed under a beam of light. The various components are designated according to the nomenclature of Matthews [8]. (b) Ultraviolet absorbance profile at 260 nm. The absorbance of the fractions around component B_{1a} and B_{2a} was determined separately after an appropriate dilution. (•———•) Absorbance at 260 nm; (○———○) CsCl density.

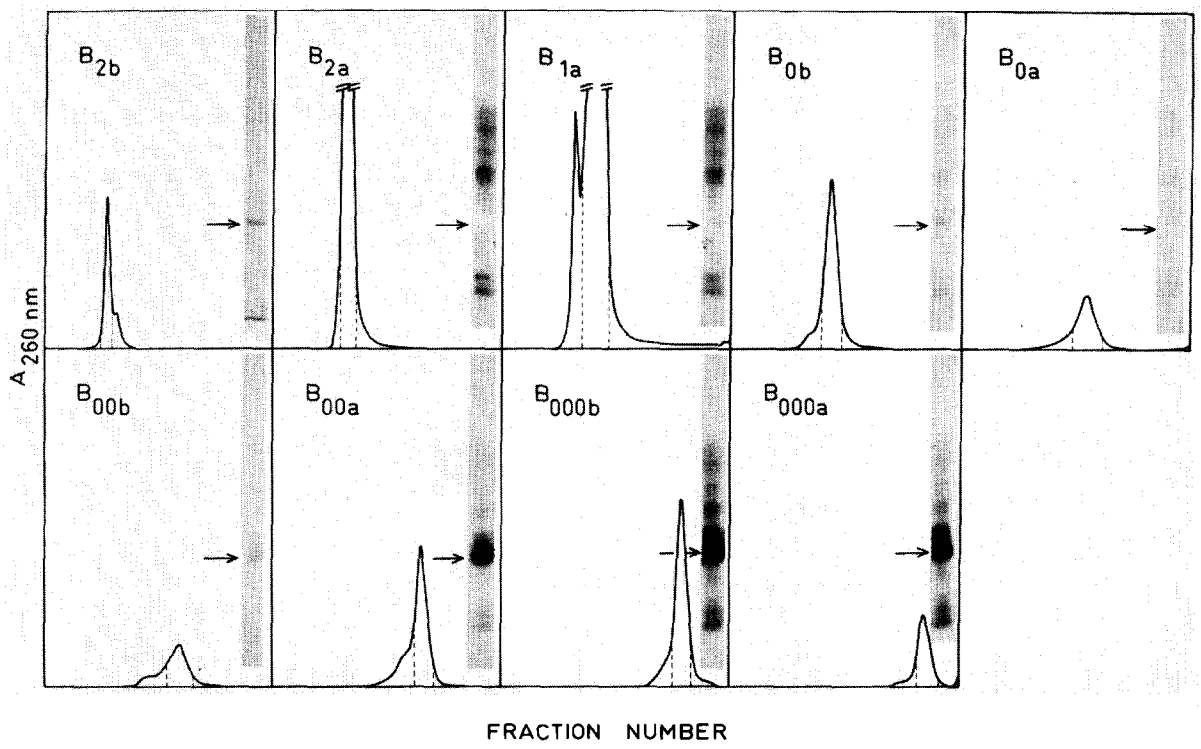

Fig.2. In vitro translation of RNA from purified components of TYMV. Purification was by four cycles of CsCl density gradient centrifugation. The UV absorbance patterns show the result for 9 components after the fourth cycle. The fractionation was as given by the dotted lines. Components B_{2b}, B_{2a} and B_{1a} were centrifuged in a CsCl solution with an initial density of 1.48 g/cc, whereas the six less dense particles were centrifuged in a CsCl solution of lower density (1.38 g/cc). The inserts represent the autoradiograms of the biosynthetic products after translation of each extracted RNA in the wheat germ cell-free system. Equal amounts (2.5 μg) were translated. The arrow shows the position of the coat protein of TYMV.

consisted of two very close bands when the tube was viewed by eye.

RNA was isolated from each component and translated in a cell-free extract of wheat germ. The autoradiograms of the biosynthetic products analyzed by SDS slab gel electrophoresis are shown in the insets of fig.2. It is clear that none or very little coat protein is synthesized with RNA from B_{1a} and B_{2a}, whereas B_{2b}-RNA yields coat protein as the sole product. Appreciable amounts of coat protein are found in the case of B_{00a} and both B_{000} particles, although surprisingly the same high molecular weight polypeptides (M_r 80 000–90 000) are synthesized as with B_{1a}- and B_{2a}- RNA.

A better separation of B_{1a} and B_{2a} is achieved when TYMV is centrifuged in a CsCl solution with a higher initial density (1.46–1.48 g/cc). Such an experiment also revealed the presence of a small amount of a component with a density higher than that of B_{2b}, which we here tentatively designate as B_{2c}. Fractionation of the gradient and translation of the extracted RNAs showed that maximal coat protein synthesis is found with fractions between B_{1a} and B_{2a} and also around B_{2b} (results not shown). This means that between B_{1a} and B_{2a} another nucleoprotein particle must be present (probably our observed B_{1b}) which contains the coat protein messenger RNA.

4. Discussion

The results described above demonstrate that the RNA isolated from the main nucleoprotein components of TYMV, B_{1a} and B_{2a} yields none or very little synthesis

of coat protein. This means that these virions do not contain the coat protein messenger RNA. Matthews [8] reported that B_{1a}, purified by three cycles of equilibrium centrifugation in CsCl gradients, is infectious which is in agreement with our observation [1] that the coat protein messenger is not needed for a successful infection. It would also imply that the coat protein messenger must originate from partial replication of genome RNA or from specific cleavage of the latter [1,11].

The RNAs from B_{1b} and B_{2b} which give rise to the synthesis of coat protein consist either of an aggregate of one genome RNA and one coat protein messenger or of an aggregate of the coat protein messenger alone. The former possibility would be in accord with the small increase in density of B_{1b} and B_{2b} of about 0.02 g/cc as compared to B_{1a} and B_{2a}, respectively. In that case one may also envision that B_{2b} is the conversion product of B_{1b}. The observation, however, that translation of B_{2b}-RNA yields coat protein as the sole biosynthetic product, argues for coat protein messenger being the sole species of RNA present in B_{2b}. Further studies are needed to substantiate this suggestion. If it is correct B_{2b} may be the source of choice for isolating pure coat protein messenger RNA.

Based on the buoyant densities, RNA contents of roughly 10% and 5% may be calculated for B_{00} and B_{000}, respectively [6], which would correspond with molecular weights of about 600 000 and 300 000 for the RNAs. Obviously, these RNA molecules would be too small in order to code proteins of 80 000–90 000 daltons. Contamination of B_{00} and B_{000} with RNA from more dense particles seems less likely to explain this phenomenon (compare fig.2) and a further characterization of these minor components seems therefore desirable.

It should be pointed out that although the results obtained by fractionating TYMV in CsCl gradients shows a rather close resemblance to those reported by Matthews [8] it is still uncertain whether the particles isolated by this authors were identical to ours. This is especially the case for components B_{1b} and B_{2b}.

It remains to be seen which biological significance can be ascribed to the occurrence of the coat protein messenger in the minor components of TYMV. The findings reported in this letter may contribute to the understanding of the nature of these components, the origin and function of which has remained unknown hitherto [7].

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