DOUBLING OF GENES IN THE RNA FRAGMENTS OF BARLEY STRIPE MOSAIC VIRUS

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1. Introduction

It has recently been demonstrated that barley stripe mosaic virus (BSMV) belongs to the group of viruses with functionally fragmented genome. Different strains of BSMV contain from two to four fragments of RNA of similar molecular weight [1,2]. The genome of the Russian strain of BSMV, contains two RNA fragments [3]. In this paper results are described suggesting that at least part of the genetic information in different RNA fragments of the Russian strain of BSMV is doubled. This conclusion has been inferrred from comparison of polypeptides synthesized upon the translation of isolated RNA fragments in a cell-free protein-synthesing system from wheat embryos.

2. Materials and methods

The Russian strain of BSMV [4] was used. RNA was isolated by phenol [5] or salt [4] deproteinization. The fragments of BSMV RNA were isolated from 3% polyacrylamide gel after electrophoresis and purified from contaminations by $CaCl_2$-precipitation [3]. Wheat embryos were obtained from wheat 'Mironovskaya 809' using the method of Johnston and Stern [6]. The fraction, containing ribosomes and other components of the protein-synthesing system (supernatant after centrifugation of embryos extract at 23 000 *g*, or S-23 fraction) was isolated in accordance with the method of Marcus [7]. The incubation mixture (0.25 ml) contained: 0.12 ml S-23 fraction, 25 mM Tris-acetate buffer, pH 8.0, 3.6 mM magnesium acetate, 50 mM KCl, 1 mM ATP, 0.2 mM GTP, 4 mM phosphoenolpyruvate, 4 μg pyruvate kinase, 2 mM dithiothreitol (Cleland's Reagent), 20 μCi of a mixture of [^{3}H]amino acids and 10 μg of RNA from BSMV (the optimal concentration of RNA was determined previously). The incubation was carried out for 45 min at 30°C. Then the mixture was supplemented with 6 M urea, 1% caseine hydrolyzate and 0.5% mercaptoethanol and incubated for 30 min at room temperature. All the treatments used thereafter were in general accordance with the procedure described by Shih and Kaesberg [8]. This was followed by electrophoresis in 10% polyacrylamide gel in 0.1 M sodium phosphate buffer with 1% SDS 16–18 h, the current being 3 mA per tube. The gels were cut into 1.1 mm disks. Only labelled peptides migrating more slowly than Bromophenol Blue were examined. The disks were placed for 16 h in 0.3% SDS. The radioactivity was assayed in a Nuclear Chicago Mark II scintillation counter. All other procedures used were exactly the same as described by us previously [9].

3. Results and discussion

Upon electrophoresis in polyacrylamide gel, RNA of the Russian strain of BSMV migrates as two zones, which were designated 1 and 2, in accordance with their electrophoretic mobility. It cannot be excluded that zone 2 contains not one but two types of RNA molecules of closely similar molecular weight (Dr L. C. Lane, personal communication). Individual fractions, RNA1 and RNA2 were isolated from the total preparation of BSMV RNA [3]. In re-electro-

phoresis RNA2 (mol. wt. approx. 1.35×10^6) migrated as a single homogeneous peak, whereas RNA1 (mol. wt. approx. 1.5×10^6) preparation contained an admixture (less than 20%) of RNA2 (or maybe the fragments of degraded RNA1).

In this work the products of translation of the following RNAs were compared: (1) a total preparation of virion RNAs of the Russian strain of BSMV (fig.1a), (2) and RNA2 fraction isolated from the total preparation of BSMV RNAs (fig.1b), (3) and RNA1 fraction isolated from the total preparation of BSMV RNAs (fig.1c), (4) an artificial BSMV RNA mixture reconstituted by mixing RNA1 and RNA2 fractions. Since RNA1 fraction contained contaminants (maybe RNA2), we believed it necessary to estimate the possible contribution of these contaminants to the translation of the RNA1 preparation. To this end, we analysed the products of the cell-free translation of an amount of RNA2 that might have been present as an admixture in the preparation of RNA1 in the incubation mixture (fig.1d).

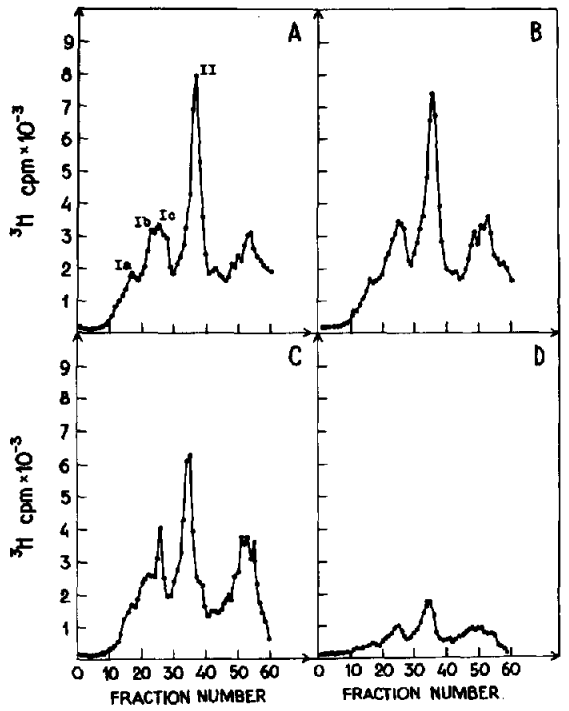

Fig.1. Radioactivity distribution in an electropherogram (SDS-polyacrylamide gel) of the polypeptides formed upon in vitro translation of BSMV RNAs: (A) total BSMV RNA (10 μg), (B) BSMV RNA2 (10 μg), (C) BSMV RNA1 (10 μg), (D) BSMV RNA2 (2 μg). For details see the text.

As a standard, RNA of bromegrass mosaic virus (BMV) was used since the products synthesized in the presence of this RNA in the cell-free system from wheat embryos had been well characterized previously [8]. The results we obtained with BMV RNA are in conformity with those of these authors, which testifies to our cell-free system having normal activity.

Previously it was demonstrated [9] that the products of translation of the total mixture of the BSMV RNA were: a BSMV coat protein (peak II) of a mol. wt. of 24 000 – 26 000 and also a set of polypeptides of a higher molecular weight (tentatively abbreviated as peaks Ia, Ib and Ic), which do not react with antiserum to BSMV (fig.1a). The polypeptides that are lighter than the coat protein (see fig.1a) are the products of the proteolytic degradation of the coat protein and, possibly, of some heavier proteins (manuscript in preparation).

In the cell-free protein-synthesizing system from wheat embryos, RNA1 and RNA2 stimulated incorporation of labelled amino acids into the polypeptides with almost the same efficiency as the total RNA mixture. In translation of RNA2, polypeptides were synthesized whose electrophoretic behaviour was similar to those of the products of translation of the total BSMV RNA (figs.1a and 1b). In the electropherogram shown in fig.1b, polypeptides can be seen with the mobility of equal the products 1a, 1b, 1c and II, shown in fig.1a. This result allows one to believe that RNA2 contains information for the synthesis of the coat protein and probably all the heavier polypeptides detected in translation of total BSMV RNA in vitro.

The profile of the translation products of RNA1 presented in fig.1c hardly differs from those of RNA2 and total preparation of BSMV RNAs (figs. 1b and 1a). RNA1 as well as RNA2 is likely to code for the virus coat protein and heavier BSMV-specific polypeptides.

Fig.1d shows the results of the experiments on translation of such an amount of RNA2 that may be present as an admixture in the preparation of RNA1 added to the cell-free system (i.e. 2 μg). The profile shown in fig.1d has the same pattern as that in fig.1b, and the low absolute content of the translation products allows one to assume that the admixture of RNA2 in the preparation of RNA1 does not con-

siderably affect the character of the distribution of products formed upon translation of RNA1. The set of products of translation of the artificial mixture of RNA1 and RNA2 taken in a ratio close to the natural one (1:6) was exactly the same as that of translation of the total BSMV RNA. This testifies to the fact that the preparations of RNA1 and RNA2 isolated by us had normal template activity.

Thus, in translation of RNA1 and RNA2 in a cell-free system from wheat embryos, no basic differences in the coding activity and specificity of these RNA species were revealed. Nevertheless, the presence of all the fragments of the BSMV genome is indispensable (in our case, RNA1 and RNA2) for preparations of BSMV RNA to display infectivity [1–3]. Thus, RNA1 and RNA2 are biologically unique, have different mol. wts, although their in vitro translation products do not reveal any considerable differences.

These observations suggest that the major part of the information in different fragments of BSMV genome must be doubled if one assumes that polypeptides 1 a–c are virus-specific proteins. If the suggestion about the doubling of the information is correct, several questions arise: (1) what distinguishes different fragments of the BSMV genome and makes their presence indispensable for infectivity to be expressed? (2) Are the genes in other strains of BSMV, containing three or four different RNA fragments, doubled? (3) What is the functional role of gene doubling?

References

[1] Jackson, A. O. and Brakke, M. K. (1973) Virology 55, 483–494.
[2] Lane, L. C. (1974) Virology 58, 323–333.
[3] Dolja, V. V., Negruk, V. I., Novikov, V. K. and Atabekov, J. G. (1976) in the press.
[4] Atabekov, J. G. and Novikov, V. K. (1966) Biokhimiya 31, 157–166.
[5] Fraenkel-Conrat, H., Singer, B. and Tsugita, A. (1961) Virology 14, 54–58.
[6] Johnston, F. B. and Stern, H. (1957) Nature 179, 160–161.
[7] Marcus, A., Efron, D. and Weeks, D. P. (1974) in: Methods in Enzymology, Nucleic Acids and Protein Synthesis (L. Grossman and K. Moldave, eds.), Academic Press, New York, 30, 749–754.
[8] Shih, D. S. and Kaesberg, P. (1973) Proc. Nat. Acad. Sci. USA 70, 1799–1803.
[9] Negruk, V. I., Novikov, V. K. and Atabekov, J. G. (1974) Proc. Acad, Sci. USSR 218, 489–493.