

## RAPID LABELLING OF A NONENCAPSULATED RNA OF BROMEGRASS MOSAIC VIRUS

G. PHILIPPS, C. GIGOT and L. HIRTH

*Groupe de Laboratoires de Strasbourg-Cronenbourg,  
Laboratoire des Applications Biologiques, 23, rue du Loess 67-Strasbourg 3  
and  
Institut de Botanique, 8, rue Goethe, Strasbourg, France*

Received 1 June 1972

### 1. Introduction

Recently Lane and Kaesberg [1] have observed that brome-grass mosaic virus (BMV) contains 5 types of RNA. The mixture of the three largest RNA's is infectious while the smallest RNA and a minor component seem to have no effect on infectivity. We report in this paper on a study of the incorporation of  $^{32}\text{P}$  into the different RNA's of BMV. We have found that the different RNA's are labelled and encapsulated at different rates.

### 2. Material and methods

BMV was inoculated to barley leaves (*Hordeum vulgare* var. Rika). The virus was prepared according to the method of Bockstahler and Kaesberg [2]. RNA was obtained after phenol extraction in the presence of bentonite and SDS. Total RNA of barley leaves was purified after phenol extraction by a method previously reported [3]. Each preparation of RNA was treated with DNAase at the concentration of 10  $\mu\text{g}/\text{ml}$  during 30 min at 20°.

Actinomycin D treatment was performed by dipping the cut end of the leaves in a solution containing 40  $\mu\text{g}/\text{ml}$  for 16 hr before labelling with  $^{32}\text{P}$ .

Polyacrylamide gel electrophoresis was performed according to the method of Loening [4]. Gels were prepared in plastic tubes of 10 mm of diameter. The solution contained a mixture of 2.4% acrylamide and

0.12% methylene bisacrylamide. The electrophoretic buffer contained 0.04 M Tris, 0.02 M Na acetate and 0.002 M EDTA at pH 7.8.

RNA was dissolved in this buffer containing 10% glycerol and layered on the gel after a pre-electrophoresis of 2 hr at 5 mA/gel at 4°. Electrophoresis was continued for 3 hr and gels were then transferred to a quartz tube and scanned in a modified Beckman DU spectrophotometer. After freezing the gels were sliced and the slices dried on filter-paper. After drying, the filter papers were put in a scintillation liquid and the radioactivity was directly determined.

The method of Peacock and Dingman [5] in which 0.5% agarose is added to the polyacrylamide was also used in some experiments. This method reduced the time of electrophoresis and avoided the freezing of the gels before slicing. In some cases the gels were stained with 0.05% toluidine blue; by this method the different RNA's produce blue bands in the gel.

### 3. Results

#### 3.1. $^{32}\text{P}$ labelling of the RNA of purified virus

Gel electrophoresis of a preparation of viral RNA shows the presence of four components: A, B, C and D (fig. 1). Component C is present in a very low quantity and appears as a shoulder in the diagram. After staining with toluidine blue, peak A appears as a doublet A-A'. We used such colored gels for molecular weight determination of components A and A'. *E. coli*

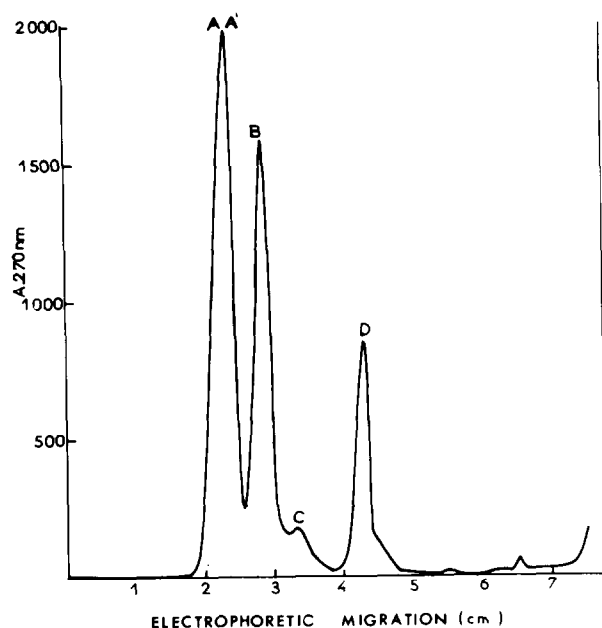


Fig. 1. Electrophoresis of BMV RNA's on a 2.4% polyacrylamide gel. Electrophoresis was performed at  $4^{\circ}$  for 2.5 hr at 5 mA per gel. After migration, gel was scanned at 270 nm with a modified Beckman DU spectrophotometer.

RNA's and TMV RNA were used as references for molecular weight determinations. We found the following values:  $A = 1.12 \times 10^6$ ;  $A' = 1.06 \times 10^6$ ;  $B = 0.73 \times 10^6$ ;  $C = 0.55 \times 10^6$ ;  $D = 0.32 \times 10^6$ . These values do not differ significantly from those determined by Lane and Kaesberg [1].

In order to study  $^{32}\text{P}$  incorporation into these different RNA's, barley leaves infected with BMV during 7 days were labelled for 1, 6 and 24 hr after a pretreatment of 16 hr with actinomycin D. After labelling the virus was purified according to the technique of Bockstahler and Kaesberg [2] and the RNA's were extracted from this purified preparation, and separated by gel electrophoresis. After a labelling of 1 hr, the radioactivity was found essentially in peak A—A' (fig. 2a). Component D was also significantly labelled. After 6 and 24 hr of incorporation, components AA', B and D were labelled, but never component C (fig. 2b—c). Specific activities of RNA's AA' and D were nearly the same and always higher than the specific activity of component B. Analogous results were reported by Hirucki [6] in the case of BMV and by Bancroft et al. [7] with CCMV.

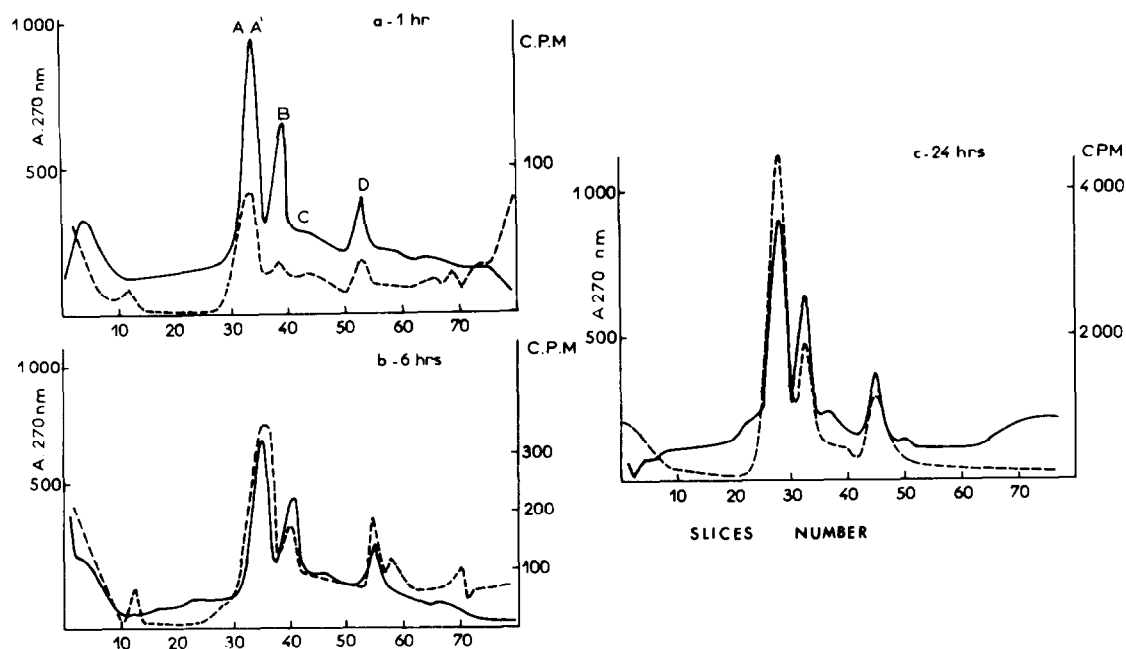


Fig. 2. Gel electrophoresis of labelled BMV RNA's. Barley leaves infected during 7 days were treated with actinomycin D and labelled with  $^{32}\text{P}$  during 1 hr (3a), 6 hr (3b) and 24 hr (3c). RNA's were extracted from purified labelled virus. After electrophoresis, gels were scanned at 270 nm, sliced and radioactivity of each disc determined as described in the text. (—): Absorbancy at 270 nm. (---):  $^{32}\text{P}$  counts per min.

### 3.2. $^{32}\text{P}$ labelling of total RNA of BMV infected barley leaves

A preparation of total RNA of infected leaves contains the 5 different viral RNA's and also the 5 plant ribosomal RNA's. Some of these RNA's having approximately the same MW, may not be separated by gel electrophoresis. Table 1 shows for example that components AA' cannot be separated from heavy chloroplastic r-RNA(ch<sub>1</sub>), component B from cytoplasmic light r-RNA(cy<sub>2</sub>) and component C from chloroplastic light r-RNA(ch<sub>2</sub>).

In order to distinguish radioactivity corresponding to viral RNA from radioactivity incorporated into r-RNA, actinomycin D treatment was used. Under our conditions (40  $\mu\text{g}/\text{ml}$  during 16 hr) actinomycin D inhibits 65% of  $^{32}\text{P}$  incorporation into r-RNA's after a labelling of 24 hr.

Table 1  
Comparative molecular weights of BMV RNA's and ribosomal RNA's from barley leaves.

| Molecular weights of ribosomal RNA's from barley leaves (according to Poulson and Beevers [8]) |                    | Molecular weights of BMV RNA's |                    |
|--|--------------------|--------------------------------|--------------------|
| Cy 1   | $1.3 \times 10^6$  | A                              | $1.12 \times 10^6$ |
| Ch 1   | $1.1 \times 10^6$  | A'                             | $1.06 \times 10^6$ |
| Cy 2   | $0.7 \times 10^6$  | B                              | $0.73 \times 10^6$ |
| Ch 2   | $0.56 \times 10^6$ | C                              | $0.55 \times 10^6$ |
| Ch 3   | $0.4 \times 10^6$  | D                              | $0.32 \times 10^6$ |

After a 1 hr  $^{32}\text{P}$  incorporation only the components A—A' and C were labelled (fig. 3a). The same profile was obtained after a 6 hr labelling (fig. 3b). When incorporation was performed during 24 hr most of the

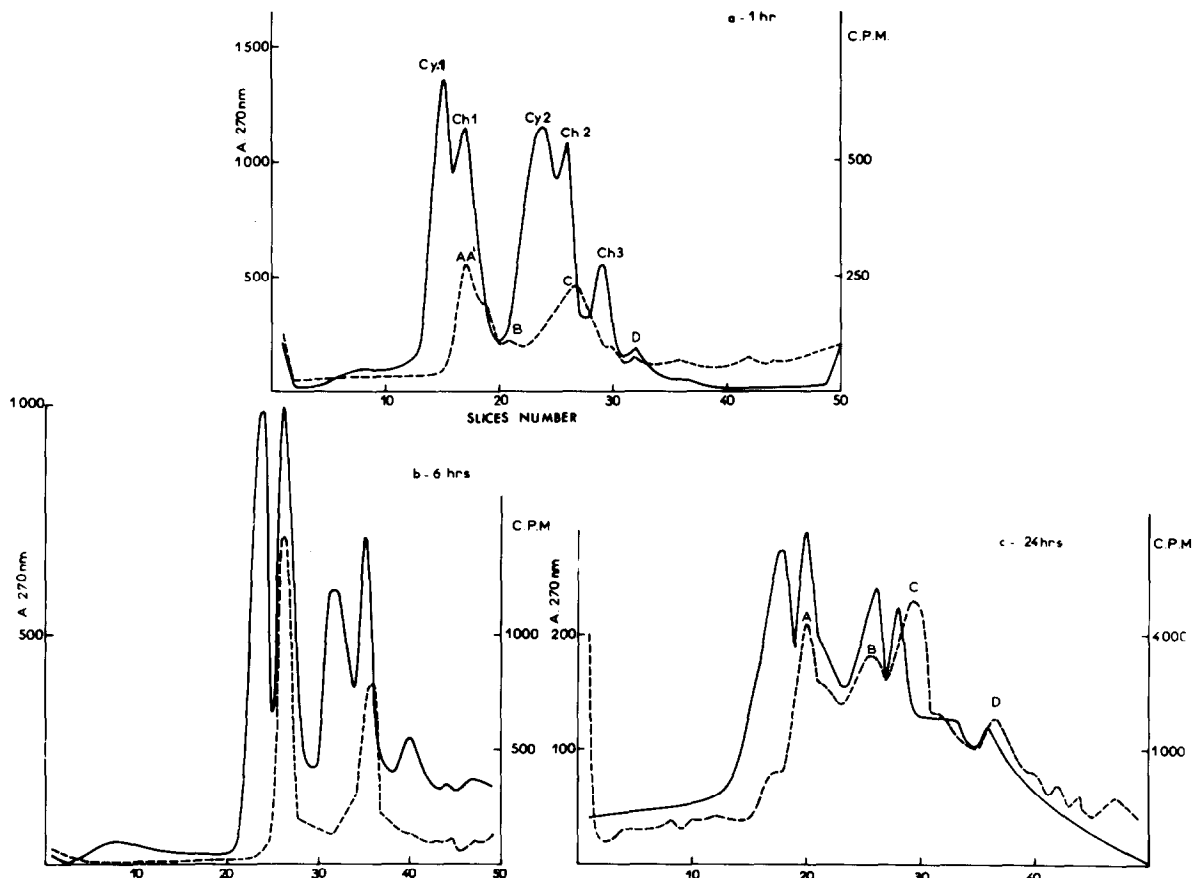


Fig. 3. Gel electrophoresis of total RNA from BMV infected barley leaves. Leaves infected during 7 days were treated with actinomycin D, labelled with  $^{32}\text{P}$  during 1 hr (4a), 6 hr (4b) and 24 hr (4c). After labelling total RNA's were extracted. (—): Absorbance at 270 nm. (---):  $^{32}\text{P}$  counts per min. Cy: cytoplasmic ribosomal RNA; Ch: chloroplastic ribosomal RNA.

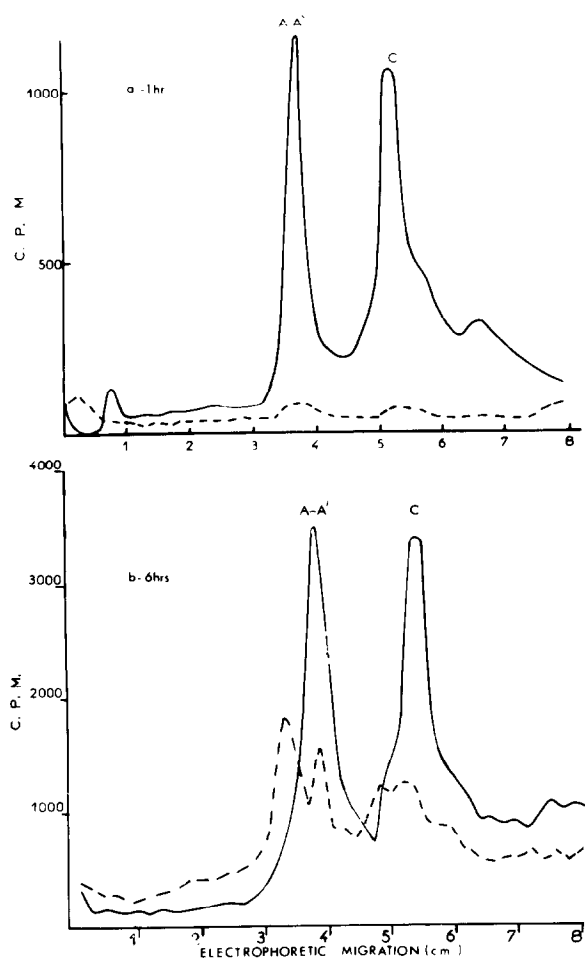


Fig. 4. Gel electrophoresis of total RNA from healthy and BMV infected barley leaves. Infected and healthy leaves were labelled during 1 and 6 hr with  $^{32}\text{P}$  without actinomycin D treatment. Figs. 4a and 5b compare  $^{32}\text{P}$  incorporation into RNA's from healthy (----) and infected (—) barley leaves after 1 and 6 hr labelling, respectively.

radioactivity was again detected in components AA' and C, but in this case components B and D were also significantly labelled (fig. 3c).

These results show that components AA' and C are rapidly and simultaneously labelled. It was possible to determine which one of these components was labelled first. In fact, even after a 15 min pulse, radioactivity was already detected in both.

In order to show whether specific  $^{32}\text{P}$  incorporation into AA' and C was not due to some effect of actinomycin D on plants, the same experiments were

repeated without this compound. In the case of barley,  $^{32}\text{P}$  was very slowly incorporated into ribosomal RNA's even without actinomycin D treatment. In contrast, viral RNA's were very rapidly labelled. Figs. (4a–b) show that after 1 and 6 hr labelling most of the radioactivity was again incorporated in components AA' and C of BMV.

#### 4. Discussion

The early results of Bockstahler and Kaesberg [9] suggested that the lightest RNA's of BMV resulted from specific breakdown of the heaviest one. On the contrary, a recent paper of Lane and Kaesberg [1] attributed specific biological functions to some of these components.

The study of  $^{32}\text{P}$  incorporation into viral RNA's shows that the different components are neither synthesized nor encapsulated at the same rate.

The experiment reported in the first paragraph shows that components AA' and D are more rapidly labelled or encapsulated than component B. The study of  $^{32}\text{P}$  incorporation into total RNA's shows that min component C is synthesized as rapidly as component AA'. Comparison of both shows that component AA' C and D are rapidly and first labelled, but RNA C is not encapsulated. Component B is synthesized more slowly.

Concerning component D, Hirucki [6] and Glitz and Eichler [10] assume that it results from a specific breakdown of component AA' or B or both. The differences between  $^{32}\text{P}$  incorporation into component B and D show that D may eventually only result from a breakdown of component A–A'. The same hypothesis could be made for component C. However, as opposed to RNA D, component C is encapsulated at an extremely slow rate, and it does not accumulate. These results suggest that this RNA could play the role of an early messenger at the beginning of the infection.

#### References

- [1] L.C. Lane and P. Kaesberg, *Nature New Biol.* 323 (1971) 40.
- [2] L.E. Bockstahler and P. Kaesberg, *Biophys. J.* 2 (1962)
- [3] C. Gigot, G. Philipps and L. Hirth, *J. Mol. Biol.* 35 (1968) 311.

- [4] U.E. Loening, *Biochem. J.* 113 (1969) 131.
- [5] A.C. Peacock and C.W. Dingman, *Biochemistry* 7 (1968) 668.
- [6] C. Hiruki, *J. Virol.* 3 (1969) 498.
- [7] J.B. Bancroft, E. Hiebert, M.W. Rees and R. Markham, *Virology* 34 (1968) 224.
- [8] R. Poulson and L. Beevers, *Plant Physiol.* 46 (1970) 315.
- [9] L.E. Bockstahler and P. Kaesberg, *J. Mol. Biol.* 13 (1965) 127.
- [10] D.G. Glitz and D. Eichler, *Biochim. Biophys. Acta* 238 (1971) 224.