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TRANSLATION OF INFECTIOUS BRONCHITIS VIRUS RNA

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1. Introduction

Infectious Bronchitis Virus (IBV) is a single-stranded RNA virus belonging to the coronavirus group. Although these viruses are important causative agents of animal and human diseases, their molecular biology is relatively unknown [1]. Recently several reports have appeared on the molecular weight and biological properties of coronavirus RNA. The molecular weight of a human coronavirus OC-43 was reported to be $6.1 \cdot 10^6$ [2] while that of IBV was $5.6 \cdot 10^6$ [3]. More recently, it was reported that IBV RNA is a single polynucleotide chain with a molecular weight of $8 \cdot 10^6$ daltons [4]. However, a major unresolved question is whether the coronaviruses are negative or positive strand viruses. A recent demonstration that the IBV RNA was infectious and polyadenylated [3–5] shows that it is a positive stranded virus. If so, then it should be translatable in a cell-free protein synthesizing system to yield virus polypeptides. This paper demonstrates that the RNA can indeed be translated in two different in vitro protein synthesizing systems into characterisable products.

2. Materials and Methods

IBV RNA was prepared from purified virions by the method of Lomniczi and Kennedy [5], and TMV virion RNA by the procedure of Marcus et al. [6]. Total cellular RNA was extracted from both IBV-infected and uninfected chick embryo kidney cells by an SDS/phenol method [7]. The micrococcal nuclease-treated rabbit reticulocyte lysate was prepared by the method that Pelham and Jackson described [8]. The technique of Roberts and Patterson [9] was used to make an in vitro protein synthesizing system from wheat germ. Conditions in the in vitro reaction mixtures have been reported elsewhere [8,10]. [^{35}S]-Methionine (650 Ci/mmol, obtained from the Radiochemical Centre, Amersham) was included in the reaction mixtures. Samples were assayed for radioactive incorporation into TCA-precipitable material by spotting 2 μl from the reaction mixture on 3 MM paper, boiling in a solution of 0.1% methionine in 5% TCA and treating as described previously [10]. Samples for analysis on polyacrylamide gel electrophoresis were processed by the published method [8]. IBV particles, labelled with [^{35}S]methionine, were grown and purified, as described by Lomniczi and Kennedy [5]. Polyacrylamide gel electrophoresis was performed under the conditions described [11].

3. Results and Discussion

In both the micrococcal nuclease-treated rabbit reticulocyte lysate system and the wheat germ system, addition of RNA extracted from the virions of IBV caused an increase in the amount of radioactivity incorporated into acid precipitable material (Table 1), although the virion RNA was not very active when compared with TMV RNA. The reasons determining the efficiency of translation of an RNA are not well understood, but in this case could be due to the large size of the IBV RNA, or because the IBV RNA has a lower affinity for ribosomes.

The products formed by in vitro translation of

TABLE 1

Translation of IBV RNA in vitro
The increase caused in acid precipitable radioactive material was measured when various RNAs were added to the in vitro systems. The total volume of the reticulocyte lysate system was 25 μl and contained 5 μCi of [^{35}S]methionine, while the wheat germ reaction mixture had a total volume of 20 μl and contained 10 μCi of [^{35}S]methionine.

Added RNA	Reticulocyte lysate system		Wheat germ system	
	μg/ml	cpm/2μl	μg/ml	cpm/2μl
none		1354		2400
IBV virion RNA	100	2277	120	41500
TMV	100	34800	250	272000
RNA from uninfected cells	400	2953	450	39000
RNA from infected cells	400	4133	450	74000

IBV RNA were analysed by polyacrylamide gel electrophoresis. When IBV virion RNA was added to the reticulocyte system only two bands, of molecular weight 44 000 and 55 000 could be detected (Fig. 1c). The larger of these co-migrated with a major capsid protein. On the other hand, multiple protein bands were found in the product formed in the wheat germ system, and none of these co-migrated with any of the virus capsid proteins (Fig. 2a).

When RNA extracted from IBV-infected and uninfected chick embryo kidney cells was added to the reticulocyte system there was a stimulation of methionine incorporation (Table 1) and a whole spectrum of polypeptides could be found in the product (Fig. 1a). Their molecular weights ranged from 20 000 to 200 000. Only one difference can be seen when the products from the system programmed with RNA from uninfected cells (Fig. 1b) were compared with the products from the system programmed by RNA from IBV infected cells (Fig. 1c). That difference is the major product of the system programmed with IBV infected cell RNA. It has a molecular weight of 55 000, co-migrates with one of the bands formed by translation of the virion RNA and is also present in the IBV capsid. We concluded that both virion RNA and intracellular RNA from infected cells were being translated to yield a virus structural protein. However,

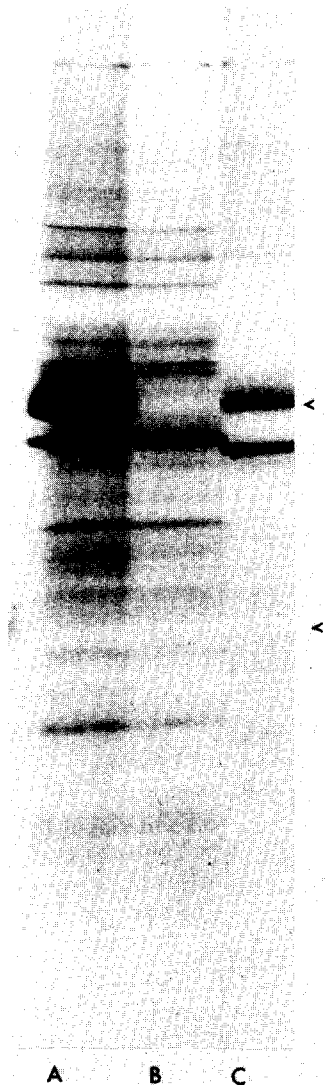

Fig. 1. Incubation mixtures from the reticulocyte lysate system (Table 1) which had been programmed with different RNAs were analysed by polyacrylamide gel electrophoresis. The arrows mark the position of the IBV virion proteins. Track A: RNA from IBV infected cells; Track B: RNA from uninfected cells; Track C; IBV virion RNA.

no trace could be detected of the smaller, 44 000 molecular weight product, which was also formed by the virion RNA in this system.

When the products formed in the wheat germ synthesis by IBV infected cellular RNA were compared with those formed by uninfected cellular RNA, two new bands could be identified, with molecular weights

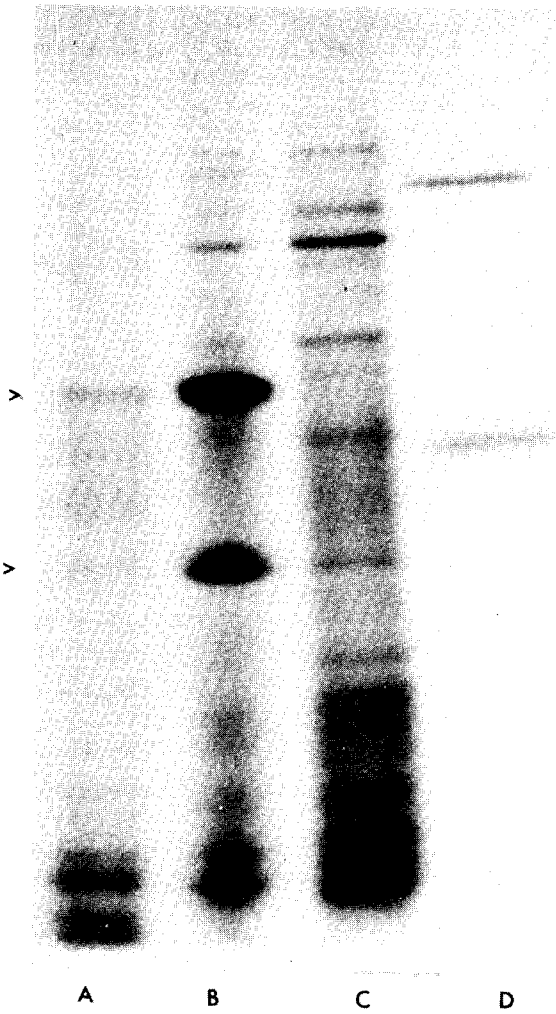

Fig. 2. Incubation mixtures from the wheat germ system (Table 1) which had been programmed by different RNAs were analysed by polyacrylamide gel electrophoresis. The arrows mark the position of the IBV specific products formed in vitro. Track A. IBV virion RNA; Track B: RNA from IBV infected cells; Track C: RNA from uninfected cells; Track D: [^{35}S]methionine-labelled IBV virion proteins.

of 22 000 and 35 000. Both of these peptides were also present in the products found in the wheat germ system when it was stimulated by IBV virion RNA, but neither was found in the virus particle.

It is clear from these results that IBV virion RNA can be translated into characterisable products, one of which is probably a virus structural protein. It must therefore be positive-stranded. In agreement with this conclusion, Schochetman et al. [3] and Lomniczi [4] have demonstrated that the RNA is infectious and that the virion does not contain any transcriptase activity (Lomniczi, unpublished results). These results clearly establish the place of IBV as a positive-stranded virus. That is, it is a member of class IV of Baltimore's classification [12].

The two different in vitro protein-synthesizing systems behave differently. In the reticulocyte system one of the products formed by the virion RNA appears to have the same molecular weight as one of the virion proteins. This product is also found when RNA from infected cells was used to stimulate the system, suggesting that is is adjacent to an initiation site. The indentity of the other product is not known. However, in the wheat germ system smaller products are made both by the virion RNA and the infected cell RNA. This could be due to premature termination, which has been reported to occur in this system [13]. However, it is a property of IBV RNA, since both influenza virus mRNA and TMV RNA are translated in this system into the correct full length products (data not shown). Addition of tRNS extracted from rabbit reticulocytes or from the wheat germ did not alter the spectrum of the products formed (Highfield and Stephenson, unpublished results).

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