TRANSLATION OF PROTEINS ACCOUNTING FOR THE FULL CODING CAPACITY OF THE SEMLIKI FOREST VIRUS 42 S RNA GENOME

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1. Introduction

Cell-free translation has been widely used to define the proteins encoded by, for example, viral messenger RNAs. In cells infected with alphaviruses (e.g., Semliki Forest and Sindbis viruses) there are two major viral RNA species, the genomic 42 S RNA (mol. wt 4–4.5 × 10^6) and the intracellular 26 S RNA (mol. wt 1.6 × 10^6) [1,2], which is a copy of the 3′ third of the 42 S RNA [3,4]. The 26 S RNA has been shown by cell-free translation to encode the structural proteins of the virion, which are synthesised as a polyprotein, p130 (mol. wt 130 000) [5–9]. This leaves about 300 000 daltons of the potential coding capacity of the 42 S RNA unaccounted for.

Using a temperature-sensitive mutant of Semliki Forest virus (ts-1), nonstructural (ns) proteins have been identified. Two precursor proteins, ns155 and ns135 (mol. wt 155 000 and 135 000, respectively) are translated sequentially, probably as a polyprotein, and cleaved to give four, more stable nonstructural proteins [10,11]. Corresponding proteins and their precursors have subsequently been detected in wild type virus infected cells [12]. If the mutant induced nonstructural precursor proteins, ns155 and ns135, have different amino acid sequences, and are in fact virus coded, they, together with the structural polyprotein, p130, would occupy virtually the full coding capacity of the SFV genome. Here we have compared the tryptic peptides derived from the mutant-induced proteins and those from the cell-free translation product directed by the wild type 42 S RNA. The results show ns155, ns135 and p130 to be distinct entities, and further that ns155 and at least part of ns135 are translated from wild type 42 S RNA in vitro.

2. Materials and methods

Growth and purification of SFV and isolation of virion 42 S RNA have been described [8]. Conditions for cell-free protein synthesis using a wheat germ extract were identical to those used earlier [13,14]. Addition of exogenous wild type 42 S RNA to the reaction resulted in a 10–20-fold increase in hot acid insoluble [^{35}S]methionine radioactivity. The labelling and purification of large amounts of proteins from SFV ts-mutant infected cells by discontinuous sodium dodecyl sulphate–polyacrylamide gel electrophoresis, and preparation of these proteins for tryptic peptide analysis is detailed elsewhere [9,11]. The cell-free protein-synthesising reactions were processed as described [8,15]. Conditions for tryptic digestion and high voltage paper elecrophoresis were as reported [11]. [^{35}S]Methionine was the radioactive label used throughout this study.

3. Results and discussion

The short-lived precursor proteins, ns155 and ns135 which have been identified previously [10], were isolated from cells infected with our SFV mutant, ts-1,

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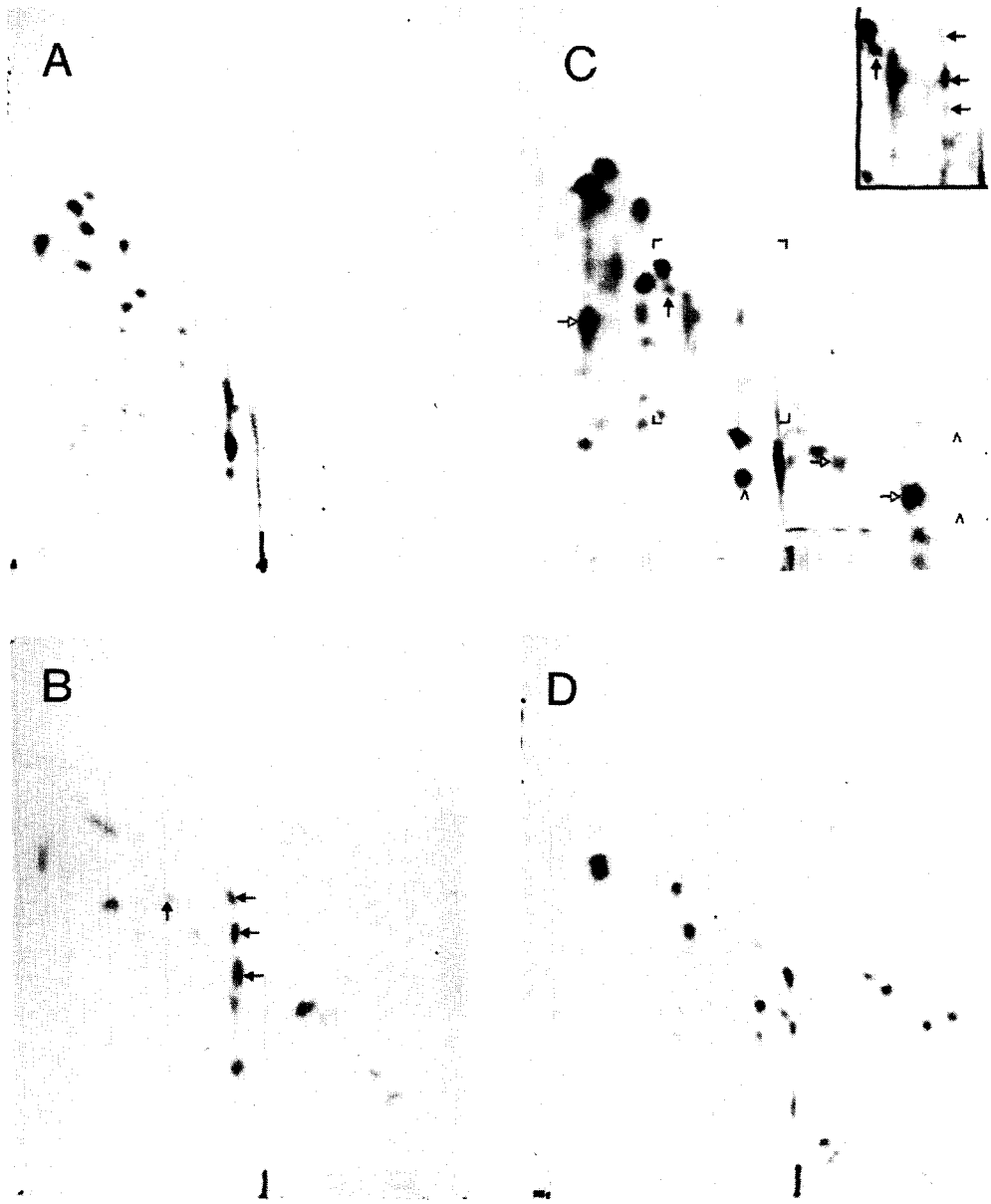

Fig.1. Tryptic peptide maps of [^{35}S]methionine containing peptides derived from SFV ts-mutant induced proteins and a cell-free protein synthesising system programmed with wild type 42 S RNA: (A) ns155 and (B) ns135 were prepared from cells infected with ts-1. (C) Peptides derived from the 42 S RNA directed in vitro product after precipitation with TCA. (D) p130 from cells infected with ts-3. Electrophoresis was on Whatman 3 MM paper, in the first dimension (horizontal) at pH 6.5, for 120 min at 40 V/cm; origin near middle, cathode at left. Electrophoresis in the second dimension (upwards) was at pH 3.5 for 90 min at 60 V/cm, cathode at top. Autoradiography was for 21 days, (A), (B) and (D), or 10 days, (C). Longer exposure (19 days) of the central part of the map indicated in (C) is shown in the inset. (——▶) Peptides, ns135-derived, detected in the in vitro product. (——▷) Possible 'lead-in' peptides found only in the in vitro product (see text). (∧) Peptides also detected in control cell-free incubations (with no added RNA).

by polyacrylamide gel electrophoresis, eluted from the gel, and digested with trypsin. The tryptic peptide maps obtained from these [^{35}S]methionine labelled proteins by high voltage paper electrophoresis are shown in fig.1(A) and (B). They are clearly different, showing the proteins to have essentially different primary structures. For comparison the structural polyprotein, p130, which has been shown to contain the peptides of the viral capsid and envelope proteins [9,16], was isolated from cells infected with another SFV mutant (ts-3) and similarly analysed. The map is shown in fig.1(D) and can be seen to be quite different from those derived from ns155 and ns135. These three proteins thus appear to be distinct entities with little or no overlapping of amino acid sequences. Since the structural proteins are encoded by the 26 S RNA [5,6,8], it follows that if the nonstructural proteins are virus coded, they must be translated from that part of the 42 S RNA which is not duplicated in the 26 S RNA.

To test this, the wild type 42 S RNA was translated in a cell-free protein synthesising system. We selected conditions used previously, under which initiation of translation on the 42 S RNA started at a single site, which was different from that on the 26 S RNA [14]. The tryptic peptide map derived from the whole protein synthesising reaction programmed with 42 S RNA is shown in fig.1(C). The map is strikingly similar to that derived from ns155 (fig.1(A)). Detailed comparison revealed that all the major peptides detected in ns155 were also present in the in vitro product, although their intensities were different. By increasing the autoradiographic exposure period, some more, but not all, of the peptides of ns135 could also be detected in the in vitro product (fig.1(C), inset). Peptides present in the control cell-free incubation (with no added mRNA) and so presumably due to the endogenous synthesis of the system, are indicated.

These results confirm that ns155 and most probably also ns135 are encoded by the virion 42 S RNA. The two nonstructural precursor proteins, together with the structural polyprotein p130, thus appear to represent translation of virtually the full coding capacity of the SFV 42 S RNA genome. Our inability to detect all the peptides of ns135 in the 42 S RNA directed in vitro product is most simply explained if ns155 and ns135 are, as proposed [10], the initial cleavage products of a giant polyprotein (mol. wt approx. 290 000). Persistent difficulty has been experienced in obtaining full translation of high molecular weight proteins in vitro [17–19]. The low intensities of some, and absence of other ns135 derived tryptic peptides would reflect the failure of the ribosomes in this cell-free system to translate the giant polyprotein fully. Conversely, the high intensities of certain other peptides in the in vitro product (indicated by (——▷) in fig.1(C), also reported in [8]), which lack counter-parts in ns155 and ns135 suggests that they may be proximal to the site for initiation of translation. They may represent a 'lead-in' sequence similar to that proposed for encephalomyocarditis virus [20], but more direct evidence for this possibility is required.

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