A tentative model of formation of structural proteins of tick-borne encephalitis virus (flavivirus)

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The time course of tick-borne encephalitis virus cell-free protein synthesis was studied by using either [^{35}S]-methionine or formyl[^{35}S]methionyl-tRNA$_f^{Met}$ as substrates, and the [^{35}S]methionine-labelled products were compared by fingerprinting tryptic peptides. An intermediate in the protein processing, the polypeptide doublet p36/33, was characterized and a tentative model for flavivirus structural protein synthesis and processing was proposed.

Flavivirus protein *Cell-free protein synthesis* *Processing* *Formyl[^{35}S]methionyl-tRNA$_f^{Met}$*

1. INTRODUCTION

Eukaryotic viruses with a unique single-stranded RNA genome of positive polarity have 2 main options to produce a set of virus-specific proteins: these may be generated by either proteolytic processing of high-molecular-mass precursors or translation of different mRNA species, including subgenomic ones, that are formed upon infection. The mode of protein synthesis used by a large group of such viruses, flaviviruses, is essentially unknown. Neither large polypeptide precursors nor subgenomic mRNAs have been detected in the flavivirus-infected cells [1]. Our approach to the problem consisted in studying protein synthesis directed in vitro by the genome of the tick-borne encephalitis virus (TBEV), a flavivirus. We had shown previously that at least 2 of the 3 known TBEV structural polypeptides, C (p13) and E (p53), could be formed in a cell-free system, that polypeptide C, compared to polypeptide E, is located more proximally to the translation initiation site and that generation of the latter (and possibly of the former) is accomplished through membrane-dependent processing of a precursor [2–4].

The more detailed study of the mode of generation of TBEV structural proteins reported here allowed us to identify some intermediates involved and to propose a tentative model for this process.

2. MATERIALS AND METHODS

The viral RNA was isolated from purified TBEV preparations [5] and translated in extracts from Krebs-2 cells in the presence of [^{35}S]methionine or formyl[^{35}S]methionyl-tRNA$_f^{Met}$ under conditions specified previously [3], except that concentrations of salts were modified so as to minimize accumulation of unfinished polypeptide chains [4].

Virus-specific polypeptides accumulating in the infected pig embryonal kidney cells were labelled with [^{35}S]methionine from 32 to 36 h postinfection under the conditions known to preferentially inhibit host cell protein synthesis [5].

Electrophoresis was performed on 15% polyacrylamide gel slabs in the presence of SDS [6]. For fingerprinting tryptic peptides method B [3] was utilized, but the concentration of TPCK-treated trypsin (Serva) was increased to 200 μg/ml, which resulted in a more simple set of peptides.

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3. RESULTS AND DISCUSSION

Our previous results suggested that p53 and p13 are formed by processing a common precursor; there were also some hints that a polypeptide with an M_r value of approx. 36000 may be an intermediate in this process [3]. To gain further insight into the mode of TBEV structural protein synthesis we have performed a more detailed study of the time course of product accumulation in the TBEV RNA-programmed extracts from Krebs-2 cells incubated in the presence of either [^{35}S]-methionine or formyl-[^{35}S]methionyl-tRNA$_f^{Met}$. The conditions used were favorable for preferential accumulation of the viral structural polypeptides. After different periods of incubation, aliquots were withdrawn from a cell-free sample, and portions of these were either fixed immediately for electrophoresis or, prior to fixation, incubated in the presence of cycloheximide ('chased') to detect possible posttranslational modifications.

The results of these experiments (fig.1) demonstrated several points. In accord with our previous observations [3], the formylmethionine label was not incorporated into p53, whereas both p13 and the p36/33 doublet were labelled by either of the substrates used. It is noteworthy that the time course of changes in the amount of the formylmethionine-labelled polypeptides appeared to mimic faithfully that of their methionine-labelled counterparts. Polypeptide p53 was produced prior to the appearance of p13 and its amount did not seem to change during the chase. The doublet p36/33 was an early product and its concentration decreased both in 'pulse' samples with time and during the chase. Polypeptide p13, a late product, accumulated in parallel with the decrease in p36/33 and primarily during the chase.

These observations allowed us to propose a tentative scheme for generation of structural proteins of TBEV (fig.2). According to the scheme, a common translation unit exists for the structural polypeptides p13 (C) and p53 (E). We shall designate the hypothetical precursor polypeptide corresponding to this translation unit as prS (for precursor of structural proteins). In prS, the amino acid sequences of p13 and p53 occupy the N-terminal and C-terminal positions, respectively. Since there is a considerable lag between the first appearances of p13 and p53, we assume that the

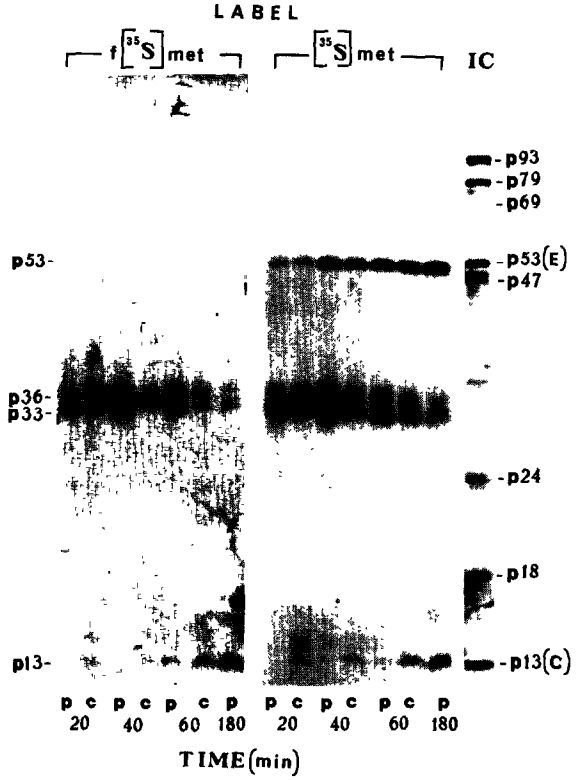

Fig.1. Time course of accumulation of polypeptides in the TBEV RNA-programmed Krebs-2 extracts, using either formyl[^{35}S]methionyl-tRNA$_f^{Met}$ or [^{35}S]methionine as substrates. At the times indicated, aliquots, 20 μl, were withdrawn from the sample translating TBEV RNA, and they were divided into 2 portions, one of which (p, pulse) was fixed immediately, whereas the other (c, chase) was further incubated in the presence of cycloheximide, 200 μg/ml. The total incubation time for the chased samples was 3 h. The electrophoretic pattern of intracellular TBEV-specific polypeptides is also shown (IC).

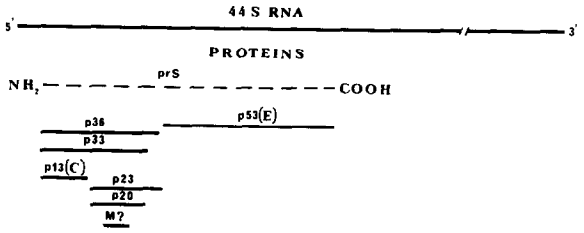

Fig.2. A tentative model of generation of TBEV structural proteins from a precursor.

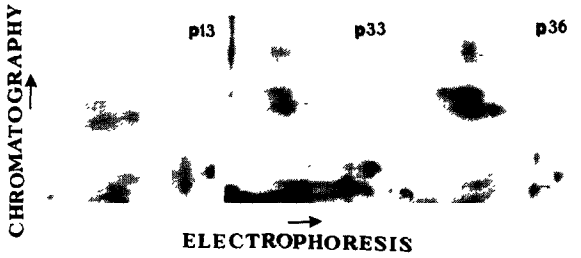

Fig.3. Fingerprints of the tryptic peptides from proteins p13, p33 and p36.

former is generated from a secondary precursor that is formed upon cleavage of p53 from prS. We propose that this precursor is identical or closely related to p36/33. This was confirmed by tryptic peptide analysis: the oligopeptides derived from p13 constituted a subset of oligopeptides derived from p36 or p33 (fig.3). Thus, the minimal estimate of the size of prS is about 90 kDa (53 + 36 kDa). The cleavage of the boundary between the amino acid sequences of p36 and p53 requires interaction of the nascent prS with membranes [4]. The cleavage itself takes place soon after interaction of the unfinished precursor with membranes, which explains why intact prS cannot be normally detected. When ribosomes completed reading the genes for structural proteins, p53 is generated without any delay. On the other hand, p36/33 is processed more slowly into its N-terminal and C-terminal fragments. The former corresponds to p13, whereas the latter should be represented by a polypeptide(s) with an M_r value of approx. 20000–23000. Although such a polypeptide could not be discerned in the radioautograph presented in fig.1 we have repeatedly observed low amounts of a doublet p23/20 in other experiments (not shown). We suggest that this doublet is unstable and undergoes rapid degradation. It is tempting to assume that the third structural protein of TBEV, the low-molecular-mass polypeptide M or (V1), which is found in virions but not inside the infected cell, is formed as a result of processing p23/20.

The model proposed here differs markedly from that suggested for Kunjin virus on the basis of UV-inactivation of the synthesis of individual virus-specific proteins [8], but it is in good agreement with the recently determined primary structures of the genomes of two other flaviviruses, yellow fever virus [9] and West Nile virus [10,11].

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