THE POST-TRANSLATIONAL PROCESSING OF SEMLIKI FOREST VIRUS STRUCTURAL POLYPEPTIDES IN PUROMYCIN TREATED CELLS

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1. Introduction

Semliki Forest virus (SFV), a member of the alpha virus group, contains four structural proteins. One of them, the C-protein (mol. wt. approx. 33 000), is a constituent of the nucleocapsid and the other three, E_1 (mol. wt. 49 000), E_2 (mol. wt. 52 000) and E_3 (mol. wt. approx. 10 000), are glycoproteins located in the virus membrane which surrounds the nucleocapsid [1]. It appears now well established that all four structural proteins are formed from a common precursor, p-130, by post-translational cleavage [2]. The C-protein seems to be located at the N-terminal and E_1 at the C-terminal end of the precursor [3–5] (fig.1). The only intermediate of the cleavage process which is relatively stable is the p-62 which yields E_2 and E_3 in a process which appears to be coupled to the final steps in the assembly of the virion [1,6]. Some other intermediates can be isolated by using amino acid analogues, inhibitors of proteolysis, or temperature sensitive mutants [6,7]. The details of the cleavage process are not known.

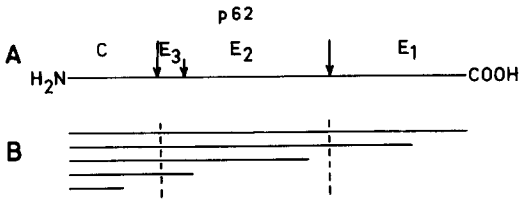

Fig.1. (A) The gene order of the structural proteins of SFV (Data from [3–5]). (B) A schematic representation of the set of released nascent polypeptide-puromycin molecules assumed to arise in the experimental conditions used. The dotted lines show the primary cleavage sites.

In the present communication an experiment is described which was designed to answer the question whether cleavage can occur at the junctions C–p-62 and p-62–E_1 on the nascent polypeptide chain, or whether most of the p-130 polypeptide must be completed prior to processing. Puromycin was used to release nascent chains from the polysomes as puromycin-peptides [8]. The concentration of puromycin was kept sufficiently low, to allow synthesis of long peptides and even completed chains. The appearance of the mature virion proteins under these conditions may yield information on the processing of the precursor (c.f. fig.1).

The results suggest that cleavage can occur between C and p-62 soon after the completion of the C-protein sequence, while E_1 must be almost completed before cleavage between p-62 and E_1 takes place.

2. Materials and methods

HeLa cells in monolayer cultures and the prototype strain of Semliki Forest virus were cultivated as described previously [9]. Labelling of virus specific polypeptides was performed by incubating the infected cells in methionine-free Eagles minimum essential medium containing 50 μCi of [^{35}S]methionine (200 Ci/mmole, Amersham) per 60 mm petri dish. Puromycin (Nutritional Biochemicals) was added to the desired concentrations 15 min prior to the isotope, and the same concentration was maintained until the cells were harvested. Whole cell extracts were prepared as described by Keränen and Kääriäinen [6]. For the determination of total [^{35}S]methionine incorporation aliquots were taken into 10% trichloroacetic acid,

heated to 90°C for 20 min and the precipitates collected on nitrocellulose filters and counted. Samples for electrophoretic analysis were treated with sodium dodecyl sulphate and mercaptoethanol at 90°C for 3 min and analyzed on 7.5% polyacrylamide gels as described [9].

3. Results and discussion

Monolayer cultures of HeLa cells were infected with SFV. 5 h after the infection the cells were exposed to different concentrations of puromycin. After a 15 min incubation period [^{35}S]methionine was added, and 15 min later unlabelled methionine to a final concentration of 1 mM. After an additional 5 min incubation period the cells were washed and whole cell extracts prepared. As expected, increasing concentrations of puromycin resulted in decreasing amounts of acid precipitable ^{35}S activity in the extracts (fig.2).

The samples obtained with 0–20 μg/ml of puromycin were then analyzed for their content of distinct virus-specific polypeptide species. Because E_1 and E_2 are difficult to resolve from each other on polyacrylamide gels, especially for quantitative purposes, a relatively short pulse was used to assure that most of the labelled E_2 was present in its precursor form p-62 [6]. The gel electrophoresis pattern of the control sample (fig.3A) shows prominent peaks of C, E (mostly E_1) and p-62 together with some p-97, a protein which contains tryptic peptides from E_1 and E_2 [5]. With increasing concentrations of puromycin a relative increase in the 'background' activity over the entire gel is observed (fig.3). The viral polypeptides and especially the C-protein could, however, be detected even if the relative abundance of the viral polypeptides was changed considerably. Their ratios were calculated from samples in which the background was not too high, i.e. up to 5 μg/ml of puromycin.

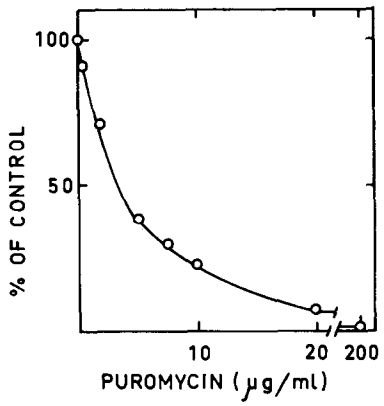

Fig.2. The inhibition of protein synthesis in SFV infected HeLa cells by puromycin. The HeLa cells were exposed to [^{35}S]methionine in the presence of varying concentrations of puromycin as described and the incorporation of ^{35}S into acid insoluble material determined.

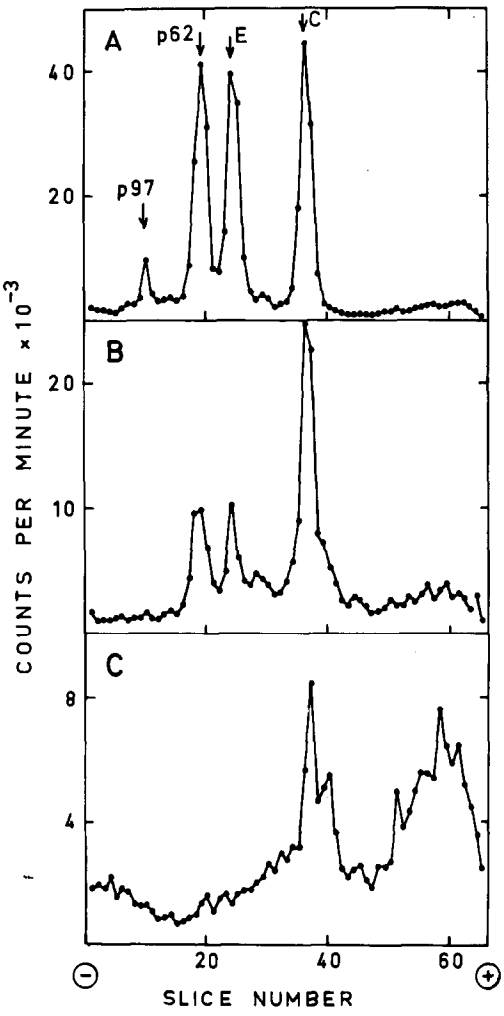

Fig.3. Infected HeLa cells were labelled with [^{35}S]methionine in the presence of (A) 0 μg/ml; (B) 2 μg/ml; (C) 10 μg/ml puromycin. Whole cell extracts were prepared and subjected to analysis on polyacrylamide gels. The viral polypeptide species are indicated by arrows; E is the position of E_1 and E_2 which are not separated in these conditions.

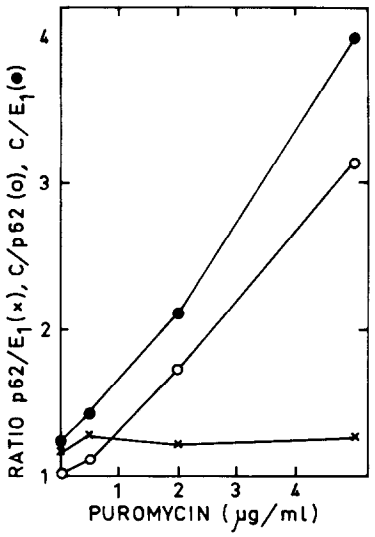

Fig.4. Samples obtained after labelling with [^{35}S]methionine in the presence of different concentrations of puromycin were analyzed on polyacrylamide gels. The ratios of p-62/E_1 (×), C/p-62 (o) and C/E_1 (•) were calculated and are presented as the function of the puromycin concentration.

Fig.4 shows that the ratios of C to E_1 and p-62 were greatly increased by puromycin treatment, while the E_1 to p-62 ratio varied only slightly.

The roughly constant p-62 to E_1 ratio suggests that whenever a p-62 molecule was released from the precursor the E_1 chain was also almost completed. C-protein could apparently be formed without a concomitant formation of the other polypeptides, i.e. it could be cleaved from the nascent precursor polypeptide before termination.

The above results were obtained in puromycin treated cells so the conditions differ distinctly from the normal. Thus the conclusions must be considered with care. It should, however, be pointed out that a cleavage scheme with 'nascent' cleavage between C and p-62 and a 'completed chain' cleavage between p-62 and E_1 appears reasonable from a biological viewpoint. The C-protein is rapidly associated with the viral RNA in the formation of the nucleocapsids [9], while the three membrane proteins are inserted into cellular membranes and glycosylated in the biogenesis of the sites on the host cell plasma membrane through which the nucleocapsids bud in the final maturation step [10,11]. In addition the kinetics of C-protein formation after synchronous initiation [4] support this scheme, and recent data by Kaluza [12] suggest that some glycosylation of a common envelope protein precursor must occur prior to further processing.

References

[1] Garoff, H. K., Simons, K. and Renkonen, O. (1974) Virology 61, 493–502.
[2] Clegg, J. C. S. and Kennedy, S. I. T. (1975) J. Mol. Biol. 97, 401–411.
[3] Clegg, J. C. S. (1975) Nature 254, 454–455.
[4] Clegg, J. C. S. and Kennedy, S. I. T. (1975) INSERM 47, 255–258.
[5] Lachmi, B.-E., Glanville, N., Keränen, S. and Kääriäinen, L., J. Virol., in the press.
[6] Keränen, S. and Kääriäinen, L. (1975) J. Virol. 16, 388–396.
[7] Morser, M. J. and Burke, D. C. (1974) J. Gen. Virol. 22, 395–409.
[8] Allen, D. W. and Zamecnik, P. C. (1962) Biochim. Biophys. Acta 55, 865–874.
[9] Söderlund, H. (1973) Intervirology 1, 354–361.
[10] Garoff, H. and Simons, K. (1974) Proc. Nat. Acad. Sci. USA 71, 3988–3992.
[11] Lenard, J. and Compans, R. W. (1974) Biochim. Biophys. Acta 344, 51–94.
[12] Kaluza, G. (1975) J. Virol. 16, 602–612.