Synthesis of Reovirus Structural Proteins (34361)

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It was previously shown that the structural proteins of the double-stranded RNA containing reoviruses could be resolved by polyacrylamide gel electrophoresis into three major (I, II, IIIb) and four minor components (m1, m2, m3, and IIIa) (1). Furthermore, it was determined that brief treatment of the complete virion with urea selectively removes the outer capsid and the resulting subviral particle (SVP) is composed of at least two protein components (I and IIIa). The present investigation employed the technique of polyacrylamide gel electrophoresis to inquire into the time course of synthesis of the individual protein components of the reovirus particle.

Materials and Methods. The procedures for the preparation of HeLa cell monolayers and for highly purified reovirus type 2 (D-5 Jones) or radioactive reovirus have been described (1, 2). Cytoplasmic fractions were obtained from HeLa cells treated for 15 min with hypotonic RSB (10^{-2} *M* Tris pH 7.4, 10^{-2} *M* NaCl and 1.5×10^{-3} *M* $MgCl_2$) and disrupted in a Dounce homogenizer (20 strokes) in such a manner that over 90% of the cells, but less than 1–2% of the nuclei, were broken. The nuclei were removed by centrifugation at 3000 rpm for 10 min and the cytoplasmic fraction was frozen until used.

Both purified virion preparations and cytoplasmic fractions were solubilized in 2% sodium dodecylsulfate (SDS), 1% 2-mercaptoethanol (2-ME) and 8 *M* urea and prepared for electrophoresis as described previously (1).

The procedures for the preparation of polyacrylamide gels (10% and the conditions of electrophoresis and staining have been described (1). Gels containing radioactive proteins were frozen, sliced, solubilized in H_2O_2, and counted (3).

Two approaches were employed to determin the time course of structural viral protein synthesis: (A) Examination of the proteins from progeny virus. In these experiments the analysis of structural viral proteins was restricted to an examination of those viral protein molecules which were actually incorporated into mature virus. Briefly, infected HeLa cultures were pulse-labeled (2 hr) with reconstituted ^{3}H-labeled protein hydrolysate at various stages of the infection cycle and then virus multiplication was allowed to proceed to completion in unlabeled medium. At the end of the infection period the viral yield was isolated, purified, and the amount of label in each of the structural protein components was measured after polyacrylamide gel electrophoresis. (B) Examination of the proteins of infected cytoplasmic fractions. In this type of analysis cells infected with reovirus were pulse-labeled (30 min) with reconstituted ^{3}H-labeled protein hydrolysate at various stages of the infection cycle. At the end of the labeling period the cells were harvested and the proteins of the cytoplasmic fraction analyzed by polyacrylamide gel electrophoresis.

In both kinds of experimental approaches the antibiotic actinomycin D (0.05 μg/ml) was present throughout the infection cycle in order to inhibit host but not viral RNA synthesis (4, 5). In procedure B the cell cultures were pretreated for 2 hr with 0.1 μg/ml of the antibiotic in order to reduce cellular metabolic activities further.

Results and Discussion. Figure 1 shows the time course of the synthesis of the sum of all the structural viral proteins which are incorporated into progeny virions. Synthesis of structural viral proteins was detected as early as 2 hr and proceeded at a rapid rate until 8–10 hr after infection. At this time protein synthesis occurred at a decreasing

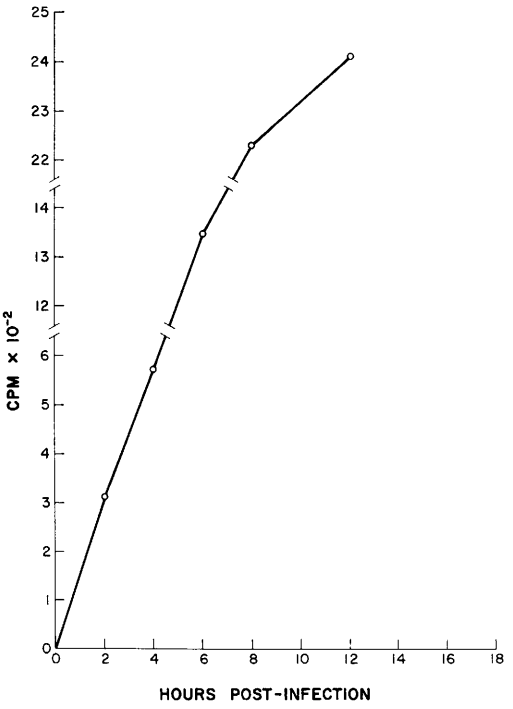

FIG. 1. Rate of formation of proteins incorporated into reovirus. Infected HeLa cells were pulse-labeled with a reconstituted mixture of 3H-labeled amino acids for 2-hr periods at various times after infection. After 30 hr, the virus yield from each culture was purified and prepared for polyacrylamide gel electrophoresis. Each interval represents the total amount of radioactivity present in various structural components of the virion.

rate and reached a maximum at 12 hr. Although not shown, small amounts of viral proteins continued to be synthesized and incorporated into virions even at 18 hr after infection.

The electrophoretic profiles at the solubilized proteins from virus yields derived by procedure A indicated that during the interval from 0 to 2 hr, structural viral proteins, comprised of major components I, II and minor component IIIa, were synthesized (Fig. 2). Major component IIIb was not synthesized until the interval between 2 to 4 hr after infection. The synthesis of the minor components was not seen at the early periods, but small amounts of minor components 2 and 3 were made during the intervals from 2 to 4 hr and 4 to 6 hr, respectively. It was often difficult to consistently resolve by the present method the synthesis of minor proteins, particularly minor component 1. By 6–8 hr all of the viral structural components were formed. The profiles of each of the major components and minor component IIIa are summarized in Fig. 3 where the amount of label incorporated during each 2-hr pulse period is plotted against time after infection. It appears that the structural proteins of reoviruses were made at different periods of the infection cycle and at different rates. Although components I, II, and IIIa were made early, both components I and IIIa reached their peak rate of synthesis at 8 hr, after which they began to decline. In contrast, component II continued to be synthesized at a rapid rate even at 12 hr after infection. The synthesis of component IIIb was not detected until 4 hr and its rate of synthesis

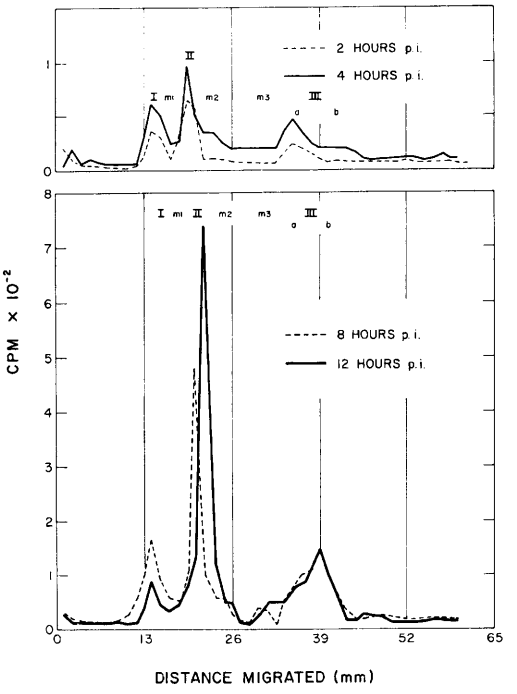

FIG. 2. Polyacrylamide gel electropherograms of dissociated proteins from viral progeny derived from HeLa cells, pulse-labeled for 2-hr periods with a reconstituted mixture of 3H-labeled amino acids at the times indicated. The various components were identified by their unique position and by coelectrophoresis with virions labeled with 14C-amino acids.

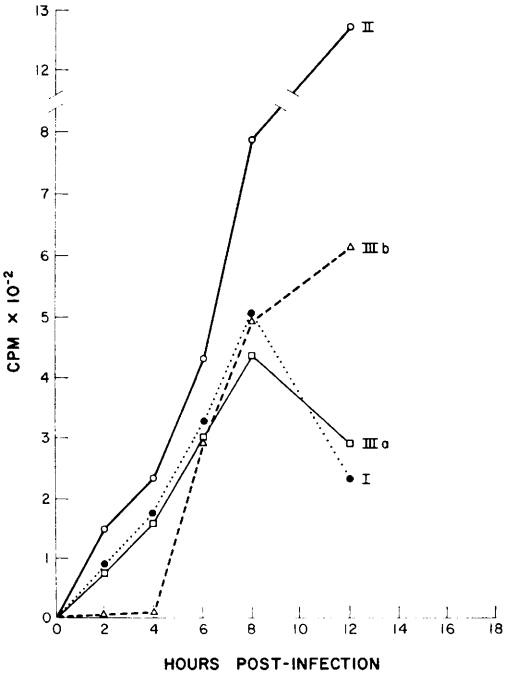

FIG. 3. Rate of formation of individual structural viral components. The amount of radioactivity in each viral component is computed from Fig. 2 and expressed here as the amount of label incorporated during each 2-hr pulse period at various times after infection. Only 4 of the viral components are shown since the minor components were difficult to resolve.

remained high even at 12 hr after infection. The rates of synthesis of both components II and IIIb decline by 18 hr postinfection.

The electrophoretic profiles of the proteins from infected cytoplasmic extracts obtained by procedure B are shown in Fig. 4. It was apparent that structural viral components I, II, and minor component IIIa were made as early as 1 hr after infection. Major component IIIb was synthesized later and together with the minor proteins were clearly seen at 5 hr after infection. Although at least 6 peaks other than structural components or cellular proteins were seen, many of these peaks were not present by 9 hr after infection. At this time, only the 7 structural viral components were seen. The identities and relationships of these "new" proteins in the synthesis of the virion remain to be established. The protein profiles from uninfected cytoplasmic extracts of 1 and 9 hr cultures remained essentially unaltered.

The amount of structural viral proteins synthesized in the infected cytoplasm is shown in Table I. Although the amount of label incorporated into total protein increased after 1 hr and remained essentially unaltered during the infection period, the proportion of structural proteins made increased with time: 32% at 1 hr after infection; 53% at 5 hr; and 73% at 9 hr. The amount of label incorporated into normal cytoplasmic extracts at 1 hr was slightly reduced by 9 hr.

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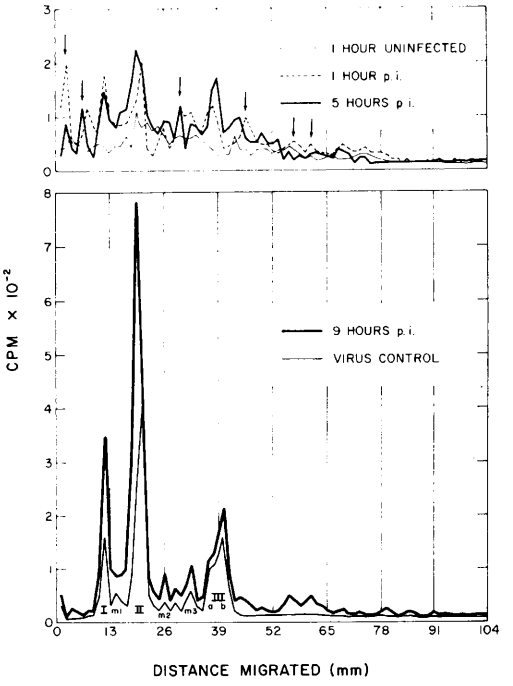

FIG. 4. Incorporation of ^{3}H-labeled amino acids into cytoplasmic proteins during 30-min pulse periods at various times after infection. Replicate cultures of HeLa cells were pretreated with 0.1 μg /ml of actinomycin D for 2 hr and then sham-infected or infected at an input multiplicity of 10. At the end of the adsorption period fresh medium containing 0.05 μg/ml of actinomycin D was introduced and at the times indicated the cell cultures were pulse-labeled for 30 min with a complete mixture of ^{3}H-labeled amino acids. Cytoplasmic fractions were prepared, solubilized, and electrophoresed on 10% polyacrylamide gels as described in the text. To facilitate identification of the viral components, each sample was coelectrophoresed with solubilized whole virus labeled with 14C-amino acids. (↓) = new peaks.

TABLE I. Protein Synthesis in Reovirus-Infected HeLa Cells.[a]

Time after infection (hr)	Total protein[b] (cpm)	Viral structural[c] protein [cpm; (%)]
1	3316	—
1 + virus	4482	1426 (31.8)
5 + virus	4373	2296 (52.5)
9	2831	—
9 + virus	4694	3410 (72.7)

[a] Cytoplasmic fractions were prepared from both infected and noninfected HeLa cells which were pulse-labeled for 30 min at the times indicated with a reconstituted mixture of ^{3}H-labeled amino acids. The cytoplasmic fractions were degraded in 2% SDS, 1% 2-ME and 8 *M* urea and subsequently electrophoresed in 10% polyacrylamide gels at 4 mA/gel for 24 hr (1). After electrophoresis the gels were frozen, sliced, solubilized in H_2O_2, and counted (3).

[b] Represents total radioactivity in the gel.

[c] Represents radioactivity present in the various structural components of the virion. The viral components were identified by coelectrophoresis with purified reovirus labeled with ^{14}C-labeled amino acids.

shown that double-stranded reovirus RNA is made as early as 4–5 hr after infection. In addition, the production of infectious virions is not detected until 5 hr after infection and is completed by 20 hr (2, 6). The present data indicate that except for major component IIIb, an outer capsid structure, most of the other protein components are made early after infection. It is interesting to note that major component II, an early protein, is one of the outer capsid proteins. Furthermore, two of the early structures, major component I and minor component IIIa represent the proteins composing the SVP. The decline in rate of synthesis of both of these proteins after 8 hr, when the rate of synthesis of other viral proteins increases, strongly suggests that a shutoff control may be in operation. However, this remains to be proved.

Summary. A study of the synthesis of structural viral proteins revealed that the majority of the major proteins were synthesized early during the infectious cycle. An exception was major component IIIb which was not made until 4 hr after infection. Analysis of the "soluble" supernatants from the cytoplasm of infected cells revealed several new nonstructural viral components.

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