INTRACELLULAR DISTRIBUTION OF POLIOVIRUS INDUCED RNA-DEPENDENT RNA POLYMERASE (REPLICASE) IN DETROIT-6 CELLS DURING THE REPLICATION CYCLE

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Infection of cultured mammalian cells with small spherical RNA viruses is known to induce the formation of an RNA-dependent RNA polymerase (replicase) (Baltimore et al., 1963; Baltimore and Franklin, 1963). Most of the enzyme is found in large particulate cytoplasmic structures (Baltimore and Franklin, 1963; Baltimore, 1964; Holland and Bassett, 1964).

Cells relatively late in the replication cycle, i.e. about 4 hours after infection, were used in all these studies. Studies by Bellett and Burness (1963) and by Levy (1961) on the intracellular distribution of new infectious material early in the replication cycle, indicate a more rapid increase in virus titre in the nuclei than in the cytoplasm. This is also found in studies carried out in this laboratory.

The present investigation deals with the kinetics of formation of the replicase in nuclei and cytoplasm of poliovirus type 3 infected Detroit-6 cells. The enzyme is first detectable in the nucleus, and about 30 minutes later in the cytoplasm. Infectivity follows the same pattern during the first 45 minutes of increase in virus titre. Later in the replication cy-

cle most of both replicase and infectivity is located in the cytoplasm.

Methods. Detroit-6 cells were grown in monolayer bottles using essentially Eagle's medium with 10 per cent calf serum (Eagle, 1955). The day before the experiments were to be carried out, the cells were transferred to spinner flasks. Cell samples taken between 2 and 6 hours after infection were sedimented and stored frozen for maximally 20 hours before use. Homogenization was accomplished by suspending the cells in 0.32 M sucrose (Dingman and Sporn, 1962) in a Potter-Elvehjem device and using a teflon plunger at 5000 rpm for 2 times $2\frac{1}{2}$ minutes. Precipitated nuclei and a few whole cells were washed once by resuspending in sucrose solution and sedimenting once more at 800 g for 5 minutes. Purification of nuclei was obtained by sedimentation through 2.39 M sucrose at 50,000 g for 2 hours.

The method of Baltimore et al. (1963) with some minor modification was used for assay of the replicase. (Table 1). Protein was determined by the biuret test (Gornall, 1949) and measurements of radioactivity were carried out in a Frieseke-Hoepfner thin window gas flow counter.

Purity of the nuclei was checked by RNA and DNA determinations (Brown, 1946; Burton, 1956). For measurements of infectivity aliquotes of the nuclear and cytoplasmic fractions were frozen and thawed 3 times and then plaqued on agar covered Detroit cell monolayers.

Results and discussion. In most of the experiments nuclei which were sedimented from the cell homogenate and washed once in 0.32 M sucrose were used. Such crude nuclei had an RNA/DNA of about 1:1, as compared with about 3:1 for whole

cells. Nuclei sedimented through 2.39 M sucrose (table 2) had a ratio of about 1:2.

The cytoplasmic fraction did not contain measurable amounts of DNA which means that very few nuclei were broken during homogenization of the cells.

Table 1

DISTRIBUTION OF REPLICASE ACTIVITY AT DIFFERENT HOURS AFTER INFECTION

	Hours after infection			
	2½	3	4½	5½
Nuclear activity per 10^8 cells	250	505	740	550
Cytoplasmic activity per 10^8 cells	30	132	338	1130
CPM/mg protein in nucleus	30	33	94	100
CPM/mg protein in cytoplasm	3	5	41	71
Nuclear activity/cytopl. activity	8.3	3.8	2.2	0.45
Nuclear sp. act./cytoplasmic sp. act.	10.0	6.6	2.3	1.4

The assay mixture contained 2.5 micrograms of actinomycin, 20 micrograms of creatine phosphokinase, 5 micromoles of phosphocreatine, 5 micromoles of magnesium acetate, 30 micromoles tris-HCL buffer pH 8.1, 0.2 ml enzyme suspension, 12.5 m micromoles of GTP-8-C^{14} (4380 CPM/m micromole) and 25 m micromoles of ATP, UTP and CTP. Final volume 0.45 ml.

Incubation took place for 15 minutes at 37°C. The samples were chilled on ice and 0.5 ml of cold 0.1 M sodium pyrophosphate and 5 ml of 0.5 M perchloric acid were added. After centrifugation the precipitate was washed 3 times with cold 0.5 M perchloric acid and once with ether-ethanol (1:1). The final precipitate was dissolved in 1.5 ml of formic acid, plated, dried under an infrared lamp and counted.

Table 1 presents results taken from 4 different experiments where the whole cell mass is harvested at the indicated hours after infection. The ratios between nuclear and cytoplasmic replicase activities decrease rapidly from about 2½ hours

after infection, when the first increase in enzyme activity is measurable, to 5½ hours after infection when maximum intracellular virus titre is obtained (Fig 1). Furthermore, a maximum in nuclear enzyme level is observed about 4½ hours after infection, while the cytoplasmic values continue to increase.

In order to study more detailed the alterations in nuclear and cytoplasmic replicase activity, an experiment was designed in which samples were taken from an infected culture every 15 minutes during the period of maximum increase of enzyme activity.

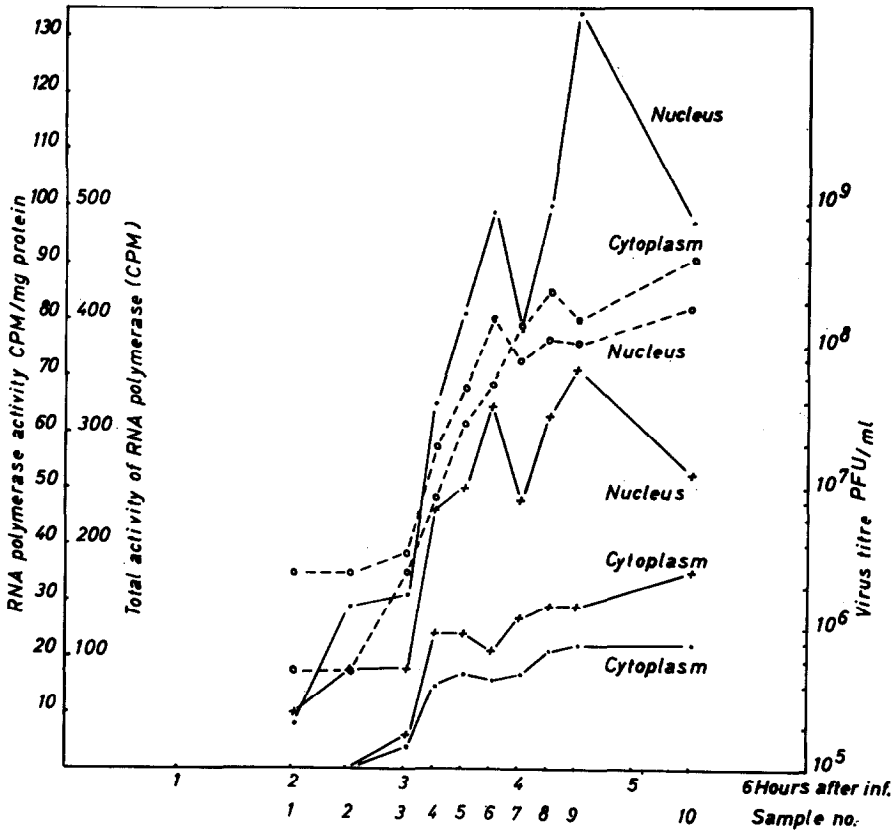

Figure 1. Kinetics of appearance of replicase and infectious material in the nuclear and cytoplasmic fractions of poliovirus infected Detroit cells.
•—•—• Sp. act of replicase. +—+—+ Total activity of replicase o--o Virus titre.

The appearance of enzyme starts 30 minutes earlier in the nucleus than in the cytoplasm and the amount of enzyme increases rapidly during the next hour, reaching a maximum 4½ hours after infection, while the cytoplasmic activity during the recorded period increases at an almost constant rate. The infectivity curves of the 2 fractions reveal a high initial increase in virus titre in the nucleus, but about 4 hours after infection most of the infectivity is found in the cytoplasm, and 5½ hours after infection 2/3 of the virus is accumulated there.

The results indicate that the replicase is synthesized in the nucleus and starts replicating virus RNA there. Our infectivity titrations measure intact virus, showing that the protein coat also is provided by the nucleus in the early phase of the replication cycle. Later, however, a steadily increasing fraction of the enzyme is found in the cytoplasm indicating a migration of the enzyme, and the bulk of the infectious material is synthesized in the cytoplasmic fraction.

There is no difference in the patterns obtained with crude and purified nuclei, indicating that the early activity measured in the nucleus is not due to cytoplasmic contaminations.

About 60 per cent of the activity was lost during purification of the nuclei, and the specific activity decreased more than the total activity. This observation strengthens our concept of the enzyme as a very mobile protein, tending to leave the nucleus shortly after synthesis and accumulate in the cytoplasm.

Attempts made to activate the replicase (Eason et al., 1963), by priming of the nuclear enzyme in the early phase of

TABLE 2

REPLICASE ACTIVITY IN NUCLEI SEDIMENTED THROUGH 2.39 M SUCROSE SOLUTION

	Hours after infection	
	3½	4½
CPM/mg protein in nuclei	18	33
CPM/mg protein in cytoplasm	11	41
Nuclear activity per 10^8 cells	460	300
Cytoplasmic activity per 10^8 cells	210	340

The nuclei were sedimented from the homogenate, washed once in 0.32 M sucrose solution and then sedimented through 2.39 M sucrose at 50,000 g for 2 hours. This procedure gives nuclei with a RNA/DNA of appr. 1:2. The nuclear pellet was suspended in a small volume of water and treated gently with a Dounce homogenizer. The suspension was then used as enzyme solution without further treatment.

the replication cycle with poliovirus RNA (PVRNA) have so far been unsuccessful. There is, however, reason to believe that template RNA i.e., either RNA complementary to PVRNA or double-stranded RNA (Baltimore et al., 1964), is a limiting factor at this phase of the replication, and that the replicase activity should be sensitive to the presence of a suitable primer

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