Inhibition of 80S Initiation Complex Formation by Infection with Poliovirus

(Accepted 12 December 1978)

SUMMARY

Anisomycin has been shown to stabilize ribosome initiation complexes containing messenger RNA and met-tRNA\textsuperscript{met} to high salt conditions. Extracts from HeLa cells treated with $5 \times 10^{-7}$ M-anisomycin for 15 min accumulate 80S initiation complexes which can be detected by their absorbance in sucrose gradients. Poliovirus-infected cells fail to form the 80S initiation complex early after infection, when inhibition of host cell protein synthesis occurs. These complexes re-form later in infection after virus RNA is synthesized. No re-formation occurs in the absence of virus replication. Thus, the step in protein synthesis inhibited by poliovirus precedes the entry of components into the 80S initiation complex.

Infection of cells with poliovirus causes a rapid and marked inhibition of cellular protein synthesis (Penman et al. 1963). The inhibited step has been narrowed down to the initiation events of protein synthesis (Leibowitz & Penman, 1971), although the precise reaction which is blocked has not been identified. Recent reports from other laboratories have clarified the temporal sequence of reactions required for initiation of protein synthesis and have identified the initiation factor requirements for these steps (Schreier et al. 1977; Trachsel et al. 1977; Benne & Hershey, 1978). Our analyses of the initiation of protein synthesis by extracts from poliovirus-infected HeLa cells performed in vitro have demonstrated that a crude initiation factor preparation from infected cells contains a messenger RNA (mRNA) discriminatory factor which is totally inactive in stimulating the initiation of protein synthesis directed by cellular mRNA, but which retains activity for stimulating translation of virus mRNA (Helentjaris & Ehrenfeld, 1978). On this basis, we suggest that host cell 'shut-off' occurs at a step in the initiation reaction which involves recognition of mRNA or of an initiation complex which contains mRNA.

In experiments described in this report, we have attempted to examine the 80S initiation complex, containing mRNA and met-tRNA\textsuperscript{met}, to determine if its formation is inhibited under conditions of virus-induced host cell shut-off. We used the drug anisomycin, which can stabilize the 80S initiation complex to high salt (0.5 M-NaCl) conditions which would dissociate ribosome complexes not containing mRNA and initiator tRNA (Lodish & Rose, 1977), to measure the amount of 80S initiation complexes in the cell. We show here that the formation of 80S initiation complexes containing cellular mRNA was inhibited by poliovirus infection and that 80S initiation complexes containing virus mRNA re-formed after synthesis of virus mRNA synthesis. We conclude that the step in protein synthesis inhibited by poliovirus precedes the entry of components into the 80S initiation complex.

Cytoplasmic extracts of HeLa cells normally contain a significant fraction of 80S ribosomes, which can be visualized by velocity sedimentation in a sucrose density gradient. However, under high salt conditions (0.5 M-NaCl, 0.03 M-Mg acetate), the absorbance profile of these extracts shows that the majority of the monosomes were dissociated into 40S and 60S ribosomal subunits. When cells were treated with $5 \times 10^{-7}$ M-anisomycin for 15 min prior to harvesting the preparation of cytoplasmic extracts, a portion of their 80S ribosomes were rendered resistant to dissociation by high salt. Fig. 1(a) shows the absor-
Fig. 1. Velocity sedimentation analysis in high salt buffer of cytoplasmic extracts from cells treated with anisomycin. 2.5 x 10⁷ HeLa cells were concentrated to 5 x 10⁶/ml and incubated with (b) or without (a) 5 x 10⁻⁷ M-anisomycin for 15 min at 37 °C. The cells were collected by centrifugation, suspended in 1 ml of 0.05 M-tris-HCl containing 0.5 M-NaCl and 0.03 M-Mg acetate and 1% Nonidet P-40. The nuclei were removed by centrifugation and the cytoplasmic extracts in high salt buffer were layered over linear 10 to 40% (w/v) sucrose gradients in the same buffer. Centrifugation was for 16 h at 17 000 rev/min in the SW 27 rotor at 4 °C. Gradients were pumped through a Gilford recording spectrophotometer measuring absorbance at 260 nm.

Absence profiles of ribosomes and ribosomal subunits from untreated cells analysed on sucrose gradients in high salt buffer. In contrast, in the presence of anisomycin, a large portion of the 80S ribosomes were stabilized to high salt (Fig. 1b). In different experiments, the absolute amount of 80S stabilized ribosomes varied according to cell density and growth rate, resulting in the most rapidly growing cells showing the highest degree of high salt stabilization of monosomes by anisomycin.

Lodish & Rose (1977) previously reported that reticulocyte ribosomes which were bound to mRNA and initiator tRNA were resistant to dissociation by high salt after anisomycin treatment in vitro. When a reticulocyte lysate was incubated with ³H-mRNA extracted from vesicular stomatitis virus-infected HeLa cells and analysed on sucrose gradients in high salt, the ³H-mRNA remained bound to ribosomes and sedimented at 80S in the presence of anisomycin whereas, in the absence of anisomycin, the 80S ribosomes bound to mRNA
Fig. 2. Sedimentation analysis of ribosomes in high salt buffer from poliovirus-infected cells treated with anisomycin. HeLa cells (5 x 10⁴/ml) were infected with 100 p.f.u./cell of poliovirus (type 1, Mahoney strain), in the absence (a to d) or presence (e to f) of 1 mM-guanidine. At the indicated times after infection, samples were removed and treated for 15 min with 5 x 10⁻⁷ M-anisomycin. The cells were collected by centrifugation and processed for sucrose density gradient sedimentation analysis in high salt buffer as described in the legend to Fig. 1. The guanidine-treated culture was incubated for 2.75 h, then the cells were washed free of guanidine and resuspended in fresh medium. At various times, samples were treated with anisomycin and analysed as above. Areas under the peaks were integrated with a Numonics Corp. 1224 graphics calculator after estimating peak overlaps. The percentage of total absorbance in ribosomal material which occurred as stabilized 80S complexes was (a) 36%, (b) 15%, (c) 35%, (d) 14%, (e) 11%, (f) 33%.
We have utilized the ability to detect 80S initiation complexes stabilized to high salt by anisomycin to determine whether or not these complexes are formed after poliovirus infection of HeLa cells. Cells were infected with 100 p.f.u./cell of the Mahoney strain of poliovirus type I and treated at various times after infection with $5 \times 10^{-7} \text{M}$-anisomycin for 15 min. Cytoplasmic extracts were prepared and analysed on sucrose gradients in high salt to determine the proportion of ribosomes which were in initiation complexes. Shortly after infection, the number of 80S initiation complexes in the infected cells decreased, reaching a minimum at approx. 1.75 h p.i. This correlates precisely with the time course of virus-induced inhibition of host cell protein synthesis (Helentjaris & Ehrenfeld, 1977). Fig. 2 shows the sucrose gradient profiles of extracts prepared at several different times after infection. By 2.75 h p.i., the number of 80S initiation complexes again begins to increase, as virus-directed translation also commences (Fig. 2c) and when the virus cycle is nearing completion, at 4 h p.i., initiation complexes again disappear (Fig. 2d). When cells were infected in the presence of 1 mM-guanidine, so that no virus replication occurred, the 80S initiation complex decreased in keeping with the kinetics of inhibition of host cell protein synthesis (Fig. 2e) and failed to reappear with further incubation. Removal of guanidine from the medium permits virus replication to proceed and 80S initiation complexes form again (Fig. 2f). Thus, the formation of cellular 80S initiation complexes is inhibited by poliovirus infection and the block to initiation of protein synthesis must occur at this or a preceding step.

Anisomycin is an inhibitor of peptidyl transferase which has been shown to bind to the large ribosomal subunit of the 80S ribosome (Barbacid & Vasquez, 1974). The concentration of anisomycin required to protect monosomes from high salt dissociation in vivo is critical. Below $5 \times 10^{-8} \text{M}$-anisomycin, almost no ribosome protection is seen. Maximum salt stabilization is observed between $5 \times 10^{-7} \text{M}$- and $1 \times 10^{-6} \text{M}$-anisomycin, but at higher concentrations stabilization is lost. The concentrations which give maximal 80S ribosome stabilization to high salt are those which just achieve full inhibition of protein synthesis. It appears that when elongation is extensively slowed, a build-up of initiation complexes occurs. The number of 80S initiation complexes observed in this way increases up to 15 min of anisomycin treatment but then remains stable with further incubation. In addition, no anisomycin-stabilized complexes were observed if the cells were treated with cycloheximide prior to or during the anisomycin treatment.

The data described in this report demonstrate that the formation of cellular 80S initiation complexes fails to occur after poliovirus infection. Recently, evidence from this laboratory has been presented (Helentjaris & Ehrenfeld, 1978) which suggests that the inhibition of host cell protein synthesis induced by poliovirus involves an mRNA recognition step of the initiation reaction. Consistent with this, ternary complex formation between GTP, eIF-2 and met-tRNA$_{\text{met}}$ appears to be normal in infected cells (Helentjaris & Ehrenfeld, 1978) and in this report we show that a later step in initiation is blocked. The suggestion put forward by Lawrence & Thach (1974) that inhibition of cellular protein synthesis results from a competition between virus mRNA and cellular mRNA for some component(s) of the initiation reaction is not supported by these data, since one would not expect a decrease in initiation complexes but merely a replacement of cellular with virus complexes. On the contrary, we find that initiation complexes disappear in infected cells and only reappear subsequent to virus mRNA synthesis. Previous work has shown that fully intact and functional mRNA can be recovered from cells in which translation is completely inhibited (Ehrenfeld & Lund, 1977; Hackett et al. 1977.) The precise step for inhibition induced by poliovirus is still not determined but it now appears to be narrowed down to an event subsequent to ternary complex formation and prior to or at the 60S junction reaction. When considered with in vitro studies showing mRNA specificity of the activity of the ribosomal
salt wash, the available data point to steps involving mRNA binding to the 40S initiation complex as the site of inhibition of host cell protein synthesis. Attempts to determine whether formation of the 40S complex containing met-tRNA, alone or the 40S complex containing met-tRNA, and mRNA is inhibited in infected cells have so far failed. It appears that once a 40S complex containing met-tRNA, and mRNA is formed, the 60S junction reaction is extremely fast, allowing no accumulation or detection of the preceding step (our unpublished observations).

This work was supported by grants from the National Institute of Health (AI 12387) and from the National Science Foundation, and by a Teacher-Scholar Award from the Dreyfus Foundation. We are grateful to Betty Brown in this laboratory who prepared the reticulocyte lysate.

Departments of Biochemistry and Microbiology
University of Utah Medical Center
Salt Lake City, Utah 84132, U.S.A.

Ellie Ehrenfeld
Sue Manis

REFERENCES


(Received 25 September 1978)