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Antibody to a Host Protein Prevents Initiation by the Poliovirus Replicase

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In vitro transcription of poliovirus RNA was catalyzed by the combination of a virus-coded polymerase and a host cell protein (host factor). Antibody to host factor inhibited template-dependent synthesis of complementary RNA where presumably RNA chain initiation occurred. On the contrary, elongation of already initiated RNA chains catalyzed by the replicase-template complex was not inhibited by anti-host factor antibody. These results strongly favored our previous notion that the host factor was needed for the initiation step of viral complementary RNA synthesis.

We have previously shown that the poliovirus replicase (RNA-dependent RNA polymerase), which initiates copying of poliovirus RNA (1), can be resolved into two activities: (i) a virus-specific polyuridylic acid [poly(U)] polymerase which copies polyadenylic acid [poly(A)]:oligo(U) (5) and (ii) a host cell protein (host factor) which is needed along with poly(U) polymerase to copy poliovirion RNA (2). Host factor appears to have a role in the initiation phase of RNA synthesis because it can be replaced by oligo(U) in the RNA-synthesizing system. Factor activity also depends on the presence of poly(A) in the viral RNA, as shown by its lack of stimulation of copying RNAs lacking poly(A) (2). More than just poly(A) is needed, however, because copying of pure poly(A) is not stimulated by host factor. We have now prepared antibodies against purified host factor to study its role in poliovirus RNA-dependent synthesis of complementary RNA. Anti-host factor antiserum inhibited RNA synthesis catalyzed by partially purified template-dependent replicase, as well as RNA synthesis catalyzed by purified poly(U) polymerase-host factor combination. On the contrary, RNA synthesis by endogenous replicase-template complex and the poly(A)·oligo(U)-dependent poly(U)-polymerase activity are not inhibited by anti-host factor antibodies. Thus, it appears that the host factor is needed for the initiation step of viral RNA synthesis. A report by Dimitrieva et al. has implicated a host factor in encephalomyocarditis virus RNA replication (3).

The infection of HeLa cells with poliovirus, purification of replicase through phosphocellulose (fraction II), poly(U)-Sepharose 4B (fraction IV), and purification of host factor were as described (1, 2). The endogenous replicase-template complex was isolated from poliovirus-infected HeLa cells according to Lundquist et al. (6). For preparation of anti-host factor antibodies 200 to 300 μg of host factor purified through DEAE-Sephacel and cibacron blue column chromatography (2) was used to prime and booster animals. A sample of normal serum was prepared from the blood drawn from the animals before the first injection, which served as the control serum. Poliovirion RNA was prepared by the method described by Spector and Baltimore (7).

A template-dependent RNA polymerase (replicase) was prepared from the cytoplasm of poliovirus-infected HeLa cells, which initiated copying of poliovirus RNA without an added primer (1). When anti-host factor antibodies were tested on this system, about 60 to 65% inhibition of RNA synthesis was observed compared with that of the control serum (Fig. 1). Addition of excess host factor in the reaction mixture before the addition of immune serum relieved this inhibition (Table 1), indicating that it was probably the host factor which was inactivated in the crude system by the immune area, thereby making the replicase incapable of synthesizing complementary RNA.

Further purification of this template-dependent replicase through a poly(U) agarose column separated the host factor from viral replicase, and the resulting replicase was completely dependent on added host factor for copying poliovirus RNA (2). When anti-host factor antiserum was tested on such a reconstituted system, almost complete inhibition of RNA synthesis was observed (Table 1).

A soluble replicase-template complex was

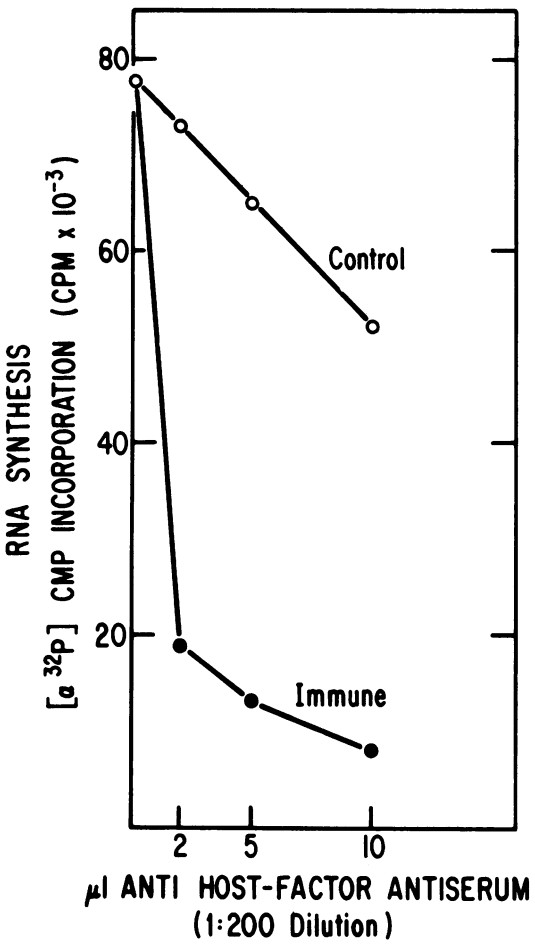

FIG. 1. Anti-host factor serum-mediated inhibition of RNA synthesis catalyzed by partially purified template-dependent poliovirus replicase. Reaction mixtures contained 10 μg of phosphocellulose-purified replicase, 1 μg of poliovirus RNA, varying amounts of either control (1:200 diluted) or immune (1:200 diluted) sera, 50 mM dithiothreitol, 10 μg of actinomycin D per ml, 5 μM [α-^{32}P]CTP, and 0.2 mM each of three other nucleoside triphosphates in a total volume of 50 μl. Incubation was for 2 h at 30°C. The labeled products were collected on membrane filters after precipitation with 7% trichloroacetic acid in the presence of 100 μg of carrier RNA. The filters were dissolved in 5 ml of Bray's solution and counted. For preparation of anti-host factor antiserum, 200 to 300 μg of host factor purified through DEAE-Sephacel and cibacron blue column chromatography (5) was used to prime the animals. Animals were boostered at day 36, and the sera were collected at day 49. For the initial inoculation, proteins were taken up in Freund complete adjuvant and injected subcutaneously. For the booster, incomplete adjuvant was used. Blood was collected from the ears of the animals, and serum was prepared by centrifugation.

prepared from poliovirus-infected HeLa cells by detergent treatment of the membrane fraction followed by precipitation of the complex with 2 M LiCl (6). The replicase-template complex does not initiate de novo RNA chains, but elongates already initiated RNA in the presence of all four ribonucleoside triphosphates. We asked whether anti-host factor antiserum would inhibit RNA synthesis catalyzed by the endogenous replicase-template complex. As shown in Table 2, no inhibition of RNA synthesis was observed at any concentration of the immune serum tested, indicating that host factor was probably not required for the completion of preinitiated RNA chains. Poly(A)·oligo(U)-directed poly(U) polymerase activity of the replicase preparation measures the elongation of an oligo(U) primer on a poly(A) template. The ability to purify poly(U) polymerase activity in the absence of replicase activity implied that host factor was not necessary for this reaction,

TABLE 1. Effect of anti-host factor antiserum on poliovirus RNA synthesis catalyzed by host factor-independent and host factor-dependent polymerases[a]

No.	Replicase	Poly(U) polymerase	Host factor	Serum	[α-^{32}P]CMP incorporation (cpm)
1	+	−	−	—[b]	55,506
2	+	−	−	Control	52,068
3	+	−	−	Immune	11,258
4	+	−	+	—	51,740
5	+	−	+	Control	46,575
6	+	−	+	Immune	39,954
7	−	+	−	—	2,001
8	−	+	+	—	30,175
9	−	+	−	Control	1,738
10	−	+	−	Immune	1,839
11	−	+	+	Control	27,297
12	−	+	+	Immune	4,530

[a] Reaction mixtures contained, in a total volume of 50 μl, the following (for tubes 1 through 6): 10 μg of phosphocellulose replicase, 0.5 μg of partially purified host factor, fraction III, 1 μg of poliovirus RNA, 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 8.0), 3.5 mM magnesium-acetate, 4 mM dithiothreitol, 10 μg of actinomycin D per ml, 5 μM [α-^{32}P]CTP (specific activity, 10,000 cpm/pmol), 0.2 mM each of ATP, UTP, and GTP, and where indicated 2.5 μl of either control (1:200 diluted) or immune (1:200 diluted) sera. Incubation was for 2 h at 30°C. The labeled products were collected on membrane filters (Millipore Corp.; 0.45 μm) after precipitation with 7% trichloroacetic acid (one third saturated with sodium pyrophosphate) in the presence of 100 μg of added carrier RNA. The filters were dissolved in 5 ml of Bray's scintillation fluid and counted. For tubes 7 through 12, reactions were carried out with 1 μg of poly(U)-Sepharose 4B-purified poly(U) polymerase and 1 μg of partially purified host factor under identical conditions as described for tubes 1 through 6.

[b] —, None.

TABLE 2. Effect of anti-host factor antiserum on RNA synthesis catalyzed by replicase-template complex isolated from poliovirus-infected HeLa cells[a]

Replicase-template complex	Control serum (μl)	Immune serum (μl)	[α-^{32}P]CMP incorporation (cpm)
+	—	—	45,669
+	4	—	45,753
+	6	—	40,538
+	—	4	44,591
+	—	6	39,273

[a] The endogenous replicase-template complex was isolated according to Lundquist et al. (6). The replicase-template complex was assayed in a total volume of 50 μl containing the following: 50 mM HEPES (pH 8.0); 8 mM magnesium-acetate; 8 μM [α-^{32}P]CTP (5,000 cpm/pmol); 1 mM each of UTP, ATP, and GTP; 4 mM phosphoenolpyruvic acid; pyruvate kinase (3 U/ml); 10 μg of actinomycin D per ml; 10 mM dithiothreitol; and where indicated various amounts of either control (1:200 diluted) or immune (1:200 diluted) sera. The incubation was at 37°C for 30 min. The labeled products were collected on membrane filters and counted. —, None.

and this point was more clearly demonstrated by showing that purified host factor would not stimulate poly(U) polymerase activity (2). As expected, no inhibition of poly(U) polymerase activity was apparent in the presence of anti-host factor antiserum (Table 3).

It is evident that the antibodies prepared against partially purified host factor can discriminate between two poliovirus-specific RNA-synthesizing systems, namely, a template-dependent replicase where, presumably, RNA chain initiation occurs and an endogenous replicase which can elongate preinitiated RNA chains. The systems which initiate RNA chains are almost completely inhibited by anti-host factor antiserum, whereas the systems which catalyze elongation of preinitiated RNA chains are not at all affected by the immune serum. These observations, along with our previous result that host factor can be replaced by oligo(U) for copying of poliovirion RNA suggest that host factor is most probably involved in the initiation phase of poliovirus complementary (minus) RNA synthesis.

How the host factor initiates RNA synthesis in this reaction is still unknown. Whether or not a primer is synthesized by host factor must yet be determined. It has been suggested that the protein found at the 5′-terminal end of poliovirus RNA (VPg) may act as a primer to initiate viral RNA synthesis (4, 8). It should now be possible to examine the mechanism of RNA synthesis initiation in vitro, using anti-host factor antibody as a probe.

The host factor preparations that we used for making antibodies are not homogeneous and contain two major proteins having approximate molecular weights of 80,000 and 60,000 and a few other minor constituents. We attempted to immunoprecipitate the host factor from [^{35}S]methionine-labeled uninfected HeLa cell extracts. The immunoprecipitates contained a major protein with an approximate molecular weight of 60,000 and other minor protein bands as expected. Although the antibodies have been helpful in understanding the role of host factor in poliovirus RNA transcription, a thorough purification of host factor to homogeneity will be required to further study the nature of host factor, as well as its role in poliovirus RNA replication, in detail.

TABLE 3. Effect of anti-host factor antiserum on poly(U) polymerase activity

Amt of poly(U) polymerase (μl)	Serum	[^{3}H]UMP incorporation (cpm)	
		Without oligo(U)	With oligo(U)
10	—	1,880	31,253
10	Control	1,789	30,245
10	Immune	1,577	29,995

[a] Poly(U)-Sepharose 4B-purified poly(U) polymerase was assayed under poly(U) polymerase assay conditions. The standard reaction mixture, in a total volume of 50 μl, contained the following: 50 mM HEPES (pH 8.0), 8 mM magnesium-acetate, 10 μg of actinomycin D per ml, 10 mM dithiothreitol, 20 μg of poly(A) per ml, 10 μg of oligo(U)$_{10\text{-}20}$ per ml, 0.08 mM [^{3}H]UTP (500 cpm/pmol), and where indicated either 4 μl of control (1:200 diluted) or 4 μl of immune (1:200 diluted) sera. Incubation was for 30 min at 30°C. The labeled products were collected on Millipore filters (after precipitation with trichloroacetic acid), and radioactivity was measured.

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