mRNA Activity of a Sindbis Virus Defective-Interfering RNA

GIOVANNI MIGLIACCIO, PATRIZIO CASTAGNOLA, ARTURO LEONE, ALFONSO CERASUOLO, AND STEFANO BONATTI*

Istituto di Biochimica Cellulare e Molecolare, II Facoltà di Medicina e Chirurgia, Università di Napoli, Naples, Italy

Received 1 November 1984/Accepted 7 May 1985

We obtained Sindbis defective-interfering particles by nine and undiluted passages of standard virus on chicken embryo fibroblasts. These particles contain a deleted 20S RNA molecule which has mRNA activity, as shown by translation in cell-free systems in vitro. In infected cells, this mRNA activity appeared to be totally inhibited except at very late times postinfection.

The Sindbis virus genome is a single-stranded RNA molecule (49S RNA) which serves as mRNA for the synthesis of the nonstructural proteins of the virus (14). Soon after infection, a full-length cRNA is made which acts as a template for the synthesis of new genomic 49S RNA and a subgenomic viral mRNA, 26S RNA (12); this 26S RNA is identical to the 3' third of the 49S RNA and codes for the structural proteins of the virus (2). Sindbis defective-interfering (DI) particles can be generated by serial high-multiplicity passage of the virus in cultured cells (13); the late particles contain deleted RNAs of about 18 to 20S whose replication and amplification depend on complementation by standard virus. The structure of these deleted RNA molecules is heterogeneous; the presence of rearrangements, repeated segments, and tRNA-like structures at the 5' end has been reported previously (6, 9, 10). A general feature of all alphavirus DI RNAs examined so far is the lack of any mRNA activity as shown by pulse-labeling in vivo, analysis of polysome profiles, and translation in cell-free systems in vitro (5, 7, 16). It has been suggested that the absence of mRNA activity confers an evolutionary advantage to these DI RNAs: they are thought to maintain only the sequences necessary for replication and packaging to better compete with the standard helper virus for both processes (11). In our laboratory we obtained a Sindbis DI particle population by nine undiluted passages (18 h each) of Sindbis standard virus on chicken embryo fibroblasts (CEF): cells infected with this DI preparation synthesized almost exclusively a 20S RNA (Fig. 1) which is encapsidated in the extracellular virions (Fig. 2 and 3). Northern analysis of the blotted RNAs against a Sindbis virus cDNA probe covering for the untranslated region at the 3' end and part of the E2 protein of the 49S RNA (A. Leone, P. Castagnola, A. Cerasuolo, and R. Cancchedda, Microbiologica, in press) suggests also that this DI 20S RNA retained the 3' end sequences which are thought to be crucial for the replication of defective as well as 49S mRNA (data not shown) (11).

A surprising result was obtained by translation experiments in vitro. The RNA extracted from the DI virus synthesized, predominantly, three polypeptides of molecular weights of 48,000, 46,000, and 44,000, respectively (Fig. 4, lane 5); as expected, no trace of these products was detectable in translation performed with standard intracellular or viral RNA (Fig. 4, lanes 2 and 3) or with total RNA extracted from uninfected CEF (data not shown). Conversely, the DI intracellular RNA synthesized these three new polypeptides as major products, in addition to the well-known 26S and 49S RNA translation products (Fig. 4, lane 4). Essentially the same results were obtained with DI intracellular RNA ex-

---

* Corresponding author.

---

FIG. 1. Electrophoretic analysis of intracellular Sindbis virus standard and DI RNAs. Monolayer cultures of CEF were infected in parallel with standard or DI virus at a multiplicity of 50 PFU per cell. After 1 h of adsorption, fresh medium containing 1 μg of actinomycin D and 10 μCi of [3H]uridine per ml was added to the cells. The medium and the cells were harvested separately, 7 h postinfection, and the RNA was extracted as described previously (1). Portions of each RNA preparation were electrophoresed in a formaldehyde-containing 1% agarose gel (8). After the run, the gel was fluorographed by methanolic PPO (2,5-diphenyloxazole). Lane 1, Standard virus RNA; lane 2, DI RNA. Prolonged exposure of the gel revealed 49S and 26S RNA in lane 2. Arrows indicate the positions of 28S and 18S rRNA markers.
extracted from 3 to 7 h postinfection with $[^3]H$]leucine instead of $[^3]S$]methionine or with the rabbit reticulocyte lysate instead of the wheat germ system. As is clearly shown in Fig. 2 and 3, the $[^3]H$]uridine-labeled DI 20S RNA corresponded to a single RNA species of $1.2 \times 10^6$ molecular weight which is totally absent in Sindbis standard virus RNA; the mRNA coding for the three new polypeptides was most probably this DI RNA, as shown by the precise comigration on a sucrose gradient of mRNA activity, a band of $1.2 \times 10^6$ molecular weight, and the 20S peak (Fig. 2, 3, and 5). CEF infected at a high multiplicity with the DI virus synthesized the three polypeptides in large amounts but only at very late times postinfection when the synthesis of the structural proteins was clearly decreasing (Fig. 6, lanes 6 to 9). No trace of the new polypeptides was detectable in cells infected in parallel with the standard virus (Fig. 6, lanes 1 to 5) or with the DI virus at a low multiplicity (0.05 PFU per cell) (data not shown); this last finding is as expected if the three polypeptides were synthesized by a viral RNA molecule incapable of autonomous replication. All these results strongly suggest that the DI 20S RNA has mRNA activity but that this activity is efficiently inhibited in the infected
cell. Although the 20S RNA is detectable in large amounts from early times postinfection, its mRNA activity is first expressed about 10 h postinfection. We cannot yet explain the molecular mechanism of this translation inhibition. Recently, Van Steeg et al. (15) reported that Semliki Forest virus C protein is the cause of host-protein-synthesis shutoff and viral 42S RNA translation inhibition but has no effect on viral 26S RNA translation. We have evidence that 20S RNA replication starts concomitantly with 49S RNA replication, clearly before any synthesis of 26S RNA is detectable in the infected cells; furthermore, the mRNA activity of the 20S RNA in vivo was higher when C protein and glycoprotein synthesis decreased. Both observations suggest that a mechanism similar to the one reported for Semliki Forest virus inhibits the synthetic activity of 20S RNA; however, because DI RNAs can have very complex structures, it is difficult to predict the molecular basis of the translation inhibition of this DI RNA.

We thank Antonella Monticelli for advice with the RNA gel, Dennis Thiele for critical reading, Ranier Cancedda for support, and Rosaria Baldoni for typing.

This work was supported in part by Consiglio Nazionale delle Ricerche grant no. 83.00363.04.

LITERATURE CITED