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Apoptosis-Inducing and Apoptosis-Preventing Functions of Poliovirus

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Data showing that an apoptotic reaction (the exit into the cytoplasm and nucleolytic internucleosomal degradation of chromosomal DNA, compaction and fragmentation of chromatin, cellular shrinkage, and cytoplasmic blebbing) developed in a subline of HeLa-S3 cells upon nonpermissive poliovirus infection with either a guanidine-sensitive poliovirus in the presence of guanidine, a guanidine-dependent mutant in the absence of guanidine, or certain temperature-sensitive mutants at a restrictive temperature are presented. Essentially, no apoptotic reaction occurred upon permissive infection of these cells. Both permissive and nonpermissive infections resulted in the inhibition of host protein synthesis. Actinomycin D or cycloheximide also elicited a rapid apoptotic reaction in uninfected cells. However, preinfection or coinfection with poliovirus prevented the apoptotic response to the addition of actinomycin D, and preinfection blocked cycloheximide-induced apoptosis as well. These data fit a model in which the cells used are prepared to develop apoptosis, with their viability due to the presence of certain short-lived mRNA and protein species. Poliovirus infection turns on two oppositely directed sets of reactions. On the one hand, the balance is driven toward apoptosis, probably via the shutoff of host macromolecular synthesis. On the other hand, viral protein exhibits antiapoptotic activity, thereby preventing premature cell death. To our knowledge, this is the first description of an antiapoptotic function for an RNA virus.

Interaction between viruses and their hosts often results in the death of infected cells. Sometimes, viruses may benefit from such a cytopathic reaction; for example, it may facilitate the exit of virus progeny from cells. On the other hand, the premature death of the host results in abortive curtailment of the reproductive cycle. Therefore, the time course of the host's death should be controlled.

Although viruses may develop specific tools for killing their hosts, they also may exploit preexisting cellular mechanisms. One such mechanism is known as programmed cell death (PCD; for recent reviews, see references 49, 50, and 53). PCD is mediated through expression of specific mRNA and protein species; it is implicated in the deletion of certain cells in processes as diverse as development (37), immune reactions (17), and virus infections (29). PCD can be induced by a variety of factors, but regardless of the causative agent, the final steps in many, though not all (41), instances of PCD proceed via apoptosis, a standard set of biochemical events accompanied by characteristic morphological changes. Among the most typical signs of apoptosis are the nucleolytic internucleosomal degradation of chromosomal DNA because of the activation of a Ca^{2+}-dependent nuclease, compaction and fragmentation of chromatin, and cellular shrinkage, as well as cytoplasmic blebbing and fragmentation, resulting in the formation of apoptotic bodies (4, 13, 15, 27, 54).

Evidence that several large DNA-containing viruses affect the balance of apoptosis-inducing and apoptosis-suppressing functions within infected cells is accumulating. Thus, adenovirus E1A and E1B genes encode products which trigger and prevent the development of apoptosis, respectively (38, 43, 52). Baculoviruses also possess both activities, and the virus genes responsible for suppression of apoptosis have been identified (3, 7, 10, 23, 44). The induction of apoptosis by a small DNA-containing virus, chicken anemia virus, has previously been demonstrated (22), and the gene responsible has already been identified (34). The antiapoptotic genes of Epstein-Barr virus (19–21, 46), cowpox virus (31, 39), African swine fever virus (32), and herpes simplex virus (6) have also been described previously. Interestingly, the ability of infected cells to develop apoptosis may, in certain cases, restrict infection and thus be advantageous for the host organism, as has been demonstrated for baculovirus infection (8). Thus, virus antiapoptotic functions may also be regarded as a tool to overcome host defense mechanisms.

Among RNA viruses, apoptosis-inducing activities have been reported for alphaviruses (25) and myxoviruses (45). In addition, immunodeficiencies caused by retroviruses (1, 12, 18, 26, 30, 36) and arenaviruses (40) may be related to apoptotic death of certain classes of lymphoid cells triggered by reactions as yet poorly understood.

Here, we describe evidence that poliovirus, a representative of picornaviruses (small unenveloped icosahedral RNA-containing viruses), encodes separate functions which trigger and suppress the development of apoptosis in infected cells.

MATERIALS AND METHODS

Cells and viruses. Two sublines of HeLa-S3 cells were used. One, designated HeLa-B, was originally obtained from the Institute of Viral Preparations (Moscow, Russia) and passaged over 20 years at our institute by using Eagle's medium supplemented with 10% bovine serum; the other subline (HeLa-F) came more recently from the Pasteur Institute (Paris, France) and was cultivated in Eagle's medium with 10% fetal bovine serum. The following polioviruses were used: Mgs (a clone of the type 1 Mahoney strain) and its temperature-sensitive (*ts*) derivatives *ts*153 (a guanidine-resistant [*gr*] mutant with *ts* lesions in capsid proteins [47]), *ts*317, and *ts*154 (both derived from an Mgs population grown in the presence of fluorouracil; in genetic crosses, the relevant *ts* mutations mapped to

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genes which encode nonstructural polypeptides [46a]); Mgd-2/4, a guanidine-dependent (*gd*) derivative of Mahoney with two amino acid substitutions in 2C (48); 452/62 3D, a type 3 strain, and its *ts* derivatives, *ts*557 and *ts*1441, whose *ts* defects mapped to regions which encode nonstructural and structural proteins, respectively (46a, 47). Generally, cells were infected at a multiplicity of infection (MOI) of 20 PFU per cell.

DNA analysis. Versenized cells, suspended in a buffer containing 20 mM EDTA and 10 mM Tris-HCl (pH 7.4), were treated with 0.5% Triton X-100 for 20 min at 0°C. After centrifugation in an Eppendorf 5415C centrifuge to remove intact chromatin (12,000 rpm, 15 min, 4°C), sodium dodecyl sulfate was added to the supernatant (final concentration, 1%) and phenol deproteinization was performed. Nucleic acids were precipitated with ethanol, dissolved in 10 μl of H_2O, and treated with RNase A (10 μg, 37°C, 30 min). When indicated, preparations were treated with a mixture of RNases A (10 μg), T_1 (2 U), and U_2 (10 U) in 0.05 M Na acetate (pH 4.7) at 37°C for 30 min. Glycerol was added to 8%, and samples were subjected to electrophoresis on 1.5% agarose gels.

Synthesis of macromolecules. At appropriate time intervals, cell monolayers were incubated at 36.5°C for 15 to 30 min with either [^{3}H]uridine (20 μCi/ml) or ^{14}C-labeled protein hydrolysate (1 μCi/ml). In the former case, cells were pretreated with actinomycin D (ActD) (0.5 μg/ml) for 30 min before the addition of the labeled nucleoside. When it was used, cycloheximide (Chi) was added to a final concentration of 25 to 100 μg/ml. The radioactivity of trichloroacetic acid-insoluble material was determined.

Electron microscopy. Cells were fixed with 2.5% glutaraldehyde and subsequently with 1% OsO_4 and embedded into EPON-812. Ultrathin sections were examined with a JEM-1200-II electron microscope.

RESULTS

Induction of apoptosis by poliovirus. Poliovirus reproduction in HeLa-B cells resulted in a typical cytopathic effect (24). This effect included nuclear changes, i.e., nuclear distortion and condensation and fragmentation of chromatin (Fig. 1B and C), but neither internucleosomal degradation (laddering) of chromosomal DNA (Fig. 2A, lanes 2 and 3) nor cytoplasmic blebbing (Fig. 3B), two very characteristic signs of apoptosis, was observed. The investigated fraction of nucleic acids from productively infected cells contained little, if any, DNA. Upon electrophoresis, some RNase A-resistant, ethidium bromide-stained material with mobilities corresponding to polynucleotides of up to several hundred base pairs was consistently observed (Fig. 2A, lane 2). The appearance of this material temporarily coincided with the accumulation of virus-specific products; it was absent from cells infected under nonpermissive conditions (see below). The material disappeared from samples treated with a mixture of RNases A, T_1, and U_2 (Fig. 2A, lane 3), demonstrating that it was composed of ribonucleotides. Its nature and origin were not further characterized, although it could perhaps contain the poly(A) moiety of virus RNA. Usually, the nucleic acid preparations destined for electrophoretic analysis were treated only with RNase A, with the accumulation of the heterogeneous material described above serving as an indirect semiquantitative indicator of the efficiency of virus reproduction.

On the other hand, a typical apoptotic pattern developed under certain conditions that restricted virus growth. Thus, both relatively high-molecular-mass DNA and oligonucleosome-sized DNA accumulated upon infection with *gd* and *gs* viruses under nonpermissive conditions (0 and 100 μg of guanidine-HCl per ml, respectively) (Fig. 2B, lanes 2 and 3 and 5 and 6, respectively) or upon infection at a restrictive temperature (39.5°C) with mutants having *ts* defects in nonstructural proteins, i.e., *ts*317, *ts*154 (both are serotype 1), and *ts*557 (serotype 3) (Fig. 2C, lanes 4 to 6); mutants with *ts* lesions in capsid proteins, *ts*153 (serotype 1) and *ts*1441 (serotype 3), failed to produce this effect (Fig. 2C, lanes 2 and 3, respectively). Under the conditions leading to DNA degradation, a significant proportion of abortively infected cells underwent shrinkage and cytoplasmic blebbing (Fig. 3C) (Table 1). At the time when apoptotic changes markedly developed, e.g., 50% or more of cells exhibited cytoplasmic blebbing, only a small percentage of them could be stained with trypan blue (not shown).

Upon electron microscopy, the majority of abortively infected cells (except cells infected with *ts*153 and *ts*1441 at the restrictive temperature) exhibited nuclear changes typical of apoptosis and differed markedly from those observed upon productive infection. In the former case, the nucleus occupied a major part of the cell (because of cytoplasmic shrinkage); nuclear membranes were injured and in some areas appeared to be completely destroyed, with the fragmentation of nuclei as a common phenomenon at late stages; chromatin was condensed in relatively large masses (Fig. 1D) and sometimes fragmented (Fig. 1E). In contrast, in productively infected cells, nuclei were severely deformed, appearing as elongated, highly compressed entities encircled by intact membranes; deformed nuclei constituted a minor portion of the cell volume, but no apoptotic fragmentation was observed. Chromatin exhibited different degrees of condensation, with a layer of the most condensed material adjoining nuclear membranes (Fig. 1B and C).

Thus, nonpermissive poliovirus infection promoted an apoptotic reaction, whereas productive infection failed to do so. Although different interpretations of these data are possible, an attractive hypothesis is as follows. Poliovirus encodes two oppositely directed functions which are capable of triggering and suppressing the development of apoptosis, respectively. The former function was preserved when reproduction was markedly (but not completely) inhibited, whereas the latter one required efficient expression of the virus genome. To verify this hypothesis, a test system in which apoptosis could be induced in a virus-independent way was needed.

Apoptosis in HeLa cells triggered by metabolic inhibitors. Inhibitors of macromolecular synthesis are known to inhibit PCD as well as the development of a class of apoptotic reactions in cultured cells (9, 11, 16). On the other hand, the same inhibitors promote apoptosis in certain cells and under certain conditions (2, 28, 33).

For our purpose, it was essential to find a cell line of human or primate origin (to be susceptible to poliovirus) that would develop inhibitor-triggered apoptosis rather rapidly, at time intervals shorter than the duration of the virus reproductive cycle; otherwise, it would be impossible to design an assay for the putative apoptosis-preventing function of poliovirus. Among several candidates, HeLa-B cells proved to be the most suitable.

In these cells, ActD at concentrations from 0.1 to 2.0 μg/ml brought about a typical apoptotic reaction, which was revealed by the accumulation of relatively high-molecular-mass DNA as well as oligonucleosomal DNA (Fig. 4, lanes 3 and 4), the appearance of characteristic nuclear changes (Fig. 5A and B), and cytoplasmic blebbing (Fig. 5A and B and 6B). The appropriate signs were clearly detected as early as 3.5 h after ActD treatment; by 4 to 5 h, some 30 to 40% of cells showed apoptotic changes (Table 1), with only a small proportion of cells (usually <5%) losing the ability to exclude trypan blue at this time (data not shown). The ActD-induced alterations in nuclear morphology (chromatin condensation within relatively large nuclei and severely damaged nuclear membranes) were very similar to those observed upon abortive poliovirus infection. At a higher magnification, cytoplasmic organelles (e.g., mitochondria) appeared to be well preserved in ActD-treated cells (data not shown), which is also a characteristic feature of apoptosis. Even the lowest concentration of ActD used, 0.1 μg/ml, resulted in rapid and significant (~70%) inhibition of cellular RNA synthesis (data not shown).

Similar DNA laddering (Fig. 4, lanes 5 and 6), nuclear

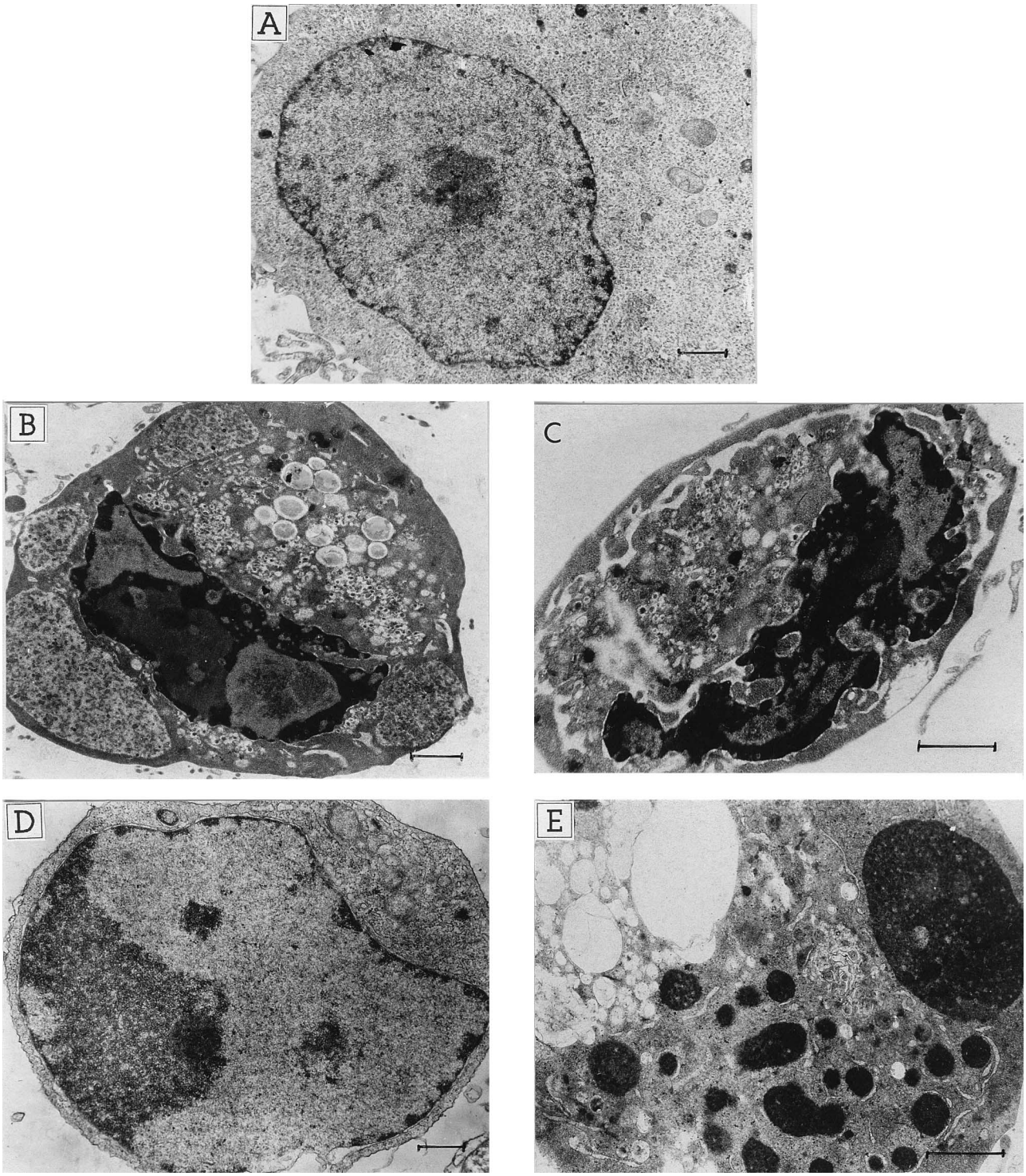

FIG. 1. Electron microscopy of HeLa-B cells productively or abortively infected with Mgs poliovirus. An uninfected cell incubated for 5 h in serum-free Eagle's medium (A); infected cells at 5 h postinfection (B and C); cells infected in the presence of 100 μg of guanidine-HCl per ml for 5 h (D) and 12 h (E). Incubation was carried out at 36.5°C. Vesicular structures corresponding to replication complexes are seen in productively (B and C) infected cells but not in abortively (D and E) infected cells. The nuclear membrane was completely destroyed and the nucleus was fragmented in the cell shown in panel E. The cell shown in panel B contains areas of destruction of unknown etiology. For other comments, see text. The bar in panel D and those in other panels correspond to 500 nm and 1 μm, respectively.

changes (Fig. 7A and B), and cytoplasmic blebbing (Fig. 8B) were observed upon the addition of Chi at 25 to 100 μg/ml (or puromycin at 10 to 40 μg/ml [data not shown]). Of note, greater accumulation of oligonucleosome-sized DNA fragments was observed when cells were preincubated in serum-free medium prior to the addition of Chi (3 h without Chi and then 2 h in its presence [Fig. 4, lane 5]) than when cells were incubated with Chi from the onset (5 h in the presence of the

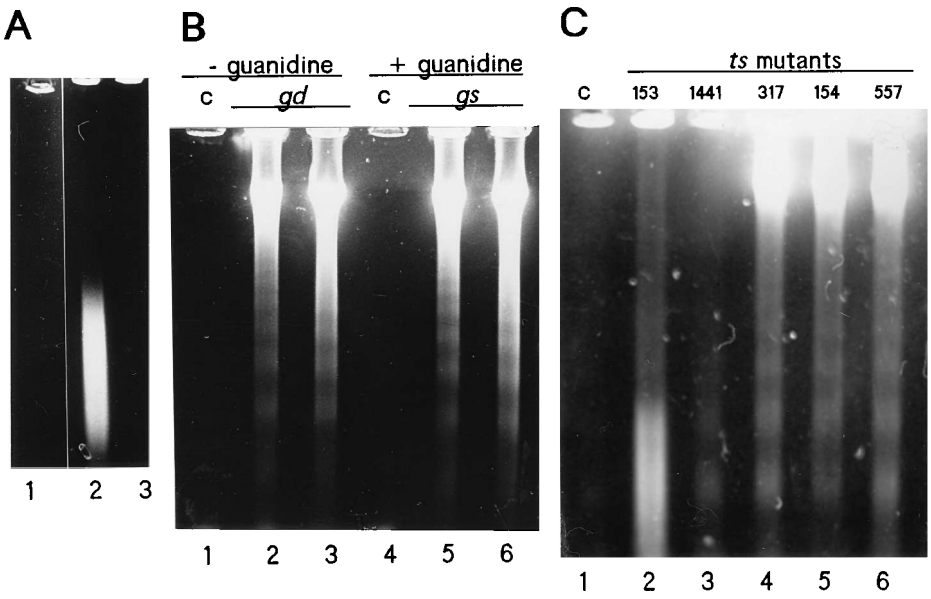

FIG. 2. Electrophoretic analysis of DNA. (A) Preparations from uninfected (lane 1) and productively infected HeLa-B cells at 5 h postinfection (lanes 2 and 3); the preparation in lane 3 was treated with a mixture of nucleases A, U_2, and T_1 as described in Materials and Methods. (B) Preparations from uninfected (C) cells incubated for 10 h in the absence (lane 1) or presence (lane 4) of guanidine-HCl (100 μg/ml) and cells abortively infected with a *gd* strain (Mgd-2/4) in the absence of guanidine (lanes 2 and 3; MOIs of 40 and 800 PFU per cell, respectively) or with a *gs* strain (Mgs) in the presence of 100 μg of guanidine-HCl per ml (lanes 5 and 6; MOIs of 80 and 1,600 PFU per cell, respectively). Infections were carried out for 10 h at 36.5°C. (C) Preparations from uninfected (C) cells (lane 1) and cells infected with *ts* poliovirus mutants (lanes 2 to 6; MOI of 20 PFU per cell) incubated for 6 h at 39.5°C.

same concentration of Chi [Fig. 4, lane 6]). Separate experiments demonstrated that serum-free preincubation sensitized HeLa-B cells to the DNA fragmentation-inducing action of ActD as well (data not shown).

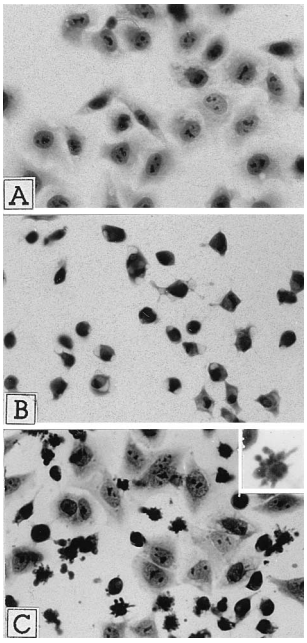

FIG. 3. Light microscopy of HeLa-B cells infected with Mgs poliovirus at an MOI of 40 PFU per cell at 8 h postinfection in the absence (B) and presence (C) of guanidine-HCl (100 μg/ml). Uninfected and untreated cells (A). Magnification, ×200. A cell displaying extensive cytoplasmic blebbing is shown in the inset (panel C) at a magnification of ×400. Hematoxylin-eosine staining was used for all panels.

Apoptosis prevention by poliovirus. The ability of poliovirus to interfere with the development of ActD- and Chi-induced apoptosis in HeLa-B cells was investigated next. The addition of ActD at 1.5 h postinfection for 4 h failed to trigger either the nuclear exit of DNA or its fragmentation (Fig. 9A, lane 4), whereas similar treatment of uninfected cells led, as expected, to a vigorous apoptotic response (Fig. 9A, lane 2). The development of biochemical signs of apoptosis was also prevented by the addition of ActD at the very beginning of infection (data not shown). The nuclear changes in cells infected in the presence of ActD (Fig. 5C and D) were typical of the virus cytopathic effect rather than ActD-induced apoptosis; the development of cytoplasmic blebbing was also prevented by infection (Fig. 6D) (Table 1). The yield of infectious virus was hardly, if at all, affected by the presence of the drug (data not shown).

Chi-induced apoptosis was similarly prevented by the prein-

TABLE 1. Proportion of cells with cytoplasmic blebbing[a]

Expt no.	Conditions	% Cells with cytoplasmic blebbing	
		Uninfected cultures	Infected cultures[b]
1	Eagle's medium (5 h)	6.6	0
	Eagle's medium + guanidine (10 h)	5.0	65.4
	Eagle's medium (3 h) and then Chi (2 h)	60.4	3.8
2	Eagle's medium (4 h)	2.0	0
	Eagle's medium + ActD (4 h)	39.9	2.6

[a] HeLa-B cells were incubated in Eagle's medium without serum as indicated. The concentrations of inhibitors were as follows: guanidine-HCl, 100 μg/ml; Chi, 100 μg/ml; ActD, 0.5 μg/ml. Monolayers were stained with hematoxylin-eosine, and the percentage of cells with blebbing was determined.

[b] Cells were infected with Mgs (MOI of 20 PFU per cell) at time zero.

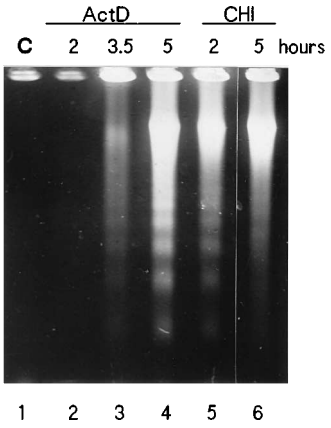

FIG. 4. Effect of metabolic inhibitors on the accumulation of fragmented DNA. Preparations from HeLa-B cells untreated (C, lane 1) or treated with 0.5 μg of ActD per ml (lanes 2 to 4) or 100 μg of Chi per ml (lanes 5 and 6) were subjected to electrophoresis. Cells were incubated for 5 h, and drugs were added 0 h (lanes 4 and 6), 1.5 h (lane 3), and 3 h (lanes 2 and 5) after the onset of incubation at 36.5°C. The duration of incubation with each drug is indicated above each lane.

fection of cells with poliovirus for at least 3 h prior to Chi treatment (Fig. 7C and D, 8D, and 9B, lane 6); shorter (2-h) preinfection failed to prevent the accumulation of partially degraded relatively high-molecular-mass DNA, an early manifestation of apoptosis (5, 35, 51), although its further fragmentation appeared to be suppressed (Fig. 9B, lane 5). On the other hand, no apparent effect of nonpermissive infection (with the *gs* virus in the presence of guanidine) on Chi-induced apoptosis was registered (data not shown). These observations suggested that a certain level of synthesis of virus proteins was essential for the prevention of apoptosis.

Effect of nonpermissive poliovirus infection on macromolecular synthesis. Since poliovirus infection brings about inhibition of host macromolecular synthesis (14, 24) and such inhibition can promote apoptosis (see above), it was important to assess the effect of poliovirus infection under nonpermissive conditions (that is, when the virus did induce apoptosis) on cellular protein synthesis. Some relevant results (e.g., the shutoff of protein synthesis upon infection with *gs* or *gd* polioviruses in the presence or absence of guanidine, respectively) have already been reported (14, 24); however, this inhibitory activity may markedly depend on a particular virus-host combination (unpublished data). In HeLa-B cells infected with *gs* virus in

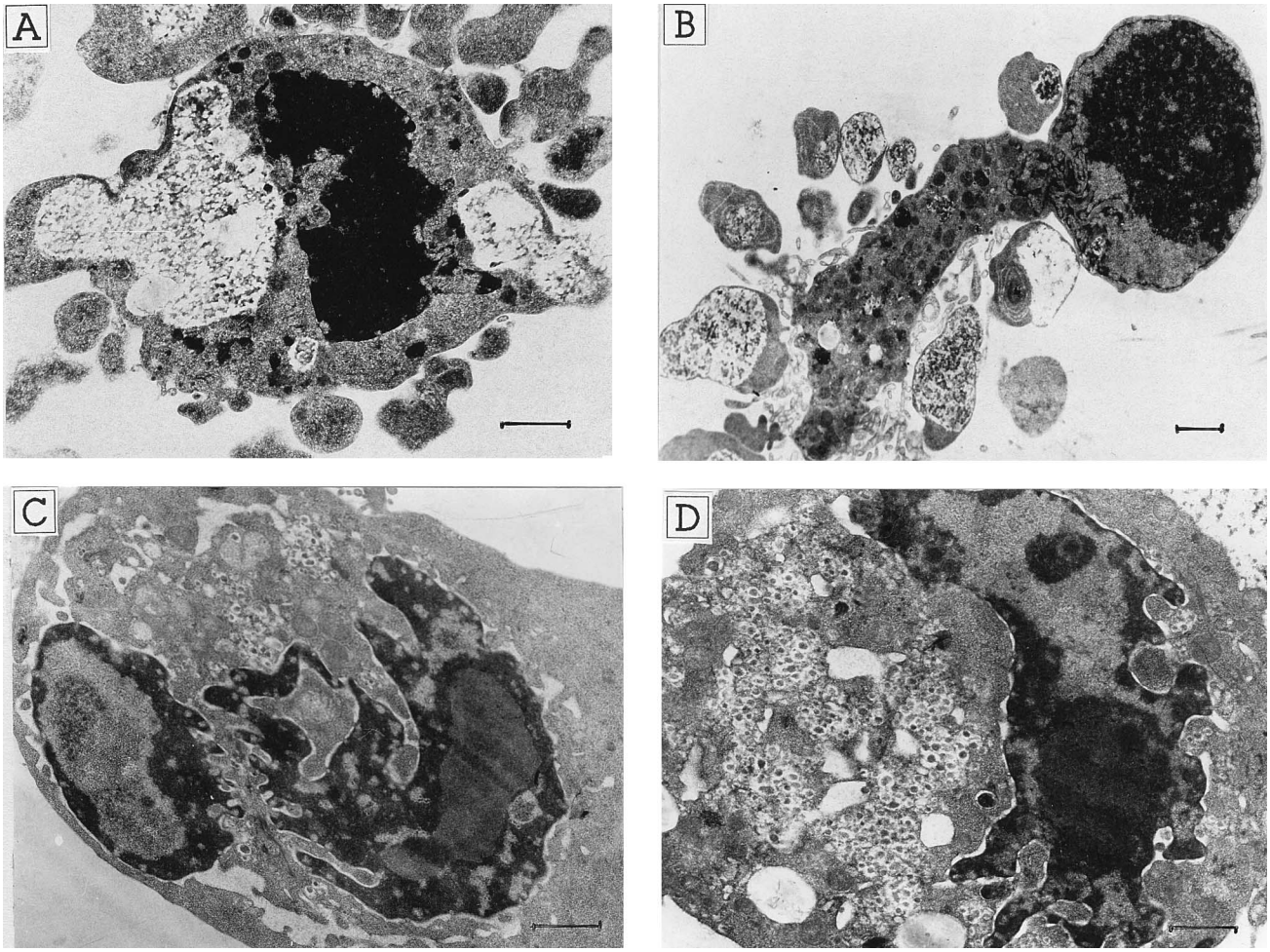

FIG. 5. Electron microscopy demonstration of ActD-induced nuclear changes and their prevention by poliovirus infection. ActD at 0.5 μg/ml was added to uninfected (A and B) and Mgs poliovirus-infected (C and D) HeLa-B cells 1 h after the onset of incubation at 36.5°C; incubation was terminated 4 h thereafter. Extensive cytoplasmic blebbing and fragmentation of nuclei are seen in panels A and B; infected cells contain vesicular replication complexes (C and D). Areas of unspecific destruction are seen in panels A and B. For other comments, see text. Bar, 1 μm.

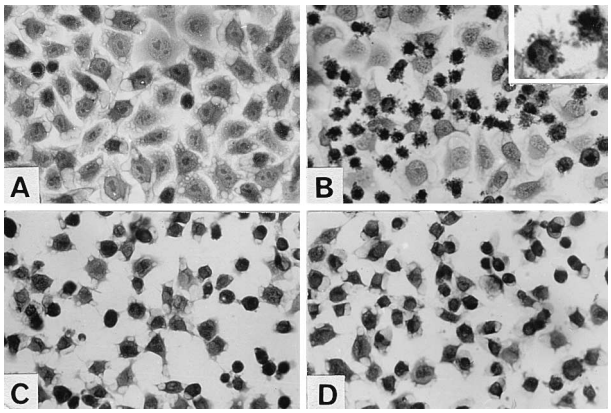

FIG. 6. Cytoplasmic blebbing induced by ActD and its prevention by poliovirus infection. Uninfected (A and B) and Mgs-infected (20 PFU per cell; C and D) HeLa-B cells were incubated for 5 h; cells were treated with ActD (0.5 μg/ml) from 1 h onward (B and D). Magnification, ×200. Two cells displaying extensive cytoplasmic blebbing are shown in the inset (panel B) at a magnification of ×400. Hematoxylin-eosine staining was used for all panels.

the presence of guanidine (Fig. 10A) or *ts* strains at a restrictive temperature (Fig. 10B), the accumulation of labeled amino acids in the acid-insoluble fraction was strongly inhibited in a time-dependent manner. A transient wave of protein synthesis activation was noted in the case of *ts*153 infection, consistent with a defect in only a late (capsid protein) function of this mutant.

Poliovirus infection and apoptosis in HeLa-F cells. In addition to being very suitable for the demonstration of the apoptosis-preventing function of poliovirus, the HeLa-B cells used in the experiments described above exhibited fairly good growth potential and supported very efficient poliovirus reproduction (up to 10^4 PFU per cell and above in 5 h). However, a significant proportion of uninfected cells contained morphological alterations of unknown etiology as manifested in the presence of vacuoles by light microscopy (Fig. 3, 6, and 8) and areas of destruction by electron microscopy (Fig. 1B, 5A and B, and 7C). To ascertain whether the abilities of poliovirus to induce and/or prevent apoptosis in these cells were related to these abnormalities, similar experiments were performed with another subline of HeLa-S3 cells, HeLa-F. These cells were obtained from another source and passaged in the laboratory in the presence of fetal bovine serum; they did not manifest the

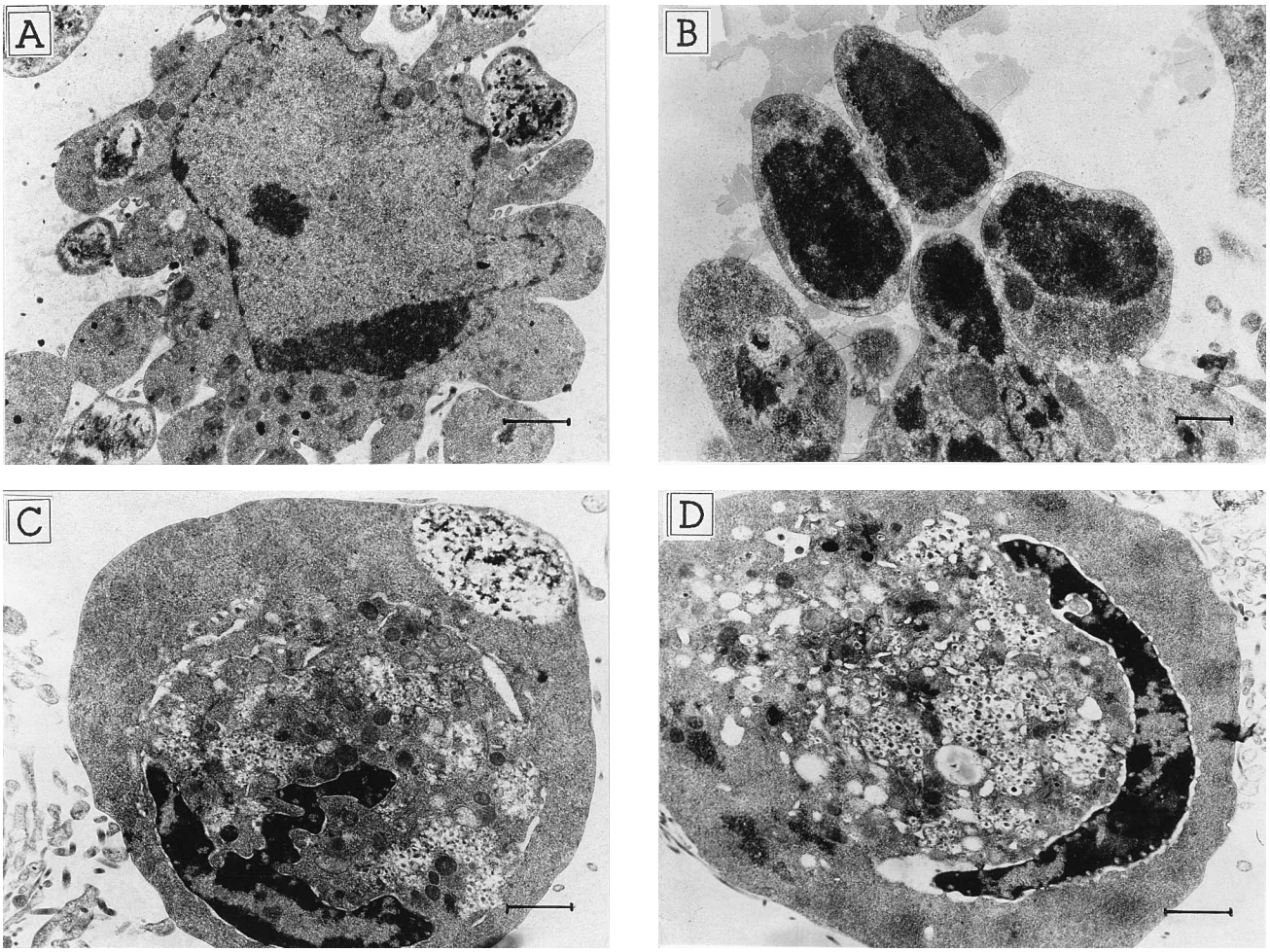

FIG. 7. Electron microscopy demonstration of Chi-induced nuclear changes and their prevention by poliovirus infection. Chi (100 μg/ml) was added to uninfected (A and B) and Mgs poliovirus-infected (C and D) HeLa-B cells 3 h after the onset of incubation at 36.5°C; incubation was terminated 2 h thereafter. Extensive cytoplasmic blebbing (A) and nuclear fragmentation (B) are seen in uninfected cells; infected cells contain vesicular replication complexes. There is an area of unspecific destruction in the cell shown in panel C. For other comments, see text. The bar in panel B and those in other panels correspond to 500 nm and 1 μm, respectively.

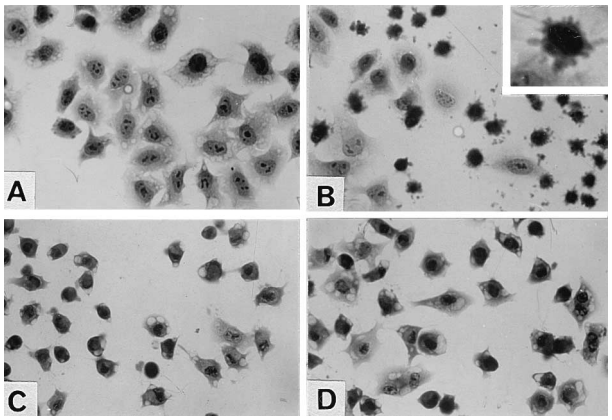

FIG. 8. Cytoplasmic blebbing induced by Chi and its prevention by poliovirus infection. Uninfected (A and B) and Mgs-infected (20 PFU per cell; C and D) HeLa-B cells were incubated for 5 h; cells were treated with Chi (100 μg/ml) from 3 h onward (B and D). Magnification, ×200. A cell displaying extensive cytoplasmic blebbing is shown in the inset (panel B) at a magnification of ×400. Hematoxylin-eosine staining was used for all panels.

abnormalities characteristic of HeLa-B cells. The principal effects observed in HeLa-B cells were reproduced in HeLa-F cells as well, although some quantitative differences in the responses of the two types of cells to both poliovirus infection and metabolic inhibitors were registered. Thus, HeLa-F cells require more time to develop inhibitor-triggered apoptosis, especially apoptosis induced by abortive poliovirus infection (data not shown). Nevertheless, the prevention of inhibitor-induced apoptosis by productive poliovirus infection was demonstrated (Fig. 11), although the necessary conditions might vary somewhat for different experiments.

DISCUSSION

As shown above, poliovirus infection may trigger the development of an apoptotic reaction. Such a reaction was observed

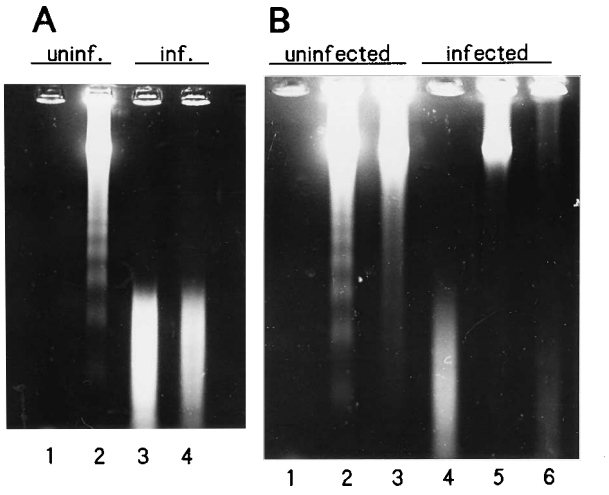

FIG. 9. Prevention of drug-induced fragmentation of DNA by productive poliovirus infection. (A) Preparations from uninfected (lanes 1 and 2) and Mgs poliovirus-infected (lanes 3 and 4) HeLa-B cells incubated at 36.5°C for 5.5 h. Lanes 2 and 4, cells treated with ActD (0.5 μg/ml) from 1.5 h onward. (B) Preparations from uninfected (lanes 1 to 3) and infected (lanes 4 to 6) cells incubated for 5 h. Lane 5, sample incubated for 4 h at 36.5°C. Chi (100 μg/ml) was added at time zero (lane 3), 2 h (lane 5), or 3 h (lanes 2 and 6) after the onset of incubation. Lanes 1 and 4, samples incubated in the absence of Chi.

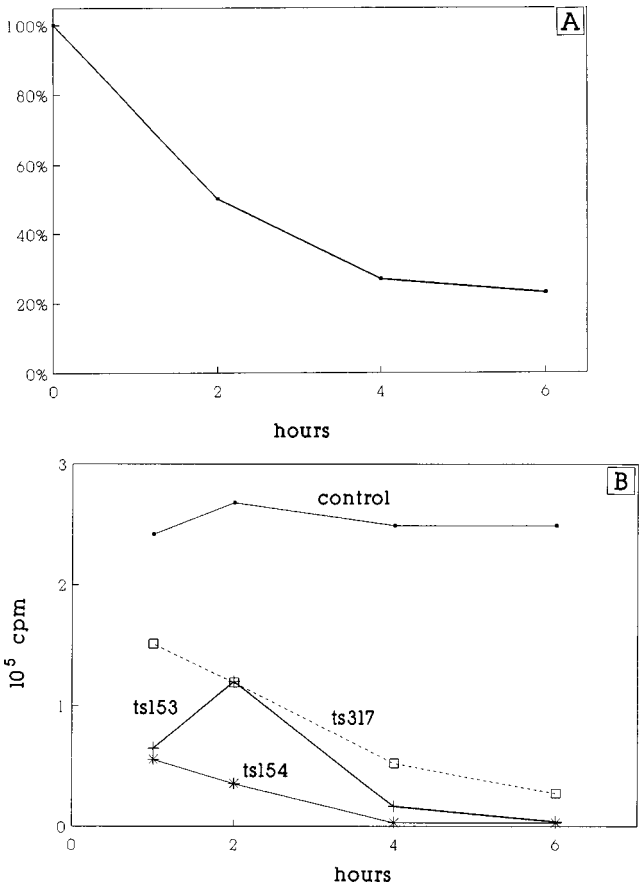

FIG. 10. Inhibition of protein synthesis upon abortive poliovirus infection. (A) HeLa-B cells infected with Mgs in the presence of guanidine-HCl (100 μg/ml). Uninfected and infected (MOI of 20 PFU per cell) cells were pulsed with ^{14}C-labeled protein hydrolysate for 15 min at the time intervals indicated. Incorporation into infected cells is presented as a percentage of that in control cultures. (B) HeLa-B cells infected with *ts* mutants at a restrictive (39.5°C) temperature. Uninfected (control) and infected (MOI of 20 PFU per cell) cells were pulsed with ^{14}C-labeled protein hydrolysate for 15 min at the time intervals indicated.

in HeLa cells under nonpermissive conditions, e.g., upon infection with *gs* or *gd* variants in the presence or absence of guanidine, respectively, as well as upon infection with certain *ts* mutants at a restrictive temperature. Poliovirus also possesses a function for suppressing its own apoptosis-inducing activity and preventing the development of apoptosis triggered by metabolic inhibitors. Although the detailed molecular mechanisms underlying these phenomena remain to be determined, all these observations fit well the following tentative model.

The investigated sublines of HeLa cells (especially HeLa-B cells) are prepared to develop an apoptotic reaction without the induction of new RNA or protein species. This follows from the fact that apoptosis can be induced by inhibitors of host RNA and protein synthesis. The rapid development of apoptosis in response to these inhibitors strongly suggests that the maintenance of the nonapoptotic status of these cells is due to the presence of some short-lived mRNA and protein species. However, incubation of uninfected HeLa-B cells in serum-free medium appears to activate an apoptosis-promoting function, as judged by a more vigorous apoptotic response to the addition of metabolic inhibitors. Thus, the interplay of cellular apoptosis-promoting and apoptosis-suppressing func-

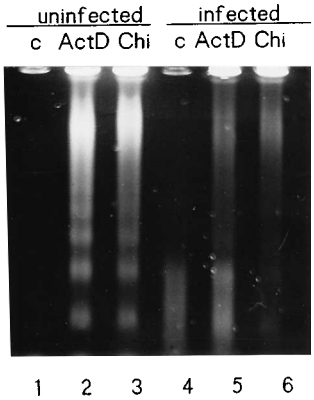

FIG. 11. Antiapoptotic effect of poliovirus infection in HeLa-F cells. Electrophoresis of DNA preparations from uninfected (lanes 1 to 3) and Mgs-infected (lanes 4 to 6) cells incubated in serum-free Eagle's medium at 36.5°C. Infections were started at time zero (lanes 4 and 6) or 1.5 h (lane 5); ActD (1 μg/ml) (lanes 2 and 5) and Chi (100 μg/ml) (lanes 3 and 6) were added 3 h after the onset of incubation for 4.5 and 3 h, respectively. Lanes C, control cultures not treated with either drug.

tions seems to be physiologically controlled. Poliovirus infection interferes with this control by turning on two sets of reactions. On the one hand, the balance is driven toward apoptosis. A likely driving force is poliovirus-induced shutoff of host protein synthesis. On the other hand, a poliovirus antiapoptotic function is concurrently switched on, probably to prolong the survival of infected cells. This function is not mediated through activation of cellular transcription (ActD-triggered apoptosis was prevented even when the drug was present from the onset of infection) but does require virus protein synthesis and is invalidated under nonpermissive conditions (remarkably, nonpermissive infections with mutants *ts*153 and *ts*1441, having *ts* lesions in capsid proteins and therefore capable of replicating and partially expressing their genomes, did not induce apoptosis). The expression of the apoptosis-inducing function under nonpermissive conditions should not be surprising, because even a very weak level of expression of the poliovirus genome is sufficient to bring about the shutoff (as exemplified by the inhibition of cellular protein synthesis by *gs* and *gd* variants in the presence and absence of guanidine, respectively).

The poliovirus antiapoptotic function appears to dominate the apoptosis-inducing one, at least upon productive infection of HeLa-B cells. This suggests that the virus antiapoptotic protein, whatever its nature, either stabilizes the putative unstable cellular antiapoptotic protein or converts the target for this protein into a state unable to promote the apoptotic response. In this regard, the antiapoptotic function of poliovirus is similar to those of the *p35* and *iap* genes of baculoviruses (10).

The virus polypeptide 2A may tentatively be suggested to be at least partially responsible for the apoptosis-inducing function because of its known involvement in virus-induced shutoff of host protein synthesis (42). On the other hand, the nature of the anti-apoptotic function of poliovirus remains to be elucidated.

Susceptible cell lines may differ from each other in the ability to respond to poliovirus infection by apoptosis and shutoff of host macromolecular synthesis (even different HeLa sublines exhibited some dissimilarity); likewise, variations were observed with respect to the cellular response to the addition of metabolic inhibitors. HeLa-B cells appear to be the most suitable for studies of poliovirus-specific apoptosis-inducing and apoptosis-preventing functions because of an exceptional combination of favorable properties, fairly rapid development of apoptosis in response to the addition of metabolic inhibitors and very efficient virus reproduction. To what extent these peculiarities are related to morphological abnormalities seen in some of these cells remains an open question. Despite these abnormalities, these cells exhibited excellent growth potential. It should be emphasized, however, that the apoptosis-inducing and apoptosis-preventing functions of poliovirus were also revealed in HeLa-F cells, in which no morphological abnormalities were observed.

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