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Final Checkpoint in the Drug-Promoted and Poliovirus-Promoted Apoptosis Is Under Post-Translational Control by Growth Factors

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Abstract The treatment of HeLa subline (HeLa-B) cells with cycloheximide or Actinomycin D resulted in a rapid (~1.5 h and ~2.5 h, respectively) development of morphological and biochemical signs of apoptosis. The addition of fetal bovine serum to the cycloheximide-treated or Actinomycin D-treated cells suppressed the apoptotic reaction, as evidenced by the postponement of the DNA fragmentation for at least 9 and 5 h, respectively. A similar suppressive effect was observed upon the serum addition to cells undergoing abortive infection with poliovirus, which died of apoptosis in the absence of the serum. The serum appeared to exert its anti-apoptotic effect without any appreciable lag and even immediately blocked further progress of ongoing DNA fragmentation. The epidermal growth factor also suppressed, although less efficiently and more transiently, the apoptotic reaction promoted by the metabolic inhibitors. It is concluded that growth factors may affect, without modulating either transcription or translation, the balance of pro-apoptotic and anti-apoptotic activities at a final checkpoint, just preceding the irreversible effector step of apoptosis.

Key words: Actinomycin D, cycloheximide, DNA degradation, chromatin fragmentation, serum factors, epidermal growth factor

The programmed cell death is a complex phenomenon involved in the normal development and immunological response [Raff et al., 1993; Vaux et al., 1994; Linette and Korsmeyer, 1994; White and Steller, 1995]; its improper functioning may result in a variety of pathologies, including malignancies [Harrington et al., 1994b; Ameisen et al., 1994; Thompson, 1995]. Schematically, the implementation of the death program includes "sensing" of the primary signals, their intracellular evaluation and treatment, and, finally, turning-on of the effector mechanism that results in irreversible nuclear and cytoplasmic alterations collectively known as apoptosis [Kerr et al., 1972; Wyllie et al., 1980; Reed, 1994; Vaux et al., 1994; Wyllie, 1995; Majno and Joris, 1995]. Among the major apoptogenic signals, the absence of trophic (growth) factors should be named [Raff, 1992; McCarthy et al., 1992]. The disengagement of appropriate receptors is believed to activate the system of secondary messengers, which in turn modulates the activity of pro-apoptotic and anti-apoptotic genes and their products [Colotta et al., 1992; McConkey and Orrenius, 1994; Oltvai and Korsmeyer, 1994; Estus et al., 1994]. The altered balance of the participants of this play results in the activation of proteolytic [Kumar, 1995; Chow et al., 1995b] and nucleolytic [Wyllie et al., 1992; Peitsch et al., 1994; Walker et al., 1994; Fraser, 1994] enzymes characteristic of the final stages of apoptosis.

The necessity of alterations in gene activities for the realization of the death program is suggested by the ability of inhibitors of transcription and translation to suppress the apoptotic response to a variety of stimuli [cf. Ghibelli et al., 1992; Chow et al., 1995a], including the withdrawal of growth factors [Martin et al., 1988; Rawson et al., 1990; McCarthy et al., 1992]. On the other hand, the treatment with such metabolic inhibitors may lead, under some conditions and in certain cells, to biochemical and morphological alterations typical of apopto-

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sis [Martin et al., 1990; Evan et al., 1992; Ledda-Columbano et al., 1992; Nicolaou et al., 1993; Polunovsky et al., 1994; Chow et al., 1995a; Lindenboim et al., 1995]. The blockage of transcription or translation in these cases is likely to result in a shift in the balance of the activities of pro- and anti-apoptotic factors due to differences in their own functional stabilities or stabilities of their mRNA templates. By studying conditions and treatments modulating cellular response to transcriptional/translational inhibitors, one may gain insight into the control of the effector stage of apoptosis.

The aim of this report is to demonstrate that fetal bovine serum (FBS) and, to a lesser extent, epidermal growth factor (EGF) are able to suppress the apoptotic response of HeLa cells to the addition of inhibitors of transcription or translation, Actinomycin D (ActD) and cycloheximide (Chi), respectively. The serum exerts a similar preventive effect on the apoptosis resulting from the abortive poliovirus infection, thought to be caused by the virus-induced shut-off of the host protein synthesis [Tolskaya et al., 1995]. Thus, in addition to their role during the most "upstream" steps of the death program, the growth factors may control the final post-translational checkpoint as well.

MATERIALS AND METHODS

Materials

FBS was purchased from Flow (Irvine, Scotland) and was heated for 30 min at 56°C. Two preparations of EGF were used: a human recombinant preparation from Sigma (St. Louis, MO) and a preparation kindly donated by Professor N.N. Nikolsky (Institute of Cytology, St. Petersburg, Russia). With regard to the apoptosis-suppressing effect, both preparations exhibited comparable activities. ActD and Chi came from Calbiochem (La Jolla, CA) and NBC (Cleveland, OH), respectively.

Cells and Virus

A subline of HeLa cells, HeLa-B [Tolskaya et al., 1995], was originally obtained from the Institute of Viral Preparations (Moscow, Russia) and was passaged over 20 years at our Institute by using Eagle's medium supplemented with 10% bovine serum. Mgs, a clonal derivative of poliovirus type 1, Mahoney strain [Tolskaya et al., 1983] was used.

Microscopic Observations

The proportion of apoptotic cells was determined in unstained preparations by counting of cells exhibiting cytoplasmic blebbing (after detachment from the substratum by versenization), as well as in monolayers using hematoxylin and eosin (H&E) staining or propidium iodide staining followed by luminescent microscopy.

DNA Analysis

Fragmentation of DNA was assayed as described previously [Tolskaya et al., 1995]. Versenized cells ($\sim 7–8 \times 10^6$), 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 centrifuge to remove intact chromatin (12,000 rpm, 15 min, 4°C), sodium dodecyl sulfate (SDS) was added to the supernatant to a final concentration of 1%, and phenol deproteinization was performed. Ethanol-precipitated nucleic acids were dissolved in 10 μl of H_2O and treated with RNase A (10 μg, 37°C, 30 min). Glycerol was added to a final concentration of 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 or ^{14}C-labeled protein hydrolysate. In the former case, the cells were pretreated with ActD (0.5 μg/ml) for 30 min before the addition of the labeled nucleoside. Chi was used at a concentration of 100 μg/ml. The radioactivity of the trichloroacetic acid (TCA)-insoluble material was determined.

RESULTS

Suppression of Apoptosis by Serum Addition to Chi- or ActD-Treated Cells

The addition of Chi or ActD to HeLa-B cultures resulted in rapid development of the biochemical and morphological signs of apoptosis in a significant proportion of the cells, with the effect augmented by a preincubation in a serum-free medium [Tolskaya et al., 1995]. In the present study, the 1-day-old confluent monolayer cultures of HeLa-B were incubated in the serum-free Eagle's medium for 3 h and then treated (if not stated otherwise) with 100 μg/ml of Chi or 0.5 μg/ml of ActD, the concentrations ensuring

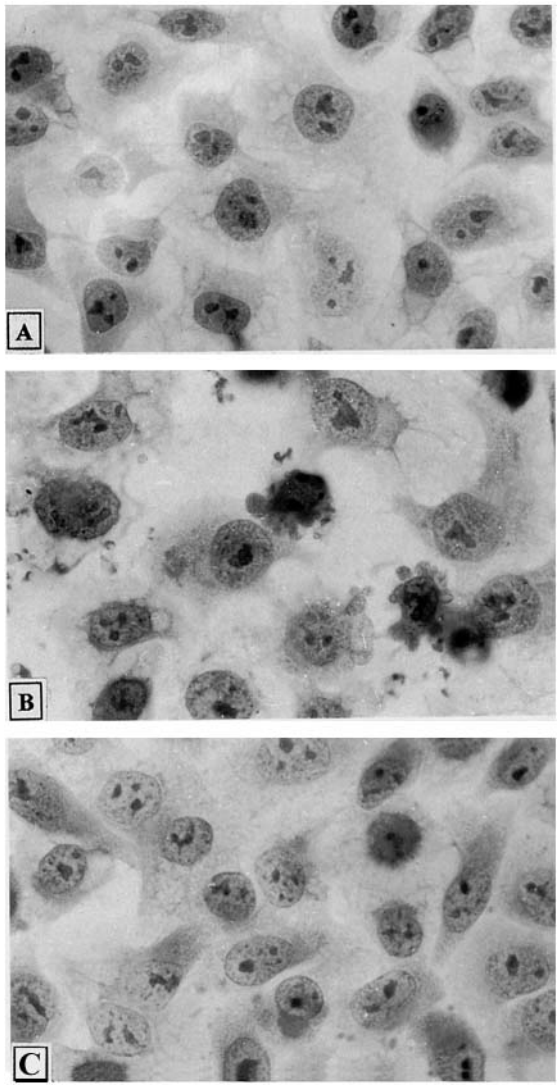

Fig. 1. Suppression of the Chi-triggered apoptosis by FBS, as revealed by microscopy of H&E-stained preparations. Control, untreated HeLa-B cells **(A)**; cells treated with Chi, 100 μg/ml, for 2 h **(B)**; cells treated with Chi as in **B**, but FBS was added 15 min after the drug. **C**: Cells exhibiting blebbing (formation of apoptosis bodies) are shown in **B**.

a nearly complete (>95%) inhibition of protein or RNA synthesis, respectively (not shown). A few hours later (approximately after 1.5 h and 2.5 h, respectively), a typical apoptotic reaction could be registered by the appearance of cells with cytoplasmic extrusions ("buds" or "blebs") (Fig. 1B) as well as condensation and fragmentation of chromatin (not shown). The chromosomal DNA was degraded, eventually to oligonucleosome-sized fragments (Fig. 2A, lanes 2–4; Fig. 2B, lane 3). All these changes took place prior to the increase in permeability to trypan blue characteristic of cell death (Table I). Qualitatively similar effects were observed upon the addition of the metabolic inhibitors to cells not preincubated in the serum-free medium; under these conditions, the apoptotic response was, however, somewhat less pronounced and less uniform.

When 5% fetal bovine serum (FBS) was added shortly (15–30 min) after the drug treatment, the apoptotic reaction was markedly suppressed, judging by microscopic observations (Fig. 1C; Table I) and by the inhibition of DNA degradation. Thus, in the experiment shown in Figure 2, the Chi-treated cultures were incubated for different time intervals with or without FBS (added 15 min after the drug). In the absence of the serum, DNA degradation started after approximately 1 h, with only high-molecular-mass (HMM) DNA fragments being detected at that time (Fig. 2A, lane 1); further accumulation of HMM species and their cleavage into nucleosome-sized fragments took place during the following hour (Fig. 2A, lanes 2 and 3). In the presence of FBS, no DNA degradation could be observed until 10 h, with only trace amounts of HMM DNA seen after such a long exposure (Fig. 2A, lanes 5–8). Upon further incubation, both HMM and oligosome DNA did accumulate (not shown). Nevertheless, cultures incubated in the presence of both Chi and FBS for 24 h could readily be passaged after removal of the drug, demonstrating that a significant proportion of the cells did survive after such a treatment.

The count of cells exhibiting cytoplasmic blebbing (Table I, exp. 2) also demonstrated the development of a marked apoptotic reaction at 1.5–2 h of incubation with Chi, whereas essentially no signs of apoptosis could be observed even by 10 h, if the drug-treated cells were incubated in the presence of FBS. We may conclude that FBS resulted in at least 9-h delay in the onset of the Chi-triggered apoptosis.

A similar experiment was carried out with the ActD-treated cells. In the absence of FBS, a typical apoptotic pattern of DNA degradation developed at 2.5–3.5 h of the drug treatment (Fig. 2B, lanes 2 and 3). In the presence of serum, a comparable level of degradation was only achieved by 8 h (Fig. 2B, lane 6), suggesting that the reaction was delayed by approximately 5 h. The proportion of cells with blebbing exhibited a similar time course (Table I, exp. 3).

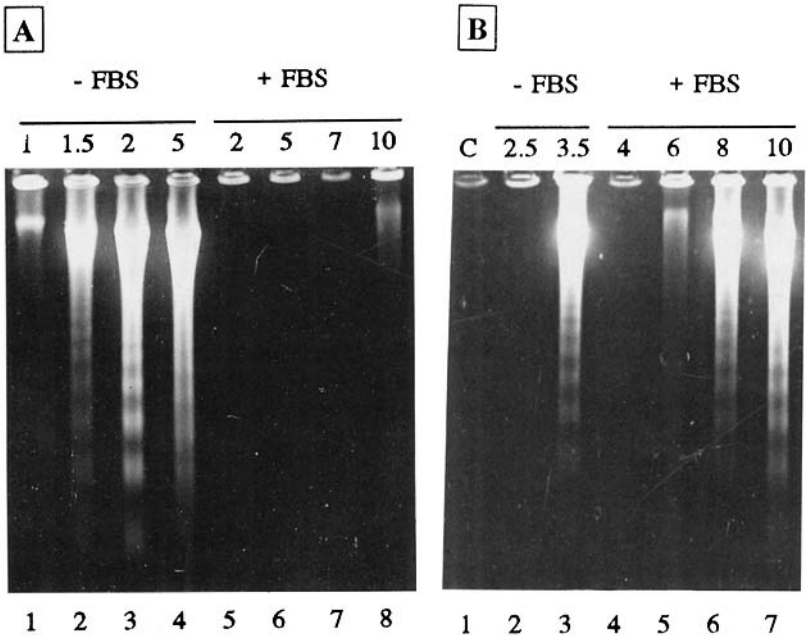

Fig. 2. Suppression of Chi- and ActD-triggered apoptosis by FBS. Monolayer cultures of HeLa-B cells were preincubated in the serum-free medium for 3 h and treated with either Chi (100 μg/ml) (**A**) or ActD (0.5 μg/ml) (**B**) for time intervals indicated. 15 min after the addition of metabolic inhibitors, FBS was added to samples marked (+FBS) to a final concentration of 5%. DNA was extracted and subjected to electrophoresis as described under Materials and Methods. C, untreated control sample preincubated in the serum-free medium for 3 h, followed by the incubation in the FBS-containing medium for 10 h.

Serum Exerts Its Anti-Apoptotic Effect at a Final Checkpoint

HeLa-B cells responded by an apoptotic reaction to the Chi or ActD addition with some lag (see above). We wanted to know how much time was needed for the implementation of the anti-apoptotic effect of FBS. In other words, did the serum act at an upstream or downstream segments of the pathway leading from the drug treatment to irreversible apoptotic alterations ("point of no return")? To answer this question, the experiments depicted in Figure 3 were carried out. No DNA fragmentation was revealed in HeLa-B cells preincubated for 3 h in the serum-free medium during the first 2 h after the addition of ActD (Fig. 3A, lanes 2–4). In the course of the next 45 min, a significant amount of fragmented, both HMM and nucleosome-sized, DNA species was accumulated (Fig. 3A, lane 5), and a further 45-min incubation resulted in a significant increase in the band intensities (Fig. 3A, lane 6). If FBS was added 120 min after ActD treatment (i.e., just before the beginning of DNA fragmentation in the absence of the serum) or earlier, no fragmented products could be detected at 3.5 h (Fig. 3A, lanes 7–9). Moreover, the addition of FBS at 165 min (i.e., during the ongoing DNA fragmentation) appeared to block further DNA degradation (cf. lane 10 with lanes 5 and 6 of Fig. 3A). We conclude that DNA fragmentation could be prevented by the addition of FBS at any time between ActD treatment and the onset of visible apoptosis. Having been added at a later time, the serum blocked further fragmentation.

Similar conclusions could be derived from the experiment with the Chi-promoted apoptosis. There were very slight signs of DNA fragmentation during the first hour of the Chi treatment (Fig. 3B, lanes 2–4), but a significant amount of the fragmented (HMM and oligonucleosome-sized) DNA accumulated during the next 30 min (Fig. 3B; lane 5), with a further increase in the band densities by 2 h (Fig. 3B; lane 6). The FBS addition 1 h after the Chi-treatment (i.e., just at the apparent onset of apoptosis in the absence of the serum) completely prevented DNA fragmentation during the next hour (Fig. 3B, lane 9). Even the addition of FBS at 90 min after the Chi treatment (when the fragmentation was in progress) appeared to arrest the process since no further accumulation of the degraded DNA could be registered during the next 30 min (cf. lane 10 with lanes 5 and 6 in Fig. 3B). Thus, the serum

TABLE I. Suppressive Effect of FBS on the Apoptosis in HeLa-B Cells, As Evidenced by Microscopic Observations*

Exp. no	Treatment	Cells (%) Apoptotic	Dead
1	Control (Eagle's medium; 24 h)	0.5	7.7
	ActD (5 h)	50.9	3.1
	ActD (5 h) + FBS	7.0	3.2
	Chi (2 h)	41.7	6.1
	Chi (2 h) + FBS	0.5	3.2
2	Control (Eagle's medium + FBS; 16 h)	0.4	3.4
	Chi (1.0 h)	4.6	nd
	Chi (1.5 h)	18.0	3.3
	Chi (2 h)	29.7	2.9
	Chi (2 h) + FBS	1.5	1.9
	Chi (5 h) + FBS	0.4	1.4
	Chi (7 h) + FBS	0.9	0.9
	Chi (10 h) + FBS	0.5	2.3
3	Control (Eagle's medium + FBS; 10 h)	<0.5	3.5
	ActD (2.5 h)	0.7	1.8
	ActD (3.5 h)	20.1	3.1
	ActD (4 h) + FBS	0.5	1.9
	ActD (6 h) + FBS	1.9	3.9
	ActD (8 h) + FBS	10.4	1.4
	ActD (10 h) + FBS	15.5	5.3

*Monolayers of HeLa-B cells were preincubated with serum-free Eagle's medium for 3 h. Incubations at 36.5°C with Chi (100 μg/ml) and ActD (0.5 μg/ml) were carried for the time indicated. FBS (5%) was added 30 min after the metabolic inhibitors. Apoptotic and dead cells corresponded to the cells with cytoplasmic blebbing and stained with trypan blue, respectively.

appeared to prevent the drug-promoted apoptosis by acting at a final (downstream) checkpoint, just preceding the DNA fragmentation.

Serum Suppress Apoptosis Triggered by Nonproductive Poliovirus Infection

Nonproductive poliovirus infection of HeLa-B cells (e.g., infection with guanidine-sensitive or -dependent virus variants in the presence or absence of millimolar concentrations of guanidine. HCl, respectively, as well as infection with certain temperature-sensitive (*ts*) mutants at a nonpermissive temperature) produces a typical apoptotic response [Tolskaya et al., 1995]. As shown in Figure 4, this response was also suppressed by the addition of FBS. The accumulation of HMM DNA was clearly evident by 8 h of abortive infection (Fig. 4, lane 3); these DNA species were further partially fragmented to yield oligonucleosome-sized products from 10 h postinfection (p.i.) onward (Fig. 4, lanes 5, 7, and 9). If, however, FBS was added 30 min p.i., no DNA fragmentation was observed at 8 h (Fig. 4, lane 4), small and moderate amounts of HMM DNA were accumulated at 10 and 14 h p.i., respectively (Fig. 4, lanes 6 and 8), and some degradation to smaller species took place by 18 h (Fig. 4, lane 10). Thus, the addition of FBS resulted in at least a 6-h delay in the development of apoptotic reaction induced by abortive poliovirus infection.

The suppressive effect of FBS on the virus-induced apoptosis could be also documented by counting of cells with cytoplasmic blebbing (data not shown). It should be noted that both biochemical (DNA fragmentation) and morphological (blebbing) signs of apoptosis were observed before the increase in permeability to trypan blue, characteristic of late steps of the viral cytopathic effect (data not shown).

The onset of apoptotic reaction in the virus-infected guanidine-treated cells could be delayed by the addition of FBS at any time before the appearance of visible signs of apoptosis, and the ongoing DNA degradation could be stopped immediately by the serum (data not shown). Thus, FBS suppressed virus-induced apoptosis by acting at the most downstream segment of the death pathway, in analogy with its effect on the apoptosis triggered by the metabolic inhibitors.

The Serum Effect Can Be Partially Mimicked by EGF

To check whether the apoptosis-preventing effect of FBS was due to growth factors, the capacity of EGF to suppress development of the drug-promoted apoptosis was studied. In the experiment depicted in Figure 5A, the time course of DNA degradation after Chi treatment was essentially similar to that shown in Figure 2A, with the appearance of HMM species after 1 h. However, no DNA fragmentation could be observed at this time, if EGF (20 ng/ml) was added 15 min after the metabolic inhibitor (Fig. 5A, lane 6), showing a delay in the onset of the apoptotic reaction. This delay was rather short, however, amounting to about half an hour.

A similar conclusion could be derived from the experiment with the ActD-treated cells. Evidence for the fragmentation of chromosomal DNA (the accumulation of HMM fragments) was seen at 150 min after the addition of the

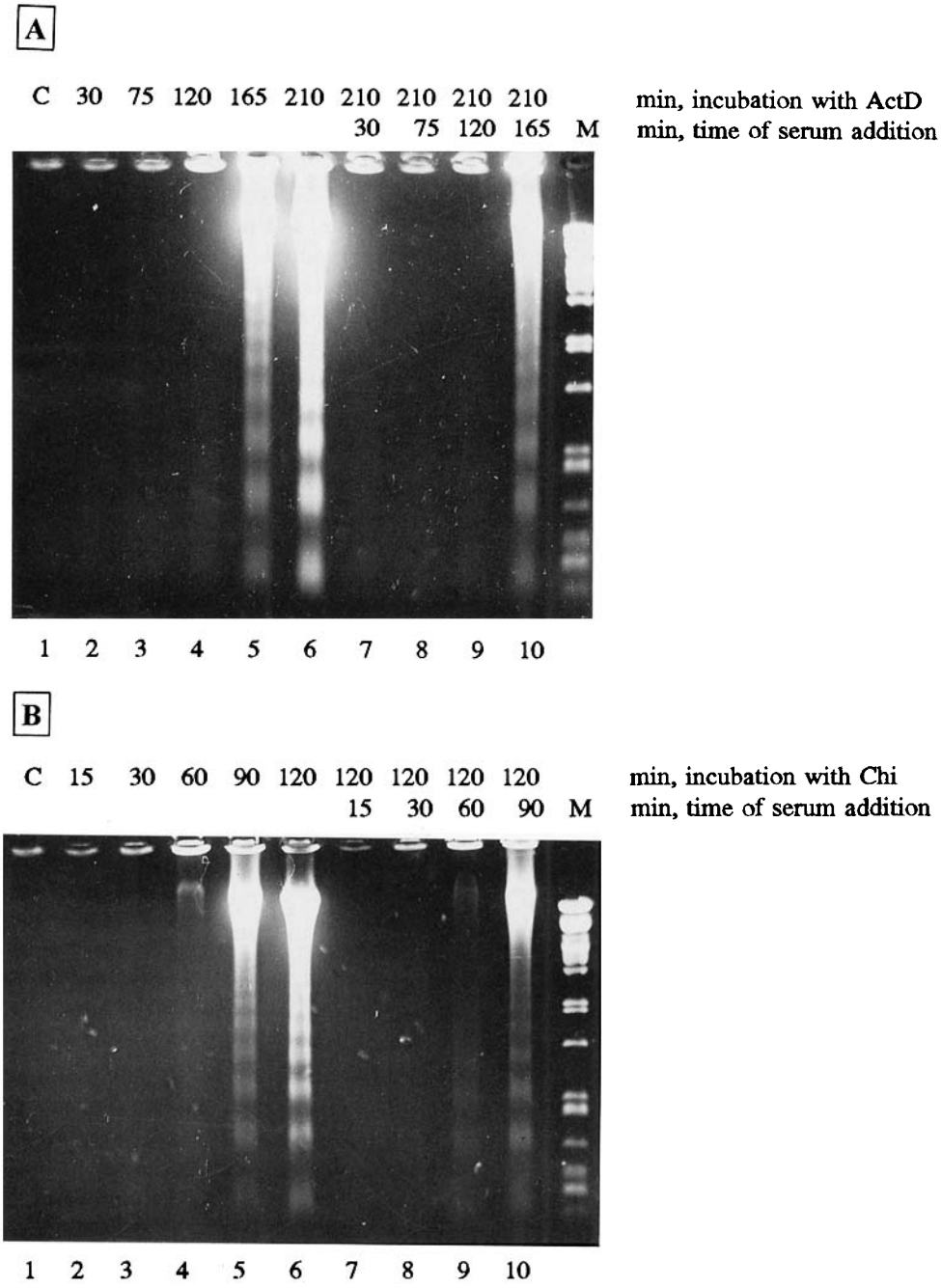

Fig. 3. Determination of the time required for the implementation of the FBS effect. After 3-h preincubation in the serum-free medium, HeLa-B cells were treated with either ActD (0.5 μg/ml for 30–210 min) **(A)**, or Chi (100 μg/ml for 15 to 120 min) **(B)**; FBS (5%) was added to appropriate drug-treated samples at the time indicated. Lane C corresponds to the untreated control cells incubated in the serum-free medium for 6.5 **(A)** and 5 **(B)** h. The electrophoretic pattern of the *Pst*I-treated phage λ DNA is shown on the lane M.

drug (Fig. 5B, lane 2). This effect was nearly completely prevented, if EGF (20 ng/ml) was added 30 min after the addition of ActD (Fig. 5B, lane 3), demonstrating that EGF did suppress the apoptotic reaction to the drug. Between 150 and 210 min of ActD treatment in the absence of EGF, there was further accumulation of large DNA fragments, as well as their degradation into oligonucleosome-sized species (Fig. 5B, lane 4). By contrast, predominantly HMM DNA accumulated during this period in the EGF-containing samples (Fig. 5B, lane 5), generating a pat-

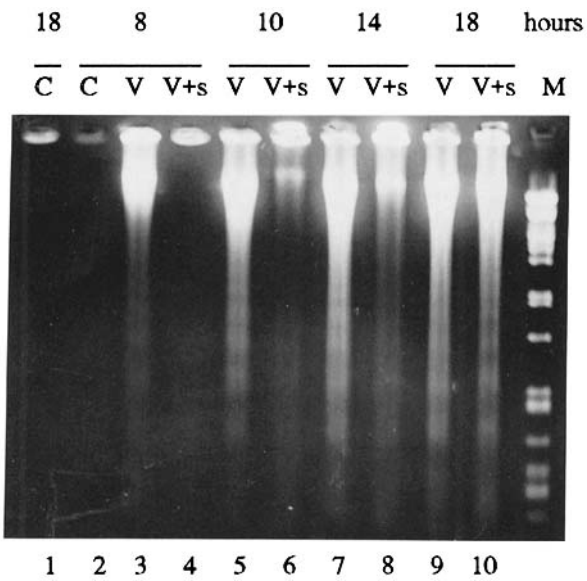

Fig. 4. Suppression by FBS of apoptosis triggered by nonproductive poliovirus infection. HeLa-B cells were infected with poliovirus in the presence of 100 μg/ml of guanidine (see Materials and Methods). HCl (samples marked V). FBS was added (to a final concentration of 5%) 30 min postinfection to appropriate samples (+s), and incubation was continued for the time indicated. C, uninfected controls incubated in the guanidine-containing serum-free medium for the time intervals indicated.

tern very similar to that observed in the absence of EGF by 150 min (Fig. 5B, lane 2). The difference between the EGF-containing and EGF-lacking samples diminished upon further incubation and seemed to disappear completely by 330 min (Fig. 5B, lanes 8 and 9). Thus, EGF-mediated suppression of the ActD-promoted apoptosis in HeLa-B cells consisted in a delay, for approximately 1 h, of the DNA fragmentation.

The suppressive effect of EGF on the time course of the apoptosis triggered by metabolic inhibitors in HeLa-B cells was confirmed by counting of cells exhibiting signs of apoptosis, cytoplasmic blebbing, and condensation and fragmentation of chromatin (data not shown).

DISCUSSION

HeLa-B cells exhibited a particularly vigorous and rapid apoptotic response to the addition of inhibitors of transcription and translation [Tolskaya et al., 1995], suggesting that their viability depends on the continuous supply of anti-apoptotic proteins. Under standard conditions, these cells grew quite well and supported efficient poliovirus reproduction. The cells were reported to contain some inclusions [Tolskaya et al., 1995], which have now been identified, by iodine staining, as a glycogen-containing material (not shown). The significance of these inclusions for the rapid reaction to ActD and Chi is unknown. Sensitization of the cells by preincubation in a serum-free medium further enhanced their responsiveness to the metabolic inhibitors [Tolskaya et al., 1995].

As in many other experimental conditions leading to apoptotic death, it is difficult to discriminate formally between the primary upstream signal and the treatments changing the cellular sensitivity to these signals. Thus, it could be argued that apoptosis in our system was triggered by nonoccupancy of the growth factor receptors caused by the preincubation in the serum-free medium. The metabolic drugs, in the framework of this reasoning, merely accelerated the inevitable development of apoptosis. It should be noted, however, that the incubation in the serum-free medium without metabolic inhibitors for up to 24 h did not result in a marked apoptotic response (cf. control samples in Figs. 3–5; unpublished observations). Therefore, it is more likely that the primary (or major) cause of apoptosis was inhibition of macromolecular synthesis, whereas preincubation only made the cells more susceptible to this metabolic alteration. In any case, the final outcome appeared to be due to a gradual shift in the balance of anti-apoptotic and pro-apoptotic factors toward the predominance of the latter. A similarity between the effects of inhibitors of transcription and translation suggests that both the putative anti-apoptotic protein(s) and its/their mRNA template(s) are metabolically unstable. An apparently more rapid development of apoptosis in response to Chi compared to ActD may be interpreted to mean that the relevant proteins turned over faster than their mRNAs.

The major finding of this study is the demonstration that the FBS addition to HeLa-B cells trated with Chi or ActD strongly suppressed apoptosis that normally occurs upon the addition of these metabolic inhibitors. The FBS addition delayed the apoptotic reaction by at least 9 and 5 h in the Chi- and ActD-treated cells, respectively. A qualitatively similar, although much more transient, suppression was provided by EGF. These results suggest that the growth factors (including EGF) could affect the intracellular balance of pro-apoptotic and anti-apoptotic activities at the post-transcriptional and post-translational levels.

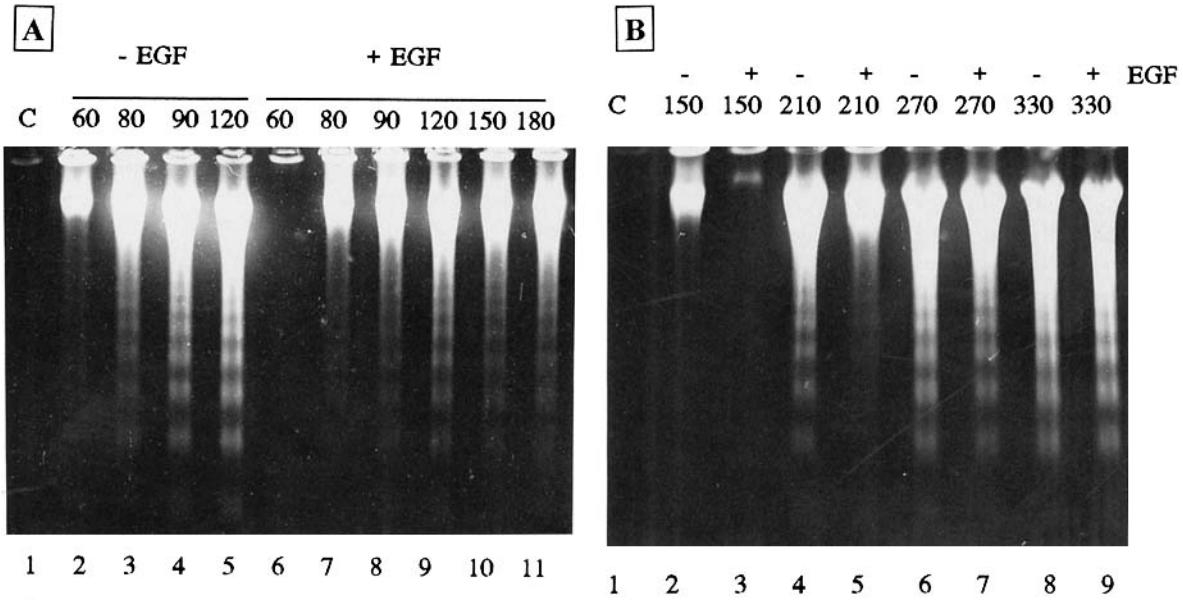

Fig. 5. Suppression by EGF of Chi-triggered (**A**) and ActD-triggered (**B**) apoptosis. The experiments were carried out as described in legend to Fig. 2 but, instead of FBS, EGF (20 ng/ml) was added at 15 and 30 min after the addition of Chi and ActD, respectively. Lanes C correspond to the untreated control cells incubated in the serum-free medium for 6 h (**A**) and 8.5 h (**B**).

The involvement of growth factors in the control of apoptosis is a firmly established fact [Raff, 1992; McCarthy et al., 1992]. Their interaction with appropriate receptors is considered to be a major factor ensuring the survival of a variety of cells. A popular view holds that appropriate receptors represent an "upstream" segment in the apoptotic pathway, and their nonoccupancy generates the primary "death signals." Such signals result in modulations of the expression of genes involved in the implementation of the apoptotic program [Colotta et al., 1992; Estus et al., 1994]. The ability of metabolic inhibitors to counteract the apoptosis-triggering effects of the withdrawal of nerve growth factor (NGF) [Martin et al., 1988], EGF [Rawson et al., 1990], or interleukin-3 (IL-3) [McCarthy et al., 1992] may be considered as a strong argument in favor of such a viewpoint.

The results reported here prove, however, that growth factors are able to affect the utmost downstream (post-transcriptional and post-translational) checkpoint of the apoptotic pathway by enhancing the relative "power" of the anti-apoptotic factor(s). The mechanism of this effect is unknown. It is tempting to speculate that it may involve ionic (e.g., Ca^{2+} or pH) changes or alterations in the concentrations of second messengers, known to occur upon the engagement of appropriate receptors [Martin et al., 1994]. Although the addition of FBS could "freeze" already ongoing DNA degradation, this fact could hardly be interpreted as evidence for direct suppression of the activity of the nuclease responsible for the degradation. It seems more likely that FBS prevented the effector step in the cells destined for apoptosis but still having not entered the "point-of-no-return" stage.

The inability of EGF to ensure an anti-apoptotic effect quantitatively comparable to that of FBS was unlikely due to the inadequate concentration of this growth factor, since no clear concentration dependence was observed within the range of 10–40 ng of EGF per ml (unpublished observations). Thus, other growth factors contained in the FBS should cooperate with EGF to achieve a maximal response. The identification of these factors and elucidation of the mechanism of their action require further studies.

It should be noted that several other systems in which growth factors appear to exert an anti-apoptotic effect at the post-transcriptional and post-translational levels were recently described. Thus, EGF (among other compounds) was reported to enhance the survival of cells of a human cancer cell line treated with Adriamycin, a DNA topoisomerase II inhibitor, in the presence of Chi [Geier et al., 1994a]. On the basis of electron microscopy and DNA electrophoresis, the authors suggested that the death of the Adriamycin-treated cells was due to a combina-

tion of apoptosis and necrosis. The same group also reported on protective effects of EGF and of insulin-like growth factor-1 (IGF-1) on the viability of certain cancer cells treated with ActD or Chi, but in this case the death of the drug-treated cells was ascribed to necrosis, rather than apoptosis [Geier et al., 1993, 1994b]. IGF-1 suppressed the development of Chi-promoted apoptosis in the serum-deprived Myc-expressing cells of a rat fibroblast line [Harrington et al., 1994a]. It was also reported that NGF prevented development of the serum deprivation-induced apoptosis in rat pheochromocytoma (PC12) cells in the presence of Chi [Batistatou and Greene, 1993] and that the pretreatment with NGF partially suppressed the apoptotic reaction of these cells triggered by Chi or ActD treatment [Lindenboim et al., 1995].

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