Independent Regulation of ppp(A2'p)nA-dependent RNase in NIH 3T3, Clone 1 Cells by Growth Arrest and Interferon Treatment*

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The regulation of ppp(A2'p)nA-(2-5A)-dependent RNase (RNase L or RNase F) was investigated in NIH 3T3, clone 1 cells using 2-5A-binding and nuclease activity assays. Minimal levels of 2-5A-dependent RNase were detected in actively dividing clone 1 cells; these levels were independently induced by growth arrest or interferon treatment. Accordingly, levels of the RNase were enhanced during growth arrest by confluency regardless of the presence or absence of interferon or antibody to interferon in the media. Measurement of 2-5A-dependent RNase was unaffected by the addition of any of six different proteinase inhibitors to the cells prior to extraction. The expression of 2-5A-dependent RNase in growth-arrested, interferon-treated cells was still relatively low (about one-third to one-half of that found in similarly treated murine Ehrlich ascites tumor cells). Although this amount of 2-5A-dependent RNase could not be detected by 2-5A-mediated ribosomal RNA cleavage, the activity was identified using a more sensitive novel assay for 2-5A-dependent RNase. In addition, introduction of 2-5A or poly(I)·poly(C) into growth-arrested, interferon-treated cells resulted in some inhibition of protein synthesis. The results indicated that the expression of 2-5A-dependent RNase in NIH 3T3, clone 1 cells is regulated under different physiological conditions and that low levels of 2-5A-dependent RNase were insufficient to significantly inhibit encephalomyocarditis virus replication.

The 2-5A-(ppp(A2'p)nA, n ≥ 2)-system (1) includes at least three types of enzymatic activities (reviewed in Ref. 2): 1) 2-5A-synthetases, double-stranded RNA-dependent enzymes that catalyze the synthesis of 2-5A from ATP, 2) 2',5' phosphodiesterase activity that degrades 2-5A to ATP and AMP, and 3) 2-5A-dependent RNase (RNase L or RNase F) which mediates effects of 2-5A in cells by cleaving single-stranded RNA at UpNp sequences, leaving 3' phosphorylated RNA (2). 

Cell Extracts

Cells were harvested by scraping and were then washed three times by centrifuging and resuspending in ice-cold phosphate-buffered saline (PBS). The cells were lysed with Nonidet P-40 containing buffer (0.5% (v/v) Nonidet P-40, 1% sodium deoxycholate, 0.1% sodium salicylate, 10 mM HEPES (pH 7.6), and 2 mM 2-mercaptoethanol). The buffer was supplemented just prior to cell extraction with 50% glycerol.

MATERIALS AND METHODS

Cell Culture

NIH 3T3, clone 1 cells (obtained from Dr. R. M. Friedman, USUHS) were grown in monolayer cultures in Eagle's MEM, containing 10% fetal bovine serum. Cells were harvested by scraping and were then washed three times by centrifuging and resuspending in ice-cold phosphate-buffered saline (PBS). The cells were lysed with Nonidet P-40 containing buffer (0.5% (v/v) Nonidet P-40, 1% sodium deoxycholate, 0.1% sodium salicylate, 10 mM HEPES (pH 7.6), and 2 mM 2-mercaptoethanol). The buffer was supplemented just prior to cell extraction with 50% glycerol.

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† The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PBS, phosphate-buffered saline; EAT, Ehrlich ascites tumor; EMCV, encephalomyocarditis virus; MEM, minimal essential medium; TDR, thymidine; CPE, cytopathic effect.
ppp(A2'p)A-dependent RNase Levels in NIH 3T3, Clone 1 Cells

100 μg/ml of leupeptin and 2 mM PMSF (in the experiments shown in Figs. 1, 2, and 5). Either complete cell lysates or postmitochondrial supernatant (centrifuged at 10,000 × g, 20 min at 4 °C) fractions were used. The protein concentrations were determined by the Bio-Rad protein assay (17).

Assays for 2-5A-dependent RNase

The Radiobinding Assay (18)—Cell extracts (200 μg of protein/assay) were incubated at 0 °C in 10 mM Tris-HCl, pH 7.6, 2 mM magnesium acetate, 0.4 mM ATP, 2% (v/v) glycerol, and between 5,000 and 15,000 cpm of ppp(A2'p)A[32P]pCp (subsequently referred to as "probe"). The probe was prepared as described previously (19) using unlabeled poly(U) (gifts from I. M. Kerr, London, or P. F. Torrence, Bethesda) as acceptor. The amount of radioactivity bound to protein was determined by its retention on nitrocellulose filters (18). The bound radioactivity from assays containing 250 μm unlabeled trimer 2-5A was taken as "background" and subtracted to obtain the results.

The level of radiobinding activity for each sample was an average of duplicate determinations.

Affinity Labeling of 2-5A-dependent RNase (20)—The probe (enough for 100,000 cpm/assay, 1 nM final concentration) was digested with bacterial alkaline phosphatase (5 units/ml) on ice for 30 min and then at 4 °C for an additional 30 min. The digested material, p(A2'p)A[pCp] (n = 1 to 3, was added to 2 mM ethylenediaminetetraacetic acid, heated to 90 °C for 5 min, and centrifuged at 10,000 × g for 5 min, and the supernatant was removed. Sodium metaperiodate (10 mM) was added to the digested probe (in the supernatant fraction) and incubated at room temperature in the dark for 60 min. This material (100,000 cpm) was incubated for 2 h at 0 °C with cell extract (200 μg of cell protein) adjusted to 2.5 mM magnesium acetate. 1.0 mM ATP, pH 7, in a final volume of 14 pl.

A further 1 h at 0 °C. Proteins were separated by electrophoresis on a sodium dodecyl sulfate-10% polyacrylamide gel and the 32P-labeled proteins were visualized by autoradiography on Kodak SB-5 or XAR-5 x-ray film.

2-5A-dependent RNase Assay Using Core (2-5A)-cellulose—The assay for 2-5A-dependent RNase using core (2-5A)-cellulose was performed as described previously (21). Briefly, postmitochondrial supernatant cell fractions (150 μg of protein/assay time point) were incubated with 25 μM (AMP equivalents) of (A2'p)A-cellulose on ice for 1 h in buffer C (11.5 mM HEPES, pH 7.6, 104 mM potassium chloride, 5.8 mM magnesium acetate, 8.8 mM 2-mercaptoethanol, 1.2 mM ATP, and 100 μg/ml of leupeptin). The 2-5A-dependent RNase-cellulose complex was then washed three times by centrifuging (700 × g for 5 min at 2 °C), dissecting the supernatant, and resuspending in greater than 10 (packed cellulose) volumes of buffer C. The washed, 2-5A-dependent RNase-cellulose was resuspended in buffer C containing 100 μm unlabeled trimer 2-5A and poly(U)-3' [32P]Cp (8 μM in phosphorus equivalents prepared as described in Ref. 21) at about 125 Ci/ml and then incubated at 30 °C. RNA breakdown was determined by pipetting aliquots (20 μl) of the incubation mixtures into 1 ml of 5% trichloroacetic acid, 0.2% pyrophosphate, plus 100 μl of 5 μg/ml of (carrier) yeast RNA on ice. Samples were filtered on No. 30 glass fiber circles (Schleicher & Schuell) and washed twice with 10 ml of ice-cold 5% trichloroacetic acid, 0.2% pyrophosphate and once with 5 ml of 95% ethyl alcohol. The radioactivity on the dried filters was determined by scintillation counting.

Assay of Protein Synthesis in Cells Treated with or without 2-5A (22) or Poly(I)-Poly(C) (23)

Cells were seeded in multiwell plates (16-mm wells) at cell densities of 100,000 cells/well in RPMI medium containing 10% calf serum. After 24 h, the medium was replaced with fresh medium containing interferon at 100 units/ml. Eighteen hours later the cells were washed with 1.0 ml of Eagle's MEM medium and incubated for 1 h in 0.45 ml of the same medium. Transfection mixtures were freshly prepared before each experiment as described previously (23): 2-5A or poly(I)-poly(C) solutions, in water, were diluted in the transfection buffer, and calcium chloride (2.5 M) was added to each dilution to a final concentration of 114 mM. After 10 min at room temperature the transfection mixtures (30 μl) were added to the wells containing 0.45 ml of MEM to give the final concentrations of 2-5A or poly(I)-poly(C) indicated in Fig. 6. The plates were incubated at 37 °C for 2 h, the transfection mixtures were aspirated, and 1 ml of MEM containing 10% calf serum was added for an additional 2 h. For protein synthesis determinations, the medium was changed to 0.5 ml of MEM minus leucine, containing 4 μCi/ml of [3H]leucine for 1 h at 37 °C. Radioactivity incorporated into trichloroacetic acid-insoluble material was determined as described previously (23).

Antiviral Activity

This was determined after incubation of the cells in multiwell plates (16-mm wells) for 20 h with interferon. The cells were then washed twice with Dulbecco's PBS and incubated for 24 h in Eagle's MEM containing 2% fetal bovine serum, 1 mM sodium pyruvate and ITS premix (Collaborative Research). The virus titers from the culture supernatants were determined by CPE using murine L-cells.

Incorporation of [3H]Thymidine into DNA

The cells were grown as previously described, in 100 × 15-mm culture dishes with 10% medium. The cells were incubated in the presence of 1.0 μCi/ml of [3H]thymidine (Amersham, 51 Ci/mmol) for 60 min at 37 °C. Each plate was rinsed with 10 ml of ice-cold PBS containing 1.0 mM unlabeled thymidine (PBS-TdR). The cell monolayers were then scraped into 10 ml of fresh ice-cold PBS-TdR and centrifuged at 400 rpm for 5 min. The cell pellets were resuspended in 1.0 ml of ice-cold PBS-TdR to which was added 1.0 ml of ice-cold 10% trichloroacetic acid. After 10 min at 0 °C the material was filtered through glass fiber filters and washed 2 times with 5.0 ml of ice-cold 5% trichloroacetic acid and then with 5.0 ml of 95% ethyl alcohol. The radioactivity on the filters was determined by scintillation counting.

RESULTS

Proteinase Inhibitors Had No Effect on Levels of 2-5A-dependent RNase Measured from Clone 1 Cells—Prior to investigating the regulation of 2-5A-dependent RNase levels in clone 1 cells, it was necessary to establish that the nuclease could be measured in the absence of interfering proteolytic activities. For instance, previous reports on clone 1 cells (16) and teratocarcinoma cells (10) indicated degradation of 2-5A-dependent RNase by PMSF- and leupeptin-sensitive proteinases, respectively. Therefore, to determine if the 2-5A-dependent RNase in clone 1 cells was degraded during or after cell lysis, six different proteinase inhibitors were added to the cell lysis and assay buffers just prior to extraction of the cells. Levels of 2-5A-dependent RNase were estimated from the ability of the nuclease to bind a radioactively labeled 2-5A derivative (probe) (19).

Addition of the proteinase inhibitors did not enhance the level of probe binding activity of 2-5A-dependent RNase measured from extracts of late-confluent clone 1 cells; about 6 to 8% of the added probe was bound in all of the assays (Table I). These levels of 2-5A-dependent RNase were low when compared to those in control murine EAT or L-cells which typically bound about 25–30% of the probe. In addition, levels of 2-5A-dependent RNase were determined by an affinity labeling method in which a radioactive 2-5A derivative is

<table>
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<th>Probe bound (%):</th>
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<tbody>
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<td>PMSF</td>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Leupeptin</td>
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</tr>
<tr>
<td>Aprotinin</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Benazamidine</td>
<td>6.6</td>
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</tr>
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</table>

* 200 μg of cell protein present in postmitochondrial supernatant fractions of late-confluent clone 1 cells was used in each assay.
covalently linked to nuclease (see Ref. 20 and “Materials and Methods”). As shown in Fig. 1, the level of 2-5A-dependent RNase (see arrow) in control EAT cells (lane 1), included for comparison, was much greater (about 10-fold as determined by densitometer scanning, not shown) than in the subconfluent clone 1 cells (lane 3). Furthermore, there was no increase in levels of 2-5A-dependent RNase from clone 1 cells with the addition of PMSF (2 mM) to the buffers (Fig. 1, compare lanes 3 and 5). The labeling of 2-5A-dependent RNase from EAT and clone 1 cells was greatly decreased by the addition of unlabeled 2-5A at 250 nM (Fig. 1, lanes 2, 4, and 6). Also, by using the affinity labeling method, there was no significant difference in levels of 2-5A-dependent RNase beween clone 1 cells that were extracted and assayed with or without pepstatin, leupeptin, aprotinin, trypsin inhibitor, or benzamidine (data not shown). In our experience, therefore, there was no significant breakdown of 2-5A-dependent RNase during or after cell lysis.

Induction of 2-5A-dependent RNase in Clone 1 Cells during Growth Arrest—It was clear from the results presented in Fig. 1 that control, actively growing clone 1 cells contained only very low levels of 2-5A-dependent RNase. It was of interest, therefore, to determine if the levels of 2-5A-dependent RNase found in clone 1 cells could be enhanced. Monolayer cultures of clone 1 cells were harvested at various times before and after reaching a quiescent state. As shown in Fig. 2A, the percent probe bound (an indirect measure of 2-5A-dependent RNase) increased about 5-fold by 7 days. The major part of the increase occurred after cell confluency on day 3. Cell growth was progressively inhibited after confluency as was [3H]thymidine incorporation into DNA (Fig. 2A). Furthermore, using cell extracts from the same experiment, a gradual increase in 2-5A-dependent RNase in clone 1 cells during growth arrest was confirmed by affinity labeling of 2-5A-dependent RNase (Fig. 2B, 2-5A-dependent RNase is indi-
with various doses of interferon (as indicated) of subconfluent (actively growing, 0) or late-confluent (growth-arrested, Δ) clone 1 cells. The cells were either untreated or treated with various doses of interferon (as indicated) for 20 h.

The experiments described thus far were performed using cytoplasmic fractions of cells. 2-5A-dependent RNase levels were, however, also determined in complete cell lysates which contained all of the cell protein. In this manner, cytoplasmic plus nuclear (24) levels of 2-5A-dependent RNase were measured using the affinity labeling method (Fig. 5). Interferon treatment clearly enhanced levels of 2-5A-dependent RNase (protein p80) at both low and high cell densities (compare lanes 5 and 7; and 9 and 11). Furthermore, 2-5A-dependent RNase levels were significantly higher in the cells grown to high cell densities as compared to those at low cell densities (Fig. 5). These results were quantified by densitometer scanning of the autoradiograph shown in Fig. 5 (Table I). It is apparent from this experiment and from Figs. 3 and 4 that the amount of induction of 2-5A-dependent RNase by interferon treatment and by growth arrest was slightly greater than additive. Moreover, the high density, interferon-treated clone 1 cells (lane 11) contained about 13-fold greater levels of 2-5A-dependent RNase than did the control, low density clone 1 cells (lane 5). However, the level of 2-5A-dependent RNase in growth-arrested, interferon-treated clone 1 cells reached only about one-half and one-third that found in control and interferon-treated EAT cells, respectively (Table II and Fig. 5).

**Fig. 3.** Induction of 2-5A-dependent RNase by interferon in actively growing and in growth-arrested clone 1 cells. Levels of 2-5A-dependent RNase were determined using the radiobinding assay in postmitochondrial extracts (200 μg of cell protein) of subconfluent (actively growing, O) or late-confluent (growth-arrested, Δ) clone 1 cells. The cells were either untreated or treated with various doses of interferon (as indicated) for 20 h.

**Induction of Functional 2-5A-dependent RNase in Clone 1 Cells by Interferon and Growth Arrest**—The effect of interferon treatment of the cells on levels of 2-5A-dependent RNase was then investigated. Clone 1 cells were cultured to subconfluent (low) or late-confluent (high) densities and then incubated for 24 h with or without interferon α + β (Fig. 3). The nuclease was induced by interferon in a dose-dependent manner in either subconfluent or late-confluent cells. Maximal induction was with about 5,000 units/ml of interferon regardless of prior induction by growth arrest (Fig. 3).

Additional experiments were then performed to determine whether the regulation of 2-5A-dependent RNase during growth arrest was independent of interferon. Therefore, the nuclease was measured prior to and during confluency from cells treated continuously with 5,000 units/ml of interferon (Fig. 4A). There was a 5-fold induction of the nuclease during the first 24 h (i.e. by day 1) of interferon treatment; thereafter, the level of the nuclease reached a plateau until cell confluency (day 5) resulted in a further 6-fold increase. The control cells showed a similar induction during growth arrest only without the initial increase on day 1 (Fig. 4A). To determine whether the induction during growth arrest could be a result of spontaneously produced interferon, cells were grown in the continuous presence of antibody to interferon α + β (1,000 neutralizing units/ml). The antibody, however, failed to prevent the induction of the nuclease during confluency (Fig. 4A). It is unlikely, therefore, that the induction was due to interferon produced by the cells. Growth curves for the three cultures were virtually indistinguishable (Fig. 4B), thus confirming an earlier report that showed clone 1 cells were resistant to the growth inhibitory effect of interferon (13).

Confirmation that the nuclease that was induced in clone 1 cells was in fact functional in the cleavage of RNA was obtained using a novel assay (see "Materials and Methods" and Ref. 21). The nuclease was immobilized and partially purified from cell extracts using core (2-5A)-cellulose. The cellulose-bound enzyme was then assayed by measuring the conversion of poly(U)-[32P]Cp to acid-soluble fragments in the presence or absence of added 2-5A. There was only slight...
**ppp(A2'p)n-A-dependent RNase Levels in NIH 3T3, Clone 1 Cells**

**Fig. 4.** Independent induction of 2-5A-dependent RNase by growth arrest or interferon treatment.

A. 2-5A-dependent RNase levels. Clone 1 cells were seeded at a subconfluent, actively growing state (time zero) and then allowed to grow to a less-confluent, growth-arrested state. 2-5A-dependent RNase was determined by radiobinding in postmitochondrial extracts (200 μg of cell protein) from cells that were untreated (○) or treated continuously with either interferon α + β (5,000 units/ml) (△) or with antibody to interferon (1000 units/ml) (□). B, cell growth curves. Cell numbers were determined for the three cultures as described (see "Materials and Methods"). Cell confluence was on day 5 (arrows) for all three cultures.

<table>
<thead>
<tr>
<th>Cell Line:</th>
<th>EAT</th>
<th>Clone 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell density:</td>
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<td>Low</td>
</tr>
<tr>
<td>IFN:</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Unlabeled 2-5A:</td>
<td>-</td>
<td>+</td>
</tr>
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<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
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<tbody>
<tr>
<td>% Probe Bound</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

**Fig. 5.** Induction of 2-5A-dependent RNase by growth inhibition and interferon treatment as measured in complete cell lysates. Cells were grown to high (3 days postconfluent) and low (subconfluent) densities and incubated for 20 h with or without

**Table II**

Induction of 2-5A-dependent RNase in NIH 3T3, clone 1 cells by interferon and/or growth arrest

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell density</th>
<th>Interferon</th>
<th>2-5A-dependent RNase</th>
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</thead>
<tbody>
<tr>
<td>Clone 1</td>
<td>Low</td>
<td>-</td>
<td>32</td>
</tr>
<tr>
<td>Clone 1</td>
<td>Low</td>
<td>+</td>
<td>147</td>
</tr>
<tr>
<td>Clone 1</td>
<td>High</td>
<td>-</td>
<td>197</td>
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<td>Clone 1</td>
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<td>+</td>
<td>410</td>
</tr>
<tr>
<td>EAT</td>
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<td>864</td>
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<tr>
<td>EAT</td>
<td>High</td>
<td>+</td>
<td>1238</td>
</tr>
</tbody>
</table>

Units are arbitrary peak area values determined by densitometer (GS 300, Hoefer Scientific Instruments) scanning of the autoradiograph shown in Fig. 5 calculated using a Shimadzu Corp. chromatopac C-RIB data processor.

2-5A-dependent RNase activity isolated from the control, subconfluent cells (Fig. 7). The level of 2-5A-dependent RNase was enhanced in cells treated with interferon or in 1,000 units/ml of interferon (IFN) as indicated. Complete cell lysates were prepared by lysing cells in an Nonidet P-40 containing buffer without subsequent centrifugation (see "Materials and Methods"). Equivalent amounts of cell lysate (200 μg of protein) were affinity-labeled with 1 nM of a 32P-labeled 2-5A derivative in the presence or absence of 250 nM unlabeled trimer 2-5A as indicated. The positions of the protein markers (numbers to the left) represent their molecular weights × 10^-5 and the 2-5A-dependent RNase (labeled p80) are indicated. An autoradiograph of the dried gel is shown.
was no detectable inhibitor of 2-5A-dependent RNase in crude extracts of clone 1 cells. Addition of 2-5A and purified mouse L-cell 2-5A-dependent RNase to an extract of clone 1 cells resulted in specific 2-5A-mediated ribosomal RNA cleavage (Fig. 6). The results are the average of four replications.

The relatively low levels of 2-5A-dependent RNase present in interferon-treated, growth-arrested clone 1 cells were, however, insufficient to produce detectable 2-5A-mediated cleavage products from ribosomal RNA (25) using either cell-free systems or intact cells after calcium phosphate co-precipitation procedures described previously (12, 22, 23). Protein synthesis (determined as described under "Materials and Methods") in control cultures treated with calcium phosphate but in the absence of the poly(I)-poly(C) or 2-5A was taken as 100% incorporation. Cell confluency occurred after about 4 days in culture (arrow). The results are the average of four replications.

Resistance of subconfluent and late-confluent NIH 3T3, clone 1 cells to the anti-EMCV activity of interferon.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>State of monolayer</th>
<th>Interferon</th>
<th>EMCV growth (log virus titer)</th>
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<tr>
<td></td>
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</tr>
<tr>
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<td>7.8</td>
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<td>7.3</td>
</tr>
<tr>
<td>Clone 1</td>
<td>Subconfluent</td>
<td>500</td>
<td>7.3</td>
</tr>
<tr>
<td>Clone 1</td>
<td>Subconfluent</td>
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<td>7.3</td>
</tr>
<tr>
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<td>Subconfluent</td>
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<td>ND</td>
</tr>
<tr>
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<tr>
<td>Clone 1</td>
<td>Late-confluent</td>
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<tr>
<td>Clone 1</td>
<td>Late-confluent</td>
<td>500</td>
<td>7.3</td>
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<td>Late-confluent</td>
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<td>Late-confluent</td>
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<td>L</td>
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*ND, not done.

The results are the average of four replicates.

**DISCUSSION**

Nilsen et al. (16) reported that addition of PMSF prior to cell homogenization facilitated detection of 2-5A-dependent RNase from NIH 3T3, clone 1 cells. In contrast, we could detect no significant differences in levels of 2-5A-dependent RNase from clone 1 cells by addition of PMSF or five other protease inhibitors to the cell lysis buffer (Table I and Fig. 1). The cell line used by Nilsen et al. (16) may, therefore, have been a variant strain of the original clone 1 cells with elevated levels of PMSF-sensitive protease (although their clone 1...
cells and ours were both obtained from the same source). In a teratocarcinoma cell line, PYS, we did, however, detect a leupeptin-sensitive proteinase that cleaved 2-5A-dependent RNase into discrete 2-5A-binding polypeptides (10).

Previous investigations have indicated that the expression of 2-5A-dependent RNase is highly regulated in certain cell lines (6, 9, 10). For instance, 2-5A-dependent RNase levels were greatly enhanced in murine embryonal carcinoma cells during cell differentiation (10). In addition, there was a 10- to 20-fold increase in 2-5A-dependent RNase levels in JLS-V9R cells in response to interferon treatment (6). The induction of enzyme during growth arrest was not due to the spontaneous production of interferon by the cells. Moreover, the nuclease by interferon treatment regardless of whether the induction involved transcriptional regulation (6). In that report, as in this study, the nuclease was obtained in JLS-V9R cells during growth inhibition by confluency (9).

In a separate study, a 6- to 8-fold induction of 2-5A-dependent RNase was obtained in JLS-V9R cells during growth inhibition by confluency (9). In that report, as in this study, the induction of enzyme during growth arrest was not due to the spontaneous production of interferon by the cells. Moreover, in clone 1 cells there was a further enhancement in levels of the nuclease by interferon treatment regardless of whether the interferon was added to the cells prior to or following growth arrest (Figs. 3-5). It is, therefore, apparent that the nuclease is independently controlled by interferon treatment or growth arrest.

The levels of 2-5A-dependent RNase were dramatically elevated in growth-arrested, interferon-treated clone 1 cells. The final level obtained was about one-third to one-half of that found in EAT or L-cells; however, there was little or no inhibition of EMCV replication in these cells (Table III). There are at least two possible explanations; either there is a threshold level of 2-5A-dependent RNase that must be achieved to obtain an antiviral state or the clone 1 cells are defective in another antiviral factor. However, clone 1 cells are not defective for 2-5A-synthetase, double-stranded RNA-dependent protein kinase, and 2'-5' phosphodiesterase (11). Therefore, any such defects would have to be in other unrecognized antiviral elements. It is clear from this report, however, that the control of 2-5A-dependent RNase levels in clone 1 cells is very complex, being linked to both the interferon system and to growth regulation.

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REFERENCES