Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5763–5767, August 1989
Biochemistry

Autoregulatory control of β-tubulin mRNA stability is linked to translation elongation

(gene expression/RNA degradation)

DAVID A. GAY*, SANGRAM S. SISODIA†, AND DON W. CLEVELAND

Department of Biological Chemistry, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

Communicated by M. Daniel Lane, May 5, 1989

ABSTRACT **Tubulin synthesis in animal cells is controlled in part by an autoregulatory mechanism that modulates the stability of ribosome-bound tubulin mRNAs. For β tubulin, the initial recognition event for this selective RNA instability has previously been shown to be a cotranslational binding (presumably by tubulin itself) to the nascent amino-terminal β-tubulin tetrapeptide just after it emerges from the ribosome. Although this "autoregulation" of tubulin expression is thus obligatorily linked to the translation process, the mechanism of how a cotranslational protein–protein binding event ultimately triggers RNA degradation is unknown. Using protein synthesis inhibitors to slow and ultimately to block translation elongation, we now show that the mRNA destabilization pathway requires ongoing ribosome translocation.**

In most animal cells, the appropriate quantitative level of tubulin expression is established in part through an autoregulatory pathway in which the apparent intracellular concentration of tubulin heterodimers (comprising one α- and one β-tubulin polypeptide) modulates the stability of tubulin mRNAs (1–7). Evidence for such a self-regulated pathway emerged initially from experiments with inhibitors that induced microtubule depolymerization: the corresponding rise in the intracellular concentration of unassembled subunits leads to a rapid and specific depression of synthesis of both α- and β-tubulin polypeptides. This decrease in synthesis [induced either by treatment with microtubule-destabilizing drugs or by direct microinjection of tubulin subunits (8)] was next shown to be the result of a posttranscriptional mechanism that alters the stability of cytoplasmic tubulin mRNAs after changes in the concentration of unpolymerized tubulin subunits (3–5, 9). The sequences that are necessary and sufficient to specify β-tubulin mRNAs as substrates for this autoregulated instability have been shown by DNA transfection experiments (6) to lie within the first 13 translated nucleotides [which encode the first four β-tubulin amino acids (Met-Arg-Glu-Ile)]. Further, by using inhibitors of protein synthesis and transfection of genes bearing premature translation termination codons, it was determined that only tubulin mRNAs that are attached to polyribosomes are destabilized by increased subunit concentrations (6, 10). Most recently, introduction of 25 different nucleotide base substitutions into this 13-base regulatory element has documented that β-tubulin RNAs are selectively targeted as substrates for destabilization not through recognition of specific RNA sequences but rather through cotranslational recognition of the amino-terminal β-tubulin tetrapeptide after its emergence from the ribosome (7).

Many important details of this mRNA instability pathway remain to be determined. For example, it is not known how the putative interaction involving the nascent tubulin polypeptide and a cellular factor(s) (presumably the tubulin heterodimer) can be transduced through the ribosome to yield enhanced degradation of the corresponding mRNA. Among the most attractive possibilities are: (*i*) the binding event could activate a cellular RNase (which itself might be a peripheral ribosome component), or (*ii*) binding could induce a transient stalling of the ribosome, which leaves the RNA in an exposed confirmation that is a better substrate for a nonspecific RNase. It is also possible that both activation of an RNase and ribosome stalling may be induced through the initial binding event to the nascent β-tubulin amino terminus. In any case, the precise role of continuing translation elongation remains unclear.

Our earlier efforts using cycloheximide to slow protein synthesis elongation rates to roughly 90–95% of their control values had indicated that slowing translation elongation enhanced the specific degradation of tubulin RNAs in response to increases in the subunit content. However, this experiment did not eliminate the possibility that translation elongation was indeed required for the mRNA destabilization if the residual (albeit reduced) translation rate was still sufficient for efficient signal transduction. This possibility became all the more likely in view of similar protein synthesis inhibition experiments done in developing sea urchin embryos (11), which found tubulin mRNA autoregulation to be disrupted by emetine, a drug that principally inhibits translation elongation.

To determine whether continuing translation elongation was required for the autoregulated destabilization of β-tubulin mRNAs, we investigated tubulin RNA stability after slowing elongation to various extents with each of three different inhibitors known to block translation elongation [cycloheximide (12, 13), anisomycin, and emetine (reviewed in ref. 14)]. Our results show that continued translation elongation is an obligatory step in the autoregulated degradation of polysome-bound β-tubulin mRNAs. This example, along with evidence linking translation and stability of other mRNAs, suggests a general model for cotranslational mRNA degradation in eukaryotes.

MATERIALS AND METHODS

Inhibitor Titrations. Mouse Ltk⁻ fibroblasts were plated in 24-well dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, penicillin, and streptomycin. When the cells were 95% confluent, the medium was replaced with 0.5 ml of fresh medium containing the appropriate concentration of each inhibitor. After incubation at 37°C for 35 min for cycloheximide and anisomycin and 105 min for emetine, the cells were washed twice in DMEM lacking L-methionine and 10% fetal bovine

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: La Jolla Cancer Center, 10901 North Torrey Pines Boulevard, La Jolla, CA 92037.
†Present address: Department of Neuropathology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

serum that had been prewarmed to 37°C. The final wash was removed, and the cells were incubated for 10 min at 37°C in 70 μl of methionine-free medium containing the appropriate amount of each inhibitor and 60 μCi (1 Ci = 37 GBq) of L-[^{35}S]methionine. The cells were washed twice in cold phosphate-buffered saline (PBS) and then lysed in 100 μl of 0.5% sodium dodecyl sulfate/50 mM Tris, pH 6.8, followed by boiling for 5 min. Samples were stored at −20°C.

Determination of Trichloroacetic Acid-Precipitable [^{35}S]-Methionine-Labeled Proteins. Boiled samples were centrifuged for 5 min in a microcentrifuge, the supernatant was removed, and equal aliquots from each sample were spotted (in triplicate) on 2.5-cm squares of 3 MM filter paper that had been pretreated in 10 mM methionine and dried. The spotted samples were allowed to dry and then precipitated for 15 min in ice-cold 5% CCl_3COOH containing 10 mM methionine followed by two 10-min washes in ice-cold 5% CCl_3COOH. The final wash was replaced with fresh 5% CCl_3COOH and boiled for 10 min. The filters were allowed to cool to room temperature, rinsed twice in 95% ethanol, once in ether, and baked until dry. The baked filters were placed in scintillation fluid and assayed in a Beckman LS 3801 scintillation counter.

Cytoplasmic RNA Analysis and Inhibitor Treatments. Mouse Ltk$^-$ fibroblasts, at 95% confluency, were pretreated for 45 min in fresh medium containing the appropriate concentration of inhibitor, followed by cotreatment with inhibitor and 10 μM colchicine for an additional 3 hr. Cytoplasmic RNA was prepared and assayed by S1 nuclease protection as described (5).

Polysomal RNA Analysis. After the appropriate drug treatments, polysomes were prepared from 100-mm dishes of Ltk$^-$ fibroblasts by scraping the monolayer in lysis buffer (10 mM Tris, pH 8.6/0.14 M NaCl/5 mM $MgCl_2$/0.5% Nonidet P-40/10 mM vanadyl–ribonucleoside complexes/100 μg of cycloheximide per ml). The lysate was mixed in a Vortex mixer for 10 sec and centrifuged for 4 min at 4°C in a microcentrifuge to remove nuclei. Equal OD_{260} units of each lysate were layered over a 15–40% linear sucrose gradient (10 mM Tris, pH 8.6/5 mM $MgCl_2$/100 mM NaCl) and centrifuged in an SW 41 rotor at 32,500 rpm for 2.0 hr. Fractions (540 μl) were collected by using an ISCO gradient fractionator and UV monitor. The fractions were immediately frozen on dry ice and stored at −70°C. RNA was isolated by the addition of sodium dodecyl sulfate and proteinase K to 0.5% and 0.2 mg/ml, respectively, followed by a 30-min incubation at 37°C. The RNA was extracted by the addition of an equal volume of 1:1 (vol/vol) phenol/chloroform and then precipitated with ethanol. The RNA was resuspended in 10 μl of diethyl pyrocarbonate-treated distilled H_2O, and equal volumes of each fraction (3 μl) were assayed by the S1 nuclease protection procedure.

RESULTS

Although previous results with cycloheximide showed an enhanced degradation of β-tubulin mRNAs when protein synthesis levels were inhibited to about 90% of control values (10), we wanted to investigate further the question of whether continuing translation elongation is obligatory for the destabilization of β-tubulin mRNAs in response to elevation in the unassembled tubulin subunit concentrations. To do this, we treated mouse L cells with increasing concentrations of each of three translation elongation inhibitors (cycloheximide, anisomycin, and emetine). The levels of remaining protein synthesis were determined by pulse-labeling with [^{35}S]-methionine (Fig. 1 *A–C*); as expected, at the highest concentrations utilized, each of the three elongation inhibitors yielded levels of >99% inhibition.

The effect on selective tubulin mRNA destabilization that results from successively reducing ribosome translocation to

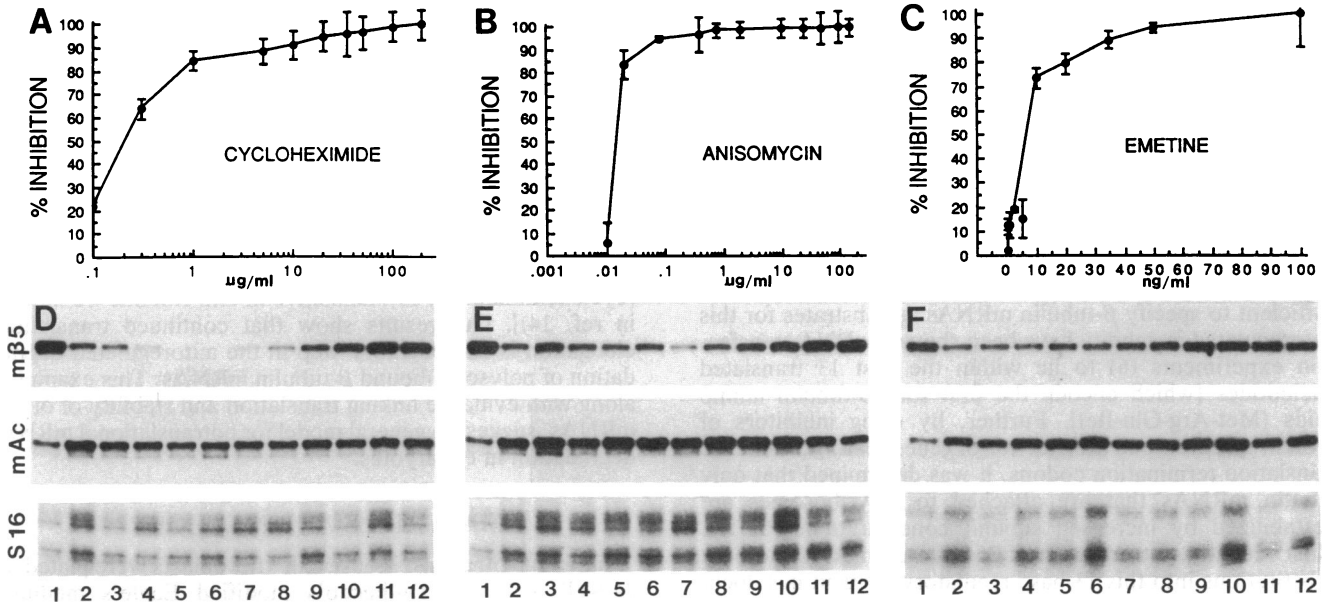

FIG. 1. Inhibition of translation elongation disrupts tubulin autoregulation. (*A–C*) Protein synthesis inhibition was measured as a function of concentration of the elongation inhibitors cycloheximide (*A*), anisomycin (*B*), and emetine (*C*). Error bars display the coefficient of variation from three determinations. (*D–F*) Levels of specific mRNAs in cells were measured after treatment with a protein synthesis inhibitor followed by addition of colchicine to elevate the unassembled tubulin subunit concentration. S1 nuclease protection analyses were used to measure levels of β tubulin (mβ5) (*Top*), actin (mAc) (*Middle*), and ribosomal protein S16 (S16) (*Bottom*). Lanes: 1, control cells not treated with inhibitors or colchicine; 2, colchicine-treated cells; and 3–12, cells pretreated with the same concentrations of inhibitor as that used in the respective titration curves (*A–C*), followed by a 3-hr cotreatment with colchicine. (*D*) For cycloheximide, the concentrations used (in μg/ml) were: 0.1 (lane 3), 0.3 (lane 4), 1.0 (lane 5), 5 (lane 6), 10 (lane 7), 20 (lane 8), 35 (lane 9), 50 (lane 10), 100 (lane 11), and 200 (lane 12). (*E*) For anisomycin the concentrations used (in μg/ml) were: 0.02 (lane 3), 0.08 (lane 4), 0.4 (lane 5), 0.8 (lane 6), 2.0 (lane 7), 10 (lane 8), 25 (lane 9), 50 (lane 10), 100 (lane 11), and 150 (lane 12). [The first point on the titration curve (0.01 μg/ml) is not shown in the RNA analysis.] (*F*) For emetine the concentrations used (in ng/ml) were: 0.25 (lane 3), 0.5 (lane 4), 1.0 (lane 5), 2.5 (lane 6), 5.0 (lane 7), 10 (lane 8), 20 (lane 9), 35 (lane 10), 50 (lane 11), and 100 (lane 12).

as low as 0.5% of the normal rate was examined by pretreating cells with each of the three drugs and then increasing the tubulin subunit concentration through colchicine-induced depolymerization of microtubules. [Since about half of cell tubulin is polymerized, this results in doubling the subunit concentration (e.g., see ref. 15).] Cytoplasmic RNA was prepared and analyzed by S1 nuclease protection for β-tubulin mRNAs, actin RNAs, or ribosomal protein S16 RNAs. Lanes 1 and 2 in Fig. 1 *D–F* show the accumulated levels of β-tubulin mRNAs in cells that synthesized proteins at normal rates and in similar control cells after a 3-hr increase of the unassembled tubulin subunit concentration. Lanes 3–12 of Fig. 1 *D–F* show the corresponding mRNA levels when translocation rates had been diminished by addition of increasing amounts of each elongation inhibitor.

As reported earlier (10), at lower concentrations of cycloheximide (concentrations that result in 90–95% inhibition of elongation), increases in the subunit concentration caused β-tubulin mRNAs to be degraded even faster than in cells translating proteins at normal rates (compare lanes 4–8 with lane 2 in Fig. 1*D*). On the other hand, while slowing elongation enhanced autoregulated instability, further inhibition of synthesis gradually restored tubulin RNA stability, until at maximal inhibition (>99.5%) β-tubulin RNAs were fully protected from colchicine-induced destabilization (compare lanes 11 and 12 with lane 1 in Fig. 1*D*). [The possibility that cycloheximide was affecting tubulin transcription rates rather than RNA stability was eliminated by treating cells only with cycloheximide. If cycloheximide induced changes in tubulin transcription, tubulin RNAs would increase; in fact, RNA levels were essentially constant (not shown).]

Moreover, the pattern of mRNA stabilization in the presence of increased tubulin subunit concentrations was similar for cells treated with anisomycin to block translation elongation. Like the cycloheximide example, inhibition of protein synthesis by low concentrations of anisomycin (e.g., 2.0 μg/ml) resulted in enhancement of autoregulated tubulin RNA destabilization (Fig. 1*E*, lane 7), whereas higher levels of inhibition of protein synthesis led to almost complete tubulin mRNA stabilization. A nearly analogous situation was found after blocking protein synthesis with emetine. Complete inhibition of translation at high drug concentrations fully protected β-tubulin mRNAs from autoregulated instability. In this case, however, no enhancement of instability was found at lower levels of protein synthesis inhibition (Fig. 1 *C* and *F*).

The observed stabilization after inhibition of protein synthesis was not due to a general stabilization of all cell mRNAs but was specific for tubulin RNAs. For example, although increasing the tubulin subunit concentration initially resulted in a slight increase in actin mRNAs (as reported earlier—ref. 16), treatment of cells with increasing amounts of any of the three elongation inhibitors did not increase stability of actin mRNAs. Similarly, the mRNA levels for ribosomal protein S16 (Fig. 1 *D–F Bottom*), and ornithine amino transferase (not shown) remained largely unchanged.

Despite the accepted modes of action of each of the inhibitors (inhibition of translation elongation without dissociation of the mRNAs from polysomes), there still existed the possibility that at the highest concentrations of inhibitor (i.e., those used to abolish all residual translation), the loss of tubulin mRNA autoregulation might not be due to inhibition of translocation. Rather, secondary effects on protein synthesis (such as inhibition of initiation) or disruption of other cell processes might be responsible. To ascertain if such effects could account for the observed stabilization of β-tubulin mRNAs in the presence of high concentrations of each elongation inhibitor, the overall polysome distribution and the location of β-tubulin mRNAs within that distribution in control and in drug-treated cells were analyzed (Fig. 2).

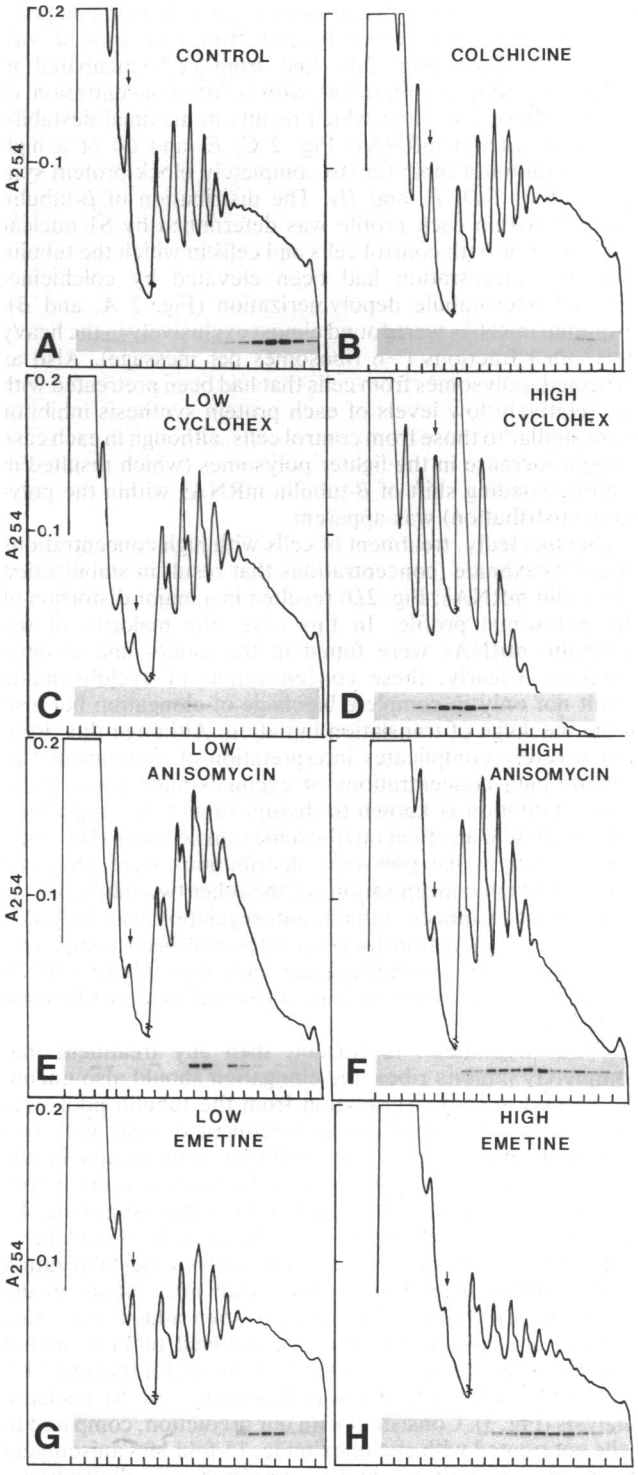

FIG. 2. β-Tubulin mRNA distribution within polysome profiles of cells treated with elongation inhibitors. Polysome profiles and the location of β-tubulin mRNAs in those profiles were analyzed for control cells (*A*) and for cells treated for 3 hr with colchicine (C-H) and a low level of cycloheximide (cyclohex; 5 μg/ml) (*C*), a low level of anisomycin (2 μg/ml) (*E*), a low level of emetine (2.5 ng/ml) (*G*), a high level of cycloheximide (200 μg/ml) (*D*), a high level of anisomycin (150 μg/ml) (*F*), and a high level of emetine (100 ng/ml) (*H*). (*B*) Polysome profile from cells treated with colchicine. (*Insets*) Levels of β-tubulin mRNAs (determined by S1 nuclease protection analysis) in each fraction from the polysome analysis. The autoradiographs for colchicine treatment (*B*) and each of the low concentrations of elongation inhibitor (*C*, *E*, and *G*) have been exposed ≈4 times longer than their experimental counterpart so that the RNAs can be visualized. An arrow indicates the position of the 80S monosome within each profile.

Sucrose gradient centrifugation was used to fractionate polysomes from control cells (Fig. 2*A*), from cells treated with colchicine alone (Fig. 2*B*), and from cells incubated in colchicine after pretreatment with a low concentration of each inhibitor (i.e., that which results in maximal destabilization of tubulin mRNAs; Fig. 2 *C*, *E*, and *G*) or a high concentration of inhibitor (to completely block protein synthesis; Fig. 2 *D*, *F*, and *H*). The distribution of β-tubulin mRNAs within each profile was determined by S1 nuclear analysis. For both control cells and cells in which the tubulin subunit concentration had been elevated by colchicine-induced microtubule depolymerization (Fig. 2 *A*, and *B*), β-tubulin mRNAs were found almost exclusively in the heavy polysomal fractions (>6 ribosomes per message). Also as expected, polysomes from cells that had been pretreated with the relatively low levels of each protein synthesis inhibitor were similar to those from control cells, although in each case a slight increase in the lighter polysomes (which resulted in a corresponding shift of β-tubulin mRNAs within the polysome distribution) was apparent.

Unexpectedly, treatment of cells with high concentrations of cycloheximide (concentrations that result in stabilization of tubulin mRNAs; Fig. 2*D*) resulted in a major distortion of the polysomal profile. In this case, the majority of the β-tubulin mRNAs were found in the mono- and disomal fractions. Clearly, these concentrations of cycloheximide result not only in complete blockage of elongation but also some blockage of translation initiation. Although this problem severely complicates interpretation of experiments involving high concentrations of cycloheximide [since polysome disruption is known to disrupt tubulin autoregulation independent of an effect on ribosome translocation (10)], only minor shifts in the polysome distributions were observed even with high concentrations of the other two inhibitors. In these latter instances, tubulin autoregulation was fully disrupted only when translocation rates had been completely blocked, strongly supporting the view that tubulin mRNA destabilization requires at least a residual rate of ribosome translocation.

If this hypothesis is correct, then any treatment that completely inhibits ribosome elongation should also uncouple tubulin mRNA degradation from the tubulin pool size. Specifically, the simultaneous treatment of cells with two inhibitors that interact with different components in the elongation process should fully inhibit translocation at concentrations lower than that required for either drug alone. To test this, we pretreated cells with the amount of cycloheximide that yields maximal tubulin mRNA destabilization (≈95% inhibition of translocation with little effect on the polysome distribution; Fig. 2*B*) and then added increasing amounts of anisomycin. The unassembled tubulin subunit concentration was increased with colchicine for the final 3 hr, and tubulin mRNA levels were determined by S1 nuclease analysis (Fig. 3). Consistent with our prediction, compared to cells not treated with cycloheximide, 15-fold-less anisomycin was necessary both to abolish completely all cellular translation activity (measured with pulse labeling; data not shown) and to inhibit the autoregulated degradation of tubulin mRNAs (compare lane 13 in Fig. 3*A* with lane 9 in Fig. 3*B*). Further, the inhibition of protein synthesis with the simultaneous use of the two inhibitors did not disrupt the polysome structure or the location of β-tubulin mRNAs within the profile (data not shown).

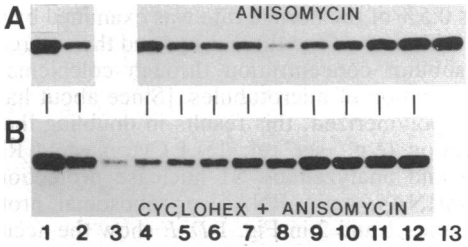

FIG. 3. Disruption of autoregulated tubulin mRNA instability by completely blocking protein synthesis by a combination of low concentrations of cycloheximide (cyclohex) and anisomycin. (*A*) Levels of β-tubulin mRNAs were measured in cells after blocking protein synthesis by addition of increasing concentrations of anisomycin (as in Fig. 1*E*). Shown for comparison is the same anisomycin titration curve for β-tubulin mRNAs as that in Fig. 1*E*. Lanes: 1, RNA isolated from control cells; 2, RNA isolated from colchicine-treated cells, and 4–13, RNAs isolated from cells pretreated with increasing concentrations of anisomycin followed by colchicine treatment (as described in Fig. 1). (*B*) Cells were pretreated for 45 min with a constant amount of cycloheximide (5 μg/ml) and various concentrations of anisomycin, followed by cotreatment with colchicine for the final 3 hr. β-Tubulin RNA levels were determined by S1 nuclease protection analysis. Lanes: 1, RNAs from control cells; 2, colchicine-treated cells; 3, cells treated with cycloheximide alone; 4–12, cells treated with cycloheximide and 0.02, 0.08, 0.4, 0.8, 2, 10, 25, 50, and 100 μg, respectively, of anisomycin per ml. The vertical lines mark the samples treated with equivalent levels of anisomycin.

DISCUSSION

Through the use of three different translation elongation inhibitors, we have demonstrated that concentrations of translation elongation inhibitors that completely disrupt cellular protein synthesis also disrupt the autoregulated degradation of tubulin mRNAs in response to increased subunit concentrations. Combined with the observations that the β-tubulin mRNAs were still found at their normal locations within polysome profiles (and therefore were capable of being recognized as substrates for degradation) and that there was no general stabilization of other cellular mRNAs after translation inhibition, we conclude that ribosome translocation is probably an obligatory step in β-tubulin mRNA degradation.

At first glance this conclusion predicts that at comparable levels of inhibition of elongation, all three inhibitors should have equivalent effects on RNA levels. This is not strictly true (Fig. 1). However, the dose–response curves for inhibition are quantitatively very different. For example, cycloheximide requires an ≈70-fold higher level to increase inhibition from 90% to 99%, but only a 2-fold increase is necessary for emetine. Thus, since each drug binds to a different translation component and since tubulin RNA degradation occurs while the RNA is ribosome-bound (7, 10), that cotranslational RNase activation will be quantitatively determined solely by the rate of elongation cannot realistically be expected.

Earlier we proposed two possible models for tubulin RNA destabilization (7). In the first, binding of the cellular factor (presumably tubulin itself) to the nascent β-tubulin polypeptide activates a ribosome-bound RNase. In the second, binding to the nascent peptide causes ribosome stalling on tubulin RNAs that are normally heavily loaded with ribosomes. This leads to a gap between ribosomes (the stalled ribosome and its adjacent 3′ unstalled counterpart). This naked stretch of RNA is then a better substrate for nonspecific RNA degradation. As shown schematically in Fig. 4, both possibilities are compatible with a requirement for continuing ribosome movement. Models dependent on selective ribosome stalling would obviously be disrupted by freezing all ribosomes, since this would prevent RNA gaps from forming as a consequence of specific stalling. For the other model, ribosome translocation would be required for transduction of the cotranslational binding event to yield activation and/or recruitment of the RNase.

There now exist an increasing number of examples of regulated RNA instability in eukaryotes. In addition to tubulin, the prominent examples include the degradation of

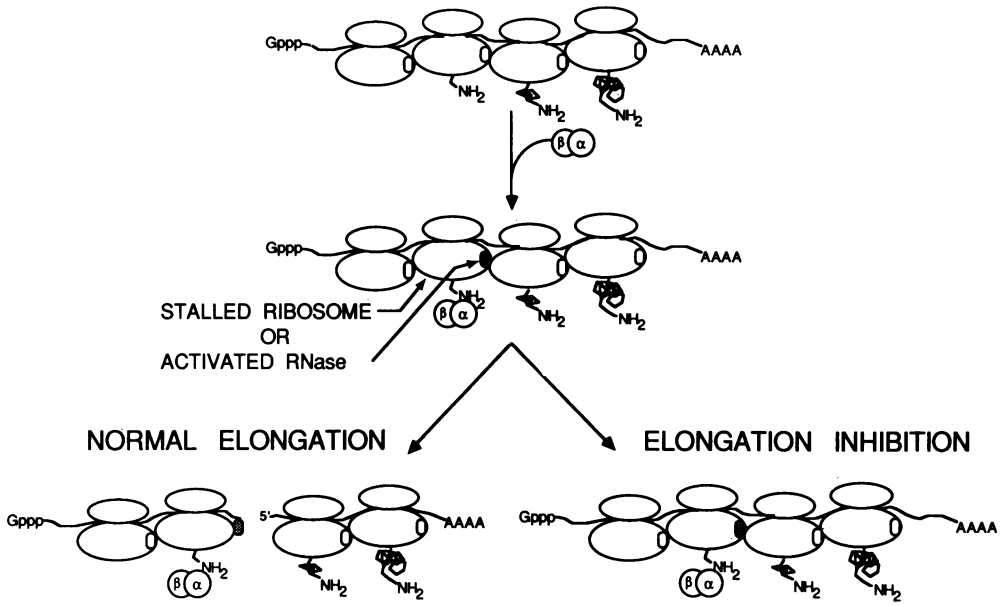

FIG. 4. Models for translation elongation-dependent destabilization of β-tubulin mRNAs. A cotranslational binding event between a cellular factor (presumably the tubulin subunit itself) and the nascent polypeptide activates a ribosome-associated nuclease or induces translational stalling. In either case, continuing translation elongation is required for RNA degradation.

histone RNAs at the cessation of DNA syntheses; the inherent instability of a series of lymphokine, cytokine, and protooncogene mRNAs; and the iron-dependent destabilization of transferrin receptor mRNA. In the most extensively studied of these, the sequences necessary and sufficient for rapid turnover of histone mRNAs have been localized to a short stem–loop structure that resides at the extreme 3′ terminus of the nonpolyadenylylated histone mRNAs (17). For the lymphokine, cytokine, and protooncogenes [as first recognized by Shaw and Kamen (18)], a 50–60 base A+U-rich domain of the 3′ untranslated region is largely responsible for mRNA instability (see also refs. 19–22). Transferrin receptor mRNAs are destabilized in the presence of high levels of iron by an ≈500-base domain in the 3′ untranslated region (23, 24). While at first glance each of these examples of mRNA instability appears to utilize a distinct control mechanism, we note that they all share a common feature: obligatory linkage of RNA instability to continuing translation. This requirement for translation was first documented for the mRNA encoding the protooncogene c-*myc* by use of inhibitors of protein synthesis (25). Translation was found to be obligatory for degradation of histone (26), transferrin receptor mRNA (24), and a variety of additional protooncogenes (e.g., c-*fos*; ref. 27).

For each of these mRNAs, protein synthesis-dependent RNA destabilization is compatible either with RNA degradation linked to translation elongation or with the action of a labile protein factor. While no data presently distinguish between these possibilities for the mRNAs carrying A+U-rich sequences or for transferrin receptor, for tubulin and histones RNA translation is certainly required. As we have documented here, an unstable effector protein cannot be needed for tubulin RNA degradation because blockage of protein synthesis to 95% actually enhances instability rather than leads to stabilization. For histones, Marzluff's group (28) has used DNA transfection to demonstrate that for degradation of histone RNAs at the end of DNA synthesis, not only must the RNA be translated, but the ribosome must translocate to within 300 bases of the terminal stem–loop structure. Obviously, the details of the initial events that target each of these mRNAs for degradation are distinct. Nevertheless, the common requirement for translation prompts us to propose that the RNA degradation pathway illustrated in Fig. 4 for tubulin may be a specific example of a general eukaryotic mechanism for regulating gene expression through translation elongation-dependent mRNA instability.

This work has been supported by Grant GM29513 from the National Institutes of Health to D.W.C. S.S.S. was the recipient of an National Institutes of Health Postdoctoral Fellowship.

1. Cleveland, D. W., Lopata, M. A., Sherline, P. & Kirschner, M. W. (1981) *Cell* **25,** 537–546.
2. Caron, J., Jones, A. L. & Kirschner, M. W. (1985) *J. Cell Biol.* **101,** 1763–1772.
3. Caron, J., Jones, A. L., Rall, L. B. & Kirschner, M. W. (1985) *Nature (London)* **317,** 648–650.
4. Pittenger, M. F. & Cleveland, D. W. (1985) *J. Cell Biol.* **101,** 1941–1952.
5. Gay, D. A., Yen, T. J., Lau, J. T. Y. & Cleveland, D. W. (1987) *Cell* **50,** 671–679.
6. Yen, T. J., Gay, D. A., Pachter, J. S. & Cleveland, D. W. (1988) *Mol. Cell. Biol.* **8,** 1224–1235.
7. Yen, T. J., Machlin, P. S. & Cleveland, D. W. (1988) *Nature (London)* **334,** 580–585.
8. Cleveland, D. W., Pittenger, M. F. & Feramisco, J. R. (1983) *Nature (London)* **305,** 738–740.
9. Cleveland, D. W. & Havercroft, J. C. (1983) *J. Cell Biol.* **97,** 919–924.
10. Pachter, J. S., Yen, T. J. & Cleveland, D. W. (1987) *Cell* **51,** 283–292.
11. Gong, Z. & Brandhorst, B. P. (1988) *Mol. Cell. Biol.* **8,** 3518–3525.
12. Hogan, B. L. M. (1969) *Biochim. Biophys. Acta* **182,** 264–266.
13. Fan, H. & Penman, S. (1970) *J. Mol. Biol.* **50,** 655–670.
14. Grollman, A. P. & Huang, M. T. (1976) in *Protein Synthesis*, ed. McConkey, E. H. (Dekker, New York), Vol. 2, pp. 125–167.
15. Lopata, M. A. & Cleveland, D. W. (1987) *J. Cell Biol.* **105,** 1707–1720.
16. Lau, J. T. Y., Pittenger, M. F. & Cleveland, D. W. (1985) *Mol. Cell. Biol.* **5,** 1611–1620.
17. Pandey, N. B. & Marzluff, W. F. (1987) *Mol. Cell. Biol.* **7,** 4557–4559.
18. Shaw, G. & Kamen, R. (1986) *Cell* **46,** 659–667.
19. Jones, T. R. & Cole, M. D. (1987) *Mol. Cell. Biol.* **7,** 4513–4521.
20. Shyu, A. B., Greenberg, M. E. & Belasco, J. G. (1989) *Genes Dev.* **3,** 60–72.
21. Kabnick, K. S. & Housman, D. W. (1988) *Mol. Cell. Biol.* **8,** 3244–3250.
22. Wilson, R. & Triesman, R. (1988) *Nature (London)* **336,** 396–399.
23. Casey, J. L., Hentze, M. W., Koeller, D. M., Caughman, S. W., Rouault, T. A., Klausner, R. D. & Harford, J. B. (1988) *Science* **240,** 924–928.
24. Muller, E. W. & Kuhn, L. C. (1988) *Cell* **53,** 815–825.
25. Linial, M., Gunderson, N. & Groudine, M. (1985) *Science* **230,** 1126–1132.
26. Sive, H. L., Heintz, N. & Roeder, R. G. (1984) *Mol. Cell. Biol.* **4,** 2723-2734.
27. Greenberg, M. E., Hermanowski, A. L. & Ziff, E. B. (1986) *Mol. Cell. Biol.* **6,** 1050-1057.
28. Graves, R. A., Pandey, N. B., Chodchoy, N. & Marzluff, W. F. (1987) *Cell* **48,** 615–626.