Turnover and Translation of in Vitro Synthesized Messenger RNAs in Transfected, Normal Cells*

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§ The abbreviations used are: GM-CSF, granulocyte-macrophage colony-stimulating factor; PBMC, peripheral blood mononuclear cells; ARE, adenosine-uridine-rich element; UTR, untranslated region; Act D, actinomycin D; DRB, 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole (DRB) (1, 6, 27). There is growing evidence that these drugs may have significant effects on mRNA turnover. The addition of Act D to NIH 3T3 cells stabilized transgenic c-fos and GM-CSF ARE-containing mRNAs (28). Also, cis elements, which normally destabilize c-fos (4), c-myc (5), or erythropoietin mRNAs (29), are nonfunctional in the presence of Act D. While the underlying mechanisms for these effects are unclear, they cast significant doubt on the validity and usefulness of mRNA decay measurements in the presence of metabolic poisons.

Several alternative methods have been developed to measure mRNA decay rates in vitro. These methods are sensitive to prolonged serum starvation and serum replenishment, which may independently affect mRNA degradation, thus reducing the general applicability of this method. Promoters activated by small, rapidly diffusible, nontoxic compounds such as tetracycline, potentially afford a reproducible on/off

We have developed a novel system to examine intracellular mRNA decay pathways in the absence of transcriptional blockade. In vitro transcribed, capped, and adenylated granulocyte-macrophage colony stimulating factor (GM-CSF) or globin RNAs were introduced by particle-mediated gene transfer into primary cultures of normal peripheral blood mononuclear cells. Transfected wild-type, human GM-CSF (hGM-AUUUA) mRNA decayed rapidly (\(t_{1/2} = 9\) min), while a mutated version lacking AUUUA repeats (hGM-AUGUA) was significantly more stable (\(t_{1/2} = 30\) min). A truncated GM-CSF mRNA lacking an entire 3′-UTR (hGM-Δ3′-UTR) was still more stable (\(t_{1/2} = 80\) min) demonstrating the existence of non-AUUUA, 3′-UTR destabilizing domains. Transfected β-globin mRNA was very stable, decaying with a half-life of \(>360\) min. Transfected mRNAs were >90% polysome associated with transgenic protein detectable within 15 min of transfection. The most stable GM-CSF mRNAs were not associated with maximal GM-CSF protein production. Agents known or hypothesized to interfere with mRNA decay, including cycloheximide, phorbol ester, or actinomycin D, stabilized both hGM-AUUA and hGM-AUGUA mRNAs. These data demonstrate the presence of 3′-UTR, destabilizing, and translational regulatory elements outside of the AUUUA repeats and unambiguously show that actinomycin D at concentrations commonly used to inhibit transcription stabilizes cytotkine mRNAs.

Transcription rates as well as mRNA stability are often tightly and coordinately regulated for transiently expressed genes such as those coding for proto-oncogenes like c-myc and c-fos and cytokines such as interleukin-2, interleukin-3, tumor necrosis factor-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF). In resting cells, transcriptional rates for these genes are low, and the cytoplasmic mRNA pools are rapidly turned over with half-lives of 20–40 min (1–6). Upon cell activation, transcriptional rates increase, and the resultant mRNAs are transiently resistant to cytoplasmic decay (1, 6–9). The combination of transcriptional up-regulation and mRNA stabilization synergistically elevate mRNA levels and subsequently the amount of protein synthesized, by as much as 50–100-fold (1, 9, 10).

Regulated mRNA stability appears to depend on the interaction between intrinsic, cis-acting elements and trans-acting factors. The former consist of either highly conserved primary sequences (3–5, 11) or stable stem-loop structures (12) located in the coding or noncoding regions. One of the few characterized cis elements is the so-called Shaw-Kamen box, AUUUA motif, or AU-rich element (ARE) which is found in the 3′-untranslated region (UTR) of many proto-oncogene and cytokine mRNAs (3, 11). To function as an instability determinant, the AUUUA motifs must be arranged in tandem, forming at least one UUAUUUAU/AU/A unit in the coding or noncoding regions. The combination of transcriptional up-regulation and mRNA stabilization synergistically elevate mRNA levels and subsequently the amount of protein synthesized, by as much as 50–100-fold (1, 9, 10).

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switch to produce a transient transcriptional pulse (30). Finally, the direct introduction of mRNA by Lipofectin (31) or electroporation (32) have been attempted. These methods have been disappointing as substantial cell damage and perturbation (electroporation) or inappropriate targeting (liposomes) have reduced their usefulness.

Recently, we and others have shown that particle-mediated gene transfer (PMGT) is an effective and versatile method for transfecting a range of normal cells and transformed cell lines irrespective of cell cycle position (10, 33). Gold beads (0.96 µm diameter) coated with expression vectors are accelerated into cells. In the aqueous, intracellular environment, nucleic acids are released and become transcriptionally active. While the efficiency is modest (5–10%) for resting, normal T lymphocytes (33), successfully transfected cells generate ample transgenic mRNA and protein. Cell death is about 20–30% and post-transfection cell physiology appears normal (10, 33). We therefore investigated if normal resting and transformed cells could be transfected with in vitro synthesized mRNAs using particle-mediated gene transfer. The decay of transgenic mRNAs could then be directly assessed in the absence of transcriptional blockage. In this study we demonstrate that PMGT can deliver intact mRNAs into the cytoplasm. These mRNAs were rapidly utilized as templates for protein synthesis. Relative decay rates were preserved between unstable and stable mRNAs and compounds with established, but mechanistically unknown effects on mRNA decay such as cycloheximide, phorbol ester (TPA), or Act D had the anticipated effects on transgenic mRNAs.

EXPERIMENTAL PROCEDURES

Plasmid Constructions—cDNA coding for a human GM-CSF was obtained from the American Type Culture Collection, Rockville, MD. Using overlap extension polymerase chain reaction, the adenosine-uridine-rich elements (AREs) of wild-type GM-CSF (GM-AUUUA) were replaced with four tandem AUGUA sequences (GM-AUGUA) as described previously (10). Polymerase chain reaction-amplified cDNA inserts of wild-type (GM-AUUUA), mutant (GM-AUGUA), and 3’-untranslated region deleted (GM-Δ3’-UTR) GM-CSF were ligated into Smal digested, T-tailed, pUC 18-based vectors (denoted pT7A90) courtesy of Richard Spritz, University of Wisconsin), downstream from a T7 RNA polymerase start site and upstream from a 90-base poly(dT) tract immediately followed by a unique Hind III site. B capped, polyadenylated mRNAs made in vitro (see “Experimental Procedures”) were selected on an oligo(dT) column and electrophoresed on a denaturing 1.2% agarose-formaldehyde gel, prior to loading onto gold beads for particle-mediated gene transfer. Lane 1, hGM-AUUUA; lane 2, hGM-AUGUA; lane 3, hGM-Δ3’-UTR; lane 4, B-globin mRNAs. Molecular size markers are on the left. C, Northern analysis of total RNA isolated from whole cell lysate, S20, polysome, and S130 fractions, 20 min after transfection of PBMC with hGM-AUGUA mRNA. Each fraction was prepared from an equivalent number of cells.

Intracellular Turnover and Translation of Transfected mRNAs

In vitro production of mRNAs for particle-mediated gene transfer and subcellular localization following transfection. A, polymerase chain reaction-amplified cDNA inserts of wild-type (hGM-AUUUA), mutant (hGM-AUGUA), 3’-UTR deleted (hGM-Δ3’-UTR) human GM-CSF, and full-length B-globin were ligated into identical Smal-digested, T-tailed, pUC 18-based vectors (pT7A90), downstream from a T7 RNA polymerase start site and upstream from a 90-base poly(dT) tract immediately followed by a unique Hind III site. B, capped, polyadenylated mRNAs made in vitro (see “Experimental Procedures”) were selected on an oligo(dT) column and electrophoresed on a denaturing 1.2% agarose-formaldehyde gel, prior to loading onto gold beads for particle-mediated gene transfer. Lane 1, hGM-AUUUA; lane 2, hGM-AUGUA; lane 3, hGM-Δ3’-UTR; lane 4, B-globin mRNAs. Molecular size markers are on the left. C, Northern analysis of total RNA isolated from whole cell lysate, S20, polysome, and S130 fractions, 20 min after transfection of PBMC with hGM-AUGUA mRNA. Each fraction was prepared from an equivalent number of cells.

RESULTS

We examined if PMGT could be used to deliver in vitro transcribed mRNAs directly into cells, whereupon their decay kinetics would be assessed without the addition of Act D or DRB. Both unstable (GM-CSF) and stable (globin) mRNAs were evaluated for decay rates and capacities for translation. Following transcription (Fig. 1A) and selection on an oligo(dT)-cellulose column, each mRNA was examined on a denaturing, formaldehyde-agarose gel and found to be full-length and intact (Fig. 1B). PBMC transfected with hGM-AUGUA mRNA were cultured for 20 min prior to isolation of whole cell lysate, S20, S130, and polysome fractions. Northern blot analysis of total RNA isolated from each of these fractions showed >90% of the transfected mRNA to be associated with polysomes and...
<5% with the S130 cytosol (Fig. 1C), consistent with a rapid mobilization of transfected mRNA onto polysomes.

Following transfection with individual mRNAs, equal volumes of transfected cells were removed at various times, RNA quantitatively isolated, and analyzed by Northern blotting (Fig. 2A). Wild-type hGM-AUUUA mRNA decayed very rapidly with a calculated half-life of approximately 9 min (Fig. 2B), which was considerably and reproducibly faster than hGM-AUGUA mRNA ($t_{1/2} = 30$ min) or hGM-$\Delta 3^\prime$-UTR ($t_{1/2} = 80$ min). $\beta$-Globin mRNA was exceedingly stable with negligible decay at 180 min and an estimated half-life greater than 360 min (Fig. 2, A and B). In a previous study (10) we determined that hGM-AUUUA and hGM-AUGUA mRNAs decayed with half-lives of 30 and 90 min, respectively, after actinomycin D transcriptional arrest. Therefore, transfected mRNAs decayed significantly faster in the absence of Act D, but maintained similar relative decay rates. The increased stability of hGM-$\Delta 3^\prime$-UTR compared with hGM-AUGUA mRNA suggests the existence of additional instability determinants in the 3'-untranslated region of GM-CSF. Such an element has been proposed by others (7, 8, 27), but has yet to be formally demonstrated.

GM-CSF mRNAs require ongoing co-translation for normal decay (3, 15–17). In the context of translational blockade with cycloheximide (3), or the absence of a start codon (17), GM-CSF mRNAs are exceedingly stable. Therefore, the rapid decay of transgenic GM-CSF mRNA was consistent with its mobilization onto polysomes. However, we sought to prove that exogenous mRNA was translated, by measuring the production of GM-CSF protein in the supernatant of transfected PBMC. To control experiments, PBMC were transfected with naked gold beads prior to Northern blotting and assaying of the cell supernatant for GM-CSF protein. Neither GM-CSF mRNA nor GM-CSF protein were detected, demonstrating that endogenous GM-CSF expression was not induced by PMGT (data not shown). Thus any GM-CSF protein detected in the supernatant after transfection must have been produced from the transgenic mRNA. We were able to detect protein production as early as 15 min after transfection (Fig. 2C). Approximately 15–20-fold more GM-CSF protein was consistently detected in the cell culture medium from PBMC transfected with the more stable hGM-AUGUA mRNA compared with those transfected with wild-type hGM-AUUUA mRNA (Fig. 2C). Interestingly, the GM-$\Delta 3^\prime$-UTR transfectants secreted only half as much GM-CSF protein as GM-AUGUA transfectants (Fig. 2C). These results imply the presence of a positive, cis-acting translational element in the 3'-UTR of GM-CSF mRNA. Transgenic mRNAs delivered intracellularly via PMGT are therefore polysi- 

come-associated, translationally active, and decay significantly more rapidly than after actinomycin D chase, but with the same rank order and relative rates as observed in the presence of Act D. Based on these data, we conclude that the measurement of mRNA decay rates after PMGT is a valid, reproducible, and reliable technique that does not require the addition of any metabolic poisons nor serum starvation.

A variety of cytokine and proto-oncogene mRNAs are stabi-

lized by translational blockade (3, 5, 15–17). This effect has been variously ascribed to a requirement for an extremely labile, destabilizing protein (36), an absolute requirement for ongoing translation (15–17) or a direct inhibitory effect of cy-

doheximide on the decay process itself. Recent data from sev-

eral laboratories have shown active translation is necessary for rapid turnover (15–17). This implies that the mRNA decay machinery is located on a polyribosome, where it interacts with translating messages. This is consistent with data showing that isolated polyribosomes faithfully mimic intracellular de-
effect were a major rationale to develop the alternative trans-
coding region determinants). Indeed, potential Act D-mediated
times indicated. Northern blotswere probed sequentially with GM-CSF
cellswere harvested starting 5 min later (zero time) and at the various
washed twice, and incubated with or without 15 μg/ml of CHX.
Thus, we wished to assess if cycloheximide
inhibition has been element-specific (4, 5, 28, 38). In some cases, the
process itself. The comparable stabilization of both GM-CSF
mRNAs (t_{1/2} > 90 min) demonstrated CHX effects do not require
intact AUUUA repeats. In simultaneously performed, control
experiments in the absence of CHX, hGM-AUUUA, and hGM-
AUGUA mRNAs decayed as expected (data not shown). Therefore,
protein synthesis blockade by CHX stabilized exogenous GM-CSF mRNAs.

Phorbol esters have also shown potent stabilizing effects on
cytokine mRNAs (1, 8). In T lymphocytes or fibroblasts, TPA
induced GM-CSF mRNA stabilization within 3–4 h after addi-
tion (1, 8, 27), although the precise kinetics remain unknown.
Under such conditions, a variety of AU-specific mRNA-binding
proteins were induced, which may interact with and stabilize
UUUA-containing mRNAs (20, 22, 24). We took advantage of the
lag in TPA-mediated stabilization of endogenous GM-CSF protein to
evaluate its effects on transfected mRNAs. After transfection,
cells were treated with TPA (20 ng/ml) for 1 h, whereupon
GM-CSF mRNA decay rates were measured. In simultaneous
control experiments, PBMC were transfected with naked gold
beads and incubated with phorbol ester for variable times.
Using Northern blot analysis of identical amounts of cellular
RNA, we were unable to detect endogenous GM-CSF mRNA within the time frame (3 h) of these experiments. Therefore, all
GM-CSF-specific mRNA signals must be from the transgene.
As shown in Fig. 4, TPA profoundly stabilized both wild-type
and AUGUA mRNAs to apparent half-lives > 120 min, which is
consistent with that observed for endogenous GM-CSF mRNA in
TPA-treated cells (1, 8). Interestingly, the mutant hGM-
AUGUA mRNA was also stabilized, suggesting an ancillary
domain (outside of the AUUUUA motifs) may be involved. Iwai et
al. (7, 8) implicated a region ~150 bases upstream from the
AUUUUA motifs in TPA-mediated stabilization of murine GM-CSF.

Over the past several years, indirect data have suggested that Act D and possibly other transcriptional inhibitors, such as
DRB, inhibit mRNA decay (4, 5, 28, 38). In some cases, the
inhibition has been element-specific (e.g. c-fos (4) and c-myc (5)
coding region determinants). Indeed, potential Act D-mediated
effects were a major rationale to develop the alternative trans-
fection technology described herein. Demonstration of Act D-
dependent modulation of mRNA stability would thus serve to
unambiguously identify this effect as well as establish a system
to investigate its mechanism. Therefore, PBMC transfected
with hGM-AUUUA or hGM-AUGUA mRNAs were treated with Act D (5 μg/ml) for 15 min prior to measurement of mRNA
decay rates by Northern blotting (Fig. 5). This concentration of
Act D is typically employed to block transcription prior to
mRNA decay measurements. As shown, both wild-type and
AUGUA mRNAs were substantially and equivalently stabi-
lized by Act D (t_{1/2} = 80–90 min). The effects were approxi-
ately comparable with those observed with cycloheximide or
phorbol ester alone. Since GM-AUGUA mRNA was also stabi-
lized, the Act D effects must also be independent of the AUUUUA motifs. Therefore, these data demonstrate that transcriptional inhibitors interfere with mRNA decay.

**DISCUSSION**

Herein we have evaluated PMGT as a method for the intra-
cellular delivery of in vitro transcribed mRNAs. As shown
previously for cDNAs, PMGT is a versatile method capable of
transfecting quiescent, normal cells or tumor cell lines. Based
on the accumulated data, we believe that mRNAs introduced
into cells by PMGT are appropriately localized, translated, and
degraded.

The decay rates measured for transfected wild-type GM-CSF
mRNA were strikingly rapid (t_{1/2} = 9 min) compared with measure-
ments made in cell lines transfected with expression vectors in
the presence of actinomycin D or DRB. Such systems typi-
cally degrade transgenic wild-type GM-CSF mRNA with a
20 to 30 min (1, 3, 27). We have shown previously that hGM-
AUUGUA mRNA transcribed from transfected expression vec-
tors was 3-4 fold more stable (t_{1/2} ~ 90 min) than wild-type
GM-CSF mRNA (10). The t_{1/2} of wild-type GM-CSF mRNA in
normal, noncytotoxic lymphocytes is unknown as the mRNA
must be up-regulated with mitogens, such as phorbol ester and
phytohemagglutinin or mitogenic, cell surface antibodies (1, 8,
9), which also stabilize it. The closest approximation to a basal
decay rate was obtained in transformed PB-3c mast cells
treated with calcium ionophore (6). After a 3-h treatment,
GM-CSF and interleukin-3 mRNA decay rates were deter-
mined after extensive washing. Under these conditions, both
AUUUUA containing mRNAs were rapidly destabilized (t_{1/2} < 15
min) compared with the measured rate with ionophore present
(t_{1/2} > 3 h). However, the precise degree to which this approxi-
mates GM-CSF mRNA decay in G0/G1 cells is unknown.

Accelerated decay seen in our system may be the result of
suboptimal or subphysiological poly(A) tail lengths on the
transgenic mRNAs. Poly(A) tail shortening appears to be the
initial step in the degradation of c-fos and possibly GM-CSF
mRNAs (28). The precise, poly(A) tail length of endogenous
GM-CSF mRNA is not known, but based on recent work by
Chen et al. (28) is likely to be about 100–150 bases or quite...
similar to the 90 base tails on the transfected mRNAs. Transfected globin mRNAs were exceedingly stable (6), demonstrating that 90-base poly(A) tails were not, a priori, destabilizing. In addition, degradation was clearly sequence-dependent, as hGM-AUGUA, hGM-Δ3'-UTR, and β-globin mRNAs all decayed with dissimilar rates which were significantly less rapid than wild-type hGM-AUUUA mRNA. If a 90-base poly(A) tail constituted a “decay intermediate” to the RNase machinery, we would expect all transfected mRNAs to show similar decay rates. The relative decay rates remain unchanged from those observed after RNA transfection followed by transcriptional blockade with Act D, further suggesting that the observed data are valid. Therefore, we propose that the decay rate for GM-CSF mRNA measured in transfected PBMC accurately represents catabolism of the endogenous mRNA.

An obvious application of mRNA PMGT is the identification of novel cis elements. While hGM-AUUUA mRNA decay is clearly dependent on the 3' AUUUA motifs, the existence of ancillary domains has been controversial. We have demonstrated that hGM-AUGUA and hGM-Δ3'-UTR, and β-globin mRNAs decayed significantly more slowly than wild-type hGM-AUUUA. Importantly, the hGM-AUGUA and hGM-Δ3'-UTR mRNAs decayed at different rates, which were substantially more rapid than that of β-globin mRNA.

Therefore, it is likely that the 3’-UTR and possibly the coding region of GM-CSF mRNA contain additional cis-acting instability elements. As the coding region of c-fos (4) and c-myc (5) contain accessory, co-dominant destabilizing regions, there is precedent for this proposal. Unlike the proto-oncogenes, however, such domains in GM-CSF mRNA cannot be co-dominant, as GM-AUGUA mRNA was far more stable than wild-type GM-CSF mRNA. Iwai et al. (7, 8) have implicated an incompletely defined region upstream of the AUUUA motifs in TPA- and ionophore-induced GM-CSF mRNA stabilization. Our data are consistent with theirs as 1) TPA stabilized both wild-type and hGM-AUGUA transgenic mRNAs to an equal extent and 2) hGM-Δ3'-UTR mRNA was significantly more stable than hGM-AUGUA mRNA. Therefore, we conclude that the ARE cannot be the TPA response element and that there must exist a second instability determinant, responsive to TPA that lies outside of the ARE.

Much data suggests that polysomes harbor the cytokine RNase machinery. We and others have shown that polysomes can degrade c-myc (2), GM-CSF (35, 37), and other mRNAs in vitro. While the absolute rates of decay were somewhat accelerated, the relative rates were maintained (2, 35). Others have shown that GM-CSF mRNAs were stabilized by start codon mutations, which prevented ribosomal loading (17), or by global interruption of protein synthesis with cycloheximide (3). Therefore, polysomal localization would strongly support our contention that transgenic mRNAs are being normally metabolized. Indeed, we have detected the bulk of transgenic mRNAs in polysomal pellets within 20 min of transfection. More importantly, GM-CSF protein, which must have been translated from transfected mRNA, can be detected intracellularly within 10 min and in the extracellular medium within 15 min of transfection. Therefore, transfected mRNAs are rapidly mobilized onto polysomes, where they direct the synthesis of immunologically detectable cytokine. Thus, transgenic mRNAs are localized to the appropriate intracellular compartment.

The rapid decay pathway observed here is clearly dependent on co-translation. Cycloheximide and puromycin have been repeatedly shown to stabilize AUUUA containing mRNAs. These effects have been variously ascribed to the loss of a labile, destabilizing protein (36) or, more likely, that rapid decay requires ribosomal movement along the mRNA (15, 16). When we blocked protein synthesis with cycloheximide, both hGM-AUUUA and hGM-AUGUA mRNAs were stabilized approximately 10-fold. Under these conditions, GM-CSF protein production was undetectable, confirming protein synthesis was completely inhibited. While we have not performed detailed kinetics, the effect was manifest within 15 min of cycloheximide addition, suggesting that decay requires ribosomal movement along the mRNA rather than the presence of a labile protein. Interestingly, both wild-type and mutant hGM-AUGUA mRNAs were stabilized, demonstrating that the AUUUA motifs were not necessary for this effect.

Kruys et al. (39) have shown previously that mRNAs containing AU-rich elements are poorly translated in oocytes. In a dosage-dependent manner, AREs from interferon-β, GM-CSF, or c-fos had equivalent effects despite the obvious sequence dissimilarities between them. Poly(A) tail-ARE interactions have been postulated to account for this regulation (40). If the AREs function as translational repressors, transfected hGM-AUGUA mRNA should encode considerably more protein per unit time than hGM-AUUUA mRNA. Such a comparison would be most valid at very early times post-transfection when transgenic mRNA pools are most equal. PBMC transfected with GM-CSF mRNA produced between 10 and 20 pg of GM-CSF/ml/10^6 cells within 15 min, while those cells transfected with wild-type hGM-AUUUA mRNA failed to produce any detectable protein. These data are thus consistent with Kruys and suggest that the ARE functions as a translational repressor in PBMC. Transgenic protein measured in the culture medium of PBMC transfected with the more stable hGM-Δ3'-UTR mRNA was about 50% of that measured with the less stable hGM-AUGUA mRNA-transfected cells (Fig. 1). These data are consistent with prior observations that short 3'-UTRs inhibit translation (41), although the deletion of a positive cis-acting translational element cannot yet be excluded.

The ability of a variety of drugs to influence mRNA decay has been well documented. Phorbol ester stabilizes many AUUUA-containing mRNAs (1, 8), possibly through the up-regulation of cytoplasmic RNA-binding proteins which mask the AUUUA domains (20, 22). The kinetics of TPA-mediated stabilization are unknown and difficult to determine in normal cells lacking constitutive, detectable expression of cytokine mRNAs. Protein synthesis inhibitors such as cycloheximide have similar stabilizing effects (3). Recently, Act D, commonly used to block transcription prior to mRNA decay determinations, has been shown to stabilize ARE containing mRNAs (28). The effects of these compounds can be directly assessed after PMGT of candidate mRNAs into normal cells. For example, we show here that CHX, TPA, and Act D all stabilize transgenic GM-CSF mRNAs. The decay of wild-type and mutant GM-CSF mRNAs were equally affected, demonstrating that the AUUUA motifs were not required for this effect. Therefore, mRNA decay rates measured in the presence of Act D are likely flawed and considerably overestimate the “true” half-lives. When normal
PBMC were transfected with cytomegalovirus-driven hGM-AUUUA expression vectors, followed by Act D treatment, the half-life of exogenous GM-AUUUA mRNA was approximately 20 min (10). The observed difference in decay between those data and that presented here may reflect a specific translational blockade (42) of transfected, cytoplasmic mRNA by Act D. Of note, the stabilization of transgenic mRNAs by the three drugs used here were not identical, with TPA being the most potent followed by CHX and finally Act D, suggesting that the underlying mechanisms for stabilization may differ. This is likely, since GM-CSF mRNA can be stabilized after protein synthesis inhibition with cycloheximide, transcriptional inhibition (Act D) with the maintenance of protein synthesis, or protein kinase C activation (TPA).

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