# ENDOTOXIN-RESPONSIVE SEQUENCES CONTROL CACHECTIN/TUMOR NECROSIS FACTOR BIOSYNTHESIS AT THE TRANSLATIONAL LEVEL

By J. HAN, T. BROWN, AND B. BEUTLER

*From The Howard Hughes Medical Institute and the Department of Internal Medicine, The University of Texas, Southwestern Medical Center, Dallas, Texas 75235*

A growing body of evidence suggests that regulation of gene expression may be accomplished through post-transcriptional mechanisms, operating within the cytosol (1–5). In most instances, however, little information exists concerning the precise mechanisms by which such regulation is achieved, and the nature of the mRNA sequences that are involved.

Certain cytokines appear to be subject to post-transcriptional control. In the case of cachectin/TNF, a macrophage-derived protein that is strongly and specifically induced by bacterial LPS (endotoxin), biosynthetic regulation seems to be exercised at several points. Endotoxin activation is accompanied by a threefold increment in transcriptional activity (6), but by approximately 100-fold increase in cellular mRNA content (6). Moreover, cachectin/TNF mRNA can exist within macrophages in a translationally inactive form (6). Cachectin/TNF protein synthesis is not detectable in quiescent cells; however cachectin/TNF is one of the major products of endotoxin-activated macrophages (7, 8). Thus, a 10,000-fold increase in the quantity of protein synthesized and secreted may occur.

Recently, much interest has focused upon the 3′-untranslated region as a potential repository of genetic elements responsible for post-transcriptional control. Some time ago, we noted that the 3′-untranslated region of various cytokines and protooncogenes contained a conserved, (U+A)-exclusive sequence (the TTATTTAT element), the function of which was unclear to us (9). Subsequent work drew attention to such sequences as elements capable of directing mRNA degradation (10, 11) and also capable of suppressing translation (12). Their role in the dynamics of gene expression has remained unclear, since no demonstration of changes in stability or translational efficiency following administration of an inducing stimulus has been presented.

If these or other 3′-untranslated sequences could indeed regulate protein synthesis, changes in mRNA stability or translational efficiency might be detected through the use of "post-transcriptional reporter" constructs. In preparing such constructs, we used a reporter gene (CAT), constitutively transcribed under the influence of a promoter unresponsive to the inducing agent, endotoxin. Varying portions of the

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Address correspondence to Dr. Bruce Beutler, The Howard Hughes Medical Institute and the Department of Internal Medicine, The University of Texas, Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9050.

3'-untranslated region of the endotoxin-responsive cDNA sequence (cachectin/TNF) were inserted downstream from the reporter coding sequence. Reporter mRNA and protein synthesis were then monitored in transfected RAW 264.7 (macrophage-derived) cells in the presence and absence of endotoxin.

## Materials and Methods

*Post-transcriptional Reporter Constructs.* The constructs produced are schematically illustrated in Fig. 1. Construct I contains a portion of the distal coding region of cachectin/TNF cDNA, as well as the entire 3'-untranslated region. Constructs II through V are successively shorter, and lack coding sequences derived from cachectin/TNF; construct V lacks the cachectin/TNF TTATTTAT element, containing only more distal sequences from the cachectin/TNF 3'-untranslated region. Construct VI also lacks the TTATTTAT element, containing only more proximal sequences from the 3'-untranslated region. Construct VII contains only the TTATTTAT element, inserted immediately downstream from the 3'-untranslated region of CAT. Construct VIII is a control for construct VII, and contains only the 3'-untranslated region of CAT, with no added sequences from cachectin/TNF. Construct IX is a control for constructs I through VI, and contains no 3'-untranslated sequences from either CAT or cachectin/TNF. Construct X is a second control for constructs I through VI, designed to ensure that only specific sequences may mediate the response to endotoxin. It contains an irrelevant cDNA sequence (representing much of the coding sequence and 3'-untranslated region of human placental ribonuclease inhibitor mRNA) (13) placed immediately downstream from the CAT coding sequence.

The constructs were produced as follows: A CAT genblock (Pharmacia Fine Chemicals, Piscataway, NJ) was blunted using the Klenow fragment of DNA polymerase I, and inserted (in correct orientation with respect to the SV40 promoter) into the Sma I site of the vector pSVL (Pharmacia Fine Chemicals). The CAT cDNA 3'-untranslated region was removed by digestion with Sca I and Sac I, and the coding sequence was repaired through the use of an oligonucleotide that began at the Sca I site, and terminated at a Sac I site immediately following the stop codon.

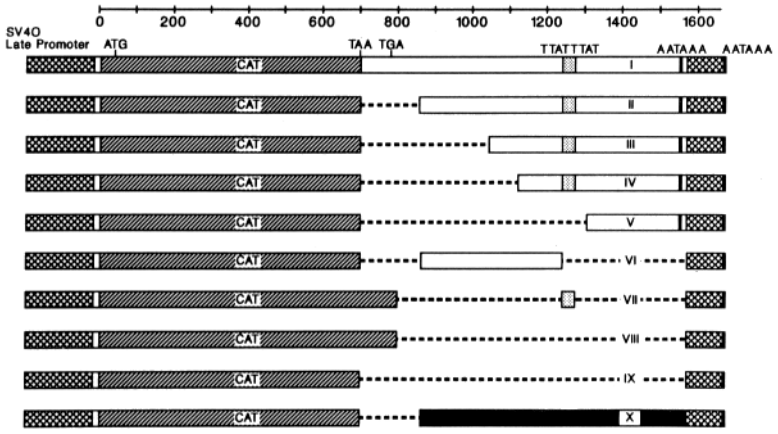


FIGURE 1. Post-transcriptional reporter constructs. Crosshatched bars represent vector sequence, diagonally shaded bars represent CAT mRNA sequence, and clear bars represent cachectin/TNF mRNA sequence, with a stippled zone (TTATTTAT) corresponding to the UpA-rich region. The solid bar represents human ribonuclease inhibitor mRNA sequence. Vertical bars represent the polyadenylation signal sequences present in cachectin/TNF mRNA and the vector, respectively. The scale is presented with reference to the first base in the 5'-untranslated region of CAT. The termination codons of CAT (TAA), and of cachectin/TNF (TGA), are as indicated. The roman numerals assigned to each of the 10 constructs shown here apply throughout the text.

A full-length human cachectin/TNF cDNA, in the vector pTZ19R, was obtained from Dr. Daniel Caput (Elf Biorecherche, Toulouse, France). The cDNA was cut with Sph I, and a series of deletions were produced using Bal-31. The ends were blunted with $S_1$ nuclease, and the 3'-untranslated region deletion fragment was recovered after digestion with Bam HI, and ligated into M13mp19 that had previously been digested sequentially with Xba I, $S_1$ nuclease, and Bam HI. The extent of deletion was determined by dideoxynucleotide sequencing, and five of the deletion fragments were excised using Sac I and Bam HI, and placed in tandem with CAT in the vector pSVL.

The scale presented in Fig. 1 refers to the 5' terminus of the CAT mRNA. With respect to the terminator U, clone I began at base −70, clone II began at base +87, clone III began at base +274, clone IV began at base +346, and clone V began at base +525. Clone VI was produced by partially digesting clone II with Nco I (which cleaves at base +425), then sequentially digesting with Sac I and $S_1$ nuclease before religation. Thus, a fragment extending between +87 and +421 was included. In clone VII, the cachectin/TNF TTATTTAT sequence was artificially synthesized, flanked by an upstream Sac I and a downstream Bam HI site; this fragment was added immediately after the 3'-untranslated region of CAT, which had already been placed in pSVL. In clone VIII (control for clone VII), no such addition was made. In clone IX (control for clones I through VI), the 3'-untranslated region of CAT was removed. In clone X (an additional control for clones I through VI), a 682 nt fragment of human ribonuclease inhibitor cDNA, corresponding to the entire 3'-untranslated region and part of the coding sequence, was introduced downstream from CAT.

*Cell Culture, Activation, and CAT Assay.* RAW 264.7 cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in DME supplemented with 5% FCS (HyClone Laboratories, Logan, UT). Cells were cotransfected with each of the constructs (or unmodified pSVL as a control) and PKO*neo* (Pharmacia Fine Chemicals) according to a calcium phosphate precipitation method (14). G418-resistant colonies generated over a 2-wk period using this antibiotic at a concentration of 1.0 mg/ml were picked at random. Transfected RAW 264.7 cell clones bearing the constructs indicated were plated at a density of $10^4$ cells/ml in 24-well plates, and incubated at 37°C for 18 h. As indicated, cultures were then activated with *E. coli* LPS (0127:B8; Difco Laboratories, Detroit, MI) at a final concentration of 1 μg/ml. At the times noted, cells were then removed from the plate by scraping into PBS, and lysed by sonication.

CAT assay was performed using the thin-layer chromatography method described by Gorman et al. (15); incubations with substrate were carried out for a period of 2 h. Numerical estimates of CAT activity were obtained by scraping the 3-acetylated form of chloramphenicol from the chromatography plate, and counting it in scintillation fluid. Since the initial concentration of $^{14}$C-chloramphenicol present in each assay system was 9.4 μM, and since the $K_M$ of *E. coli* transposon 9 CAT for chloramphenicol is 6.1 μM under the assay conditions applied (16), lysates were diluted so as to allow for the consumption of <40% of the substrate during the 2-h incubation period.

*Quantitation of Cytoplasmic CAT mRNA.* Total cytoplasmic RNA was isolated from cells that were cultured in 6-cm plates alongside the smaller cultures used for CAT assay. As in the matched smaller cultures, activation was accomplished using LPS at a concentration of 1 μg/ml. Cells were scraped from the plate, suspended in an ice-cold isotonic buffer (containing 0.14 M NaCl, 1.5 mM $MgCl_2$, 10 mM Tris, pH 8.6, and 100 U/ml heparin), and lysed by the addition of an equal volume of this buffer, containing 0.5% NP-40 detergent. The nuclei were pelleted, and the cytoplasmic extract was twice extracted with phenol, once extracted with chloroform, and precipitated with ethanol and ammonium acetate.

Total cytoplasmic RNA was carefully quantitated by subjecting it to electrophoresis in a methylmercury gel. Equal amounts of RNA were then applied to a formaldehyde gel, electrophoresed, stained with ethidium bromide, and transferred to nitrocellulose. The equality of transfer was assessed by examination of the nitrocellulose membrane; in all instances, the quantity of RNA transferred was reflective of the quantity applied to the gel, and approximately equal from lane to lane.

An antisense RNA probe for CAT was used to identify the various CAT mRNAs. After autoradiography, relative quantitation of hybridized mRNAs was achieved by liquid scintil-

lation counting of the bands, which were cut from the nitrocellulose filters over their autoradiographic images.

mRNA stability was assessed by adding actinomycin D, 10 μg/ml, to the cultures (either activated by LPS or left unactivated), which were then harvested at the times indicated for isolation of cytoplasmic RNA, and again examined by Northern blotting.

## Results

At least four clonal isolates bearing each construct were examined by CAT assay. Varying degrees of CAT expression were directed by each construct in clonal isolates before cell activation, presumably indicating variation in the number and location of inserted elements. However, the quantity of CAT activity expressed was readily detectable in all instances. As a generalization, those clones containing constructs programming the synthesis of longer mRNA molecules (including clones containing construct X) expressed CAT mRNA and protein at a lower level than those containing constructs encoding shorter mRNA molecules.

Representative clones were examined to determine the level of CAT mRNA and CAT protein expression, in a nonactivated state (Table I). While CAT mRNA levels are generally lower in cells transfected with constructs containing extensive portions of the 3′-untranslated region derived from cachectin/TNF, the expression of CAT protein is disproportionately depressed, suggesting that mRNAs possessing 3′-untranslated regions derived from cachectin/TNF are inefficiently translated. The relative efficiency of translation was quantitated by examining the ratio of CAT activity produced to cytoplasmic CAT mRNA expressed in each clone, yielding a "relative repression index." Thus, it emerged that the cachectin/TNF 3′-untranslated region present in construct II suppresses translation ~280-fold, with respect to the efficiency witnessed in the case of construct IX (which contains no 3′-untranslated region derived from cachectin/TNF). Indeed, all mRNAs derived from constructs containing the TTATTTAT element were poorly translated. Interestingly, the translation of mRNA derived from construct X was also somewhat inefficient, though more efficient than the translation of mRNAs that contained the TTATTTAT ele-

TABLE I

*Relative Repression of CAT Reporter mRNA Translation in Nonactivated RAW 264.7 Cells*

| | Construct | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| mRNA* | 1.0 | 2.0 | 2.5 | 2.4 | 4.6 | ND | 2.0 | 17 | 7.8 | 1.1 |
| CAT activity | 1.0 | 0.8 | 2.4 | 2.5 | 180 | ND | 56 | 4,200 | 880 | 20 |
| Ratio (CAT activity:mRNA) | 1.0 | 0.4 | 0.96 | 1.0 | 39 | - | 28 | 250 | 110 | 18 |
| Relative repression index‡ | 250 | 630 | 260 | 250 | 6.4 | - | 8.9 | 1.0 | 2.3 | 14 |

* mRNA level and CAT activity associated with unstimulated cells bearing construct I were arbitrarily assigned a value of 1.0, and quantities of mRNA and CAT activity associated with each of the other constructs tested are presented with reference to this value.

‡ To calculate the "relative repression index," the ratio of CAT activity to CAT mRNA was calculated for each clone. The ratio determined for construct VIII (which, according to these measurements, was the mRNA most efficiently translated) was then divided by the ratio determined for each of the other constructs. Thus, the relative translational repression of each mRNA was approximated, with reference to the efficiency measured for mRNA produced by construct VIII.

ment. Moreover, the mRNA derived from construct I (which contained a portion of the cachectin/TNF coding sequence) was translated more efficiently that the mRNA derived from construct II (which was shorter, and lacked coding sequence).

*CAT Activity and CAT mRNA Expression After Stimulation by Endotoxin.* Noting that the 3'-untranslated region derived from cachectin/TNF would strongly suppress translation, we sought to determine whether this region could also mediate a response to endotoxin in RAW 264.7 cells. CAT activity was measured in lysates prepared from cells containing construct II and from cells containing construct IX at various intervals after activation by endotoxin. Strong, time-dependent induction of CAT activity by endotoxin was observed in cells transfected with construct II (containing most of the cachectin/TNF 3'-untranslated region). An increase exceeding 200-fold was measured 9 h after endotoxin activation. However, no induction of the control CAT construct (IX) was observed, reflecting the insensitivity of the SV40 promotor, and sequences present in CAT mRNA or the vector, to the effects of endotoxin (Fig. 2 *a*).

CAT mRNA levels remained unchanged over several hours after cell activation by endotoxin (Fig. 2 *b*). Thus, the rise in CAT activity could not be attributed to a rise in the level of CAT mRNA.

In view of the fact that CAT mRNA levels remained static during endotoxin activation, we thought it unlikely that activation led to a change in mRNA stability. Nonetheless, since the TTATTTAT element is known to be destabilizing under some circumstances (10), we wished to exclude the possibility that cell activation by endotoxin could modulate the stability of the reporter mRNAs. Cells containing constructs II, V, and IX were treated with the transcriptional inhibitor actinomycin D and activated by endotoxin (or not activated in the case of control cultures; Fig. 2 *c*). Each reporter construct tested (including those containing the cachectin/TNF TTATTTAT sequence) gave rise to mRNA that was entirely stable over a period of 3 h. No change in stability was observed after endotoxin induction of actinomycin-treated cells.

*Localization of Endotoxin-responsive Sequence(s).* The dissociation of CAT mRNA levels and CAT expression suggested that the response to endotoxin conferred by the cachectin/TNF 3'-untranslated region was caused by modulation of translational efficiency. In an effort to identify which part of the cachectin/TNF 3'-untranslated region was responsive to endotoxin, cells bearing each of the constructs were stimulated with endotoxin, or left unstimulated (Fig. 3). Cells containing the control constructs (VIII and IX) exhibited little or no response to endotoxin (1.7-fold and 1.5-fold induction, respectively). However, CAT expression was strongly induced in cells containing constructs I through IV. Invariably, construct II proved to be the most inducible: a 124-fold increase in CAT activity was observed in the experiment shown. For reasons that remain unclear, construct I, which contained a distal segment of the cachectin/TNF coding sequence, was consistently less inducible (comparable in responsiveness to constructs III and IV).

CAT expression was induced far more weakly (9.5-fold) in cells containing construct V, and still more weakly (2.3-fold) in cells bearing construct VI. Thus, just as it is important in suppressing translation, the TTATTTAT element appears to be critical for the response to endotoxin. However, by itself, the TTATTTAT element could confer only a rather weak (7.3-fold) response, as witnessed by the inducibility of CAT activity in cells bearing construct VII. Thus, the context within which

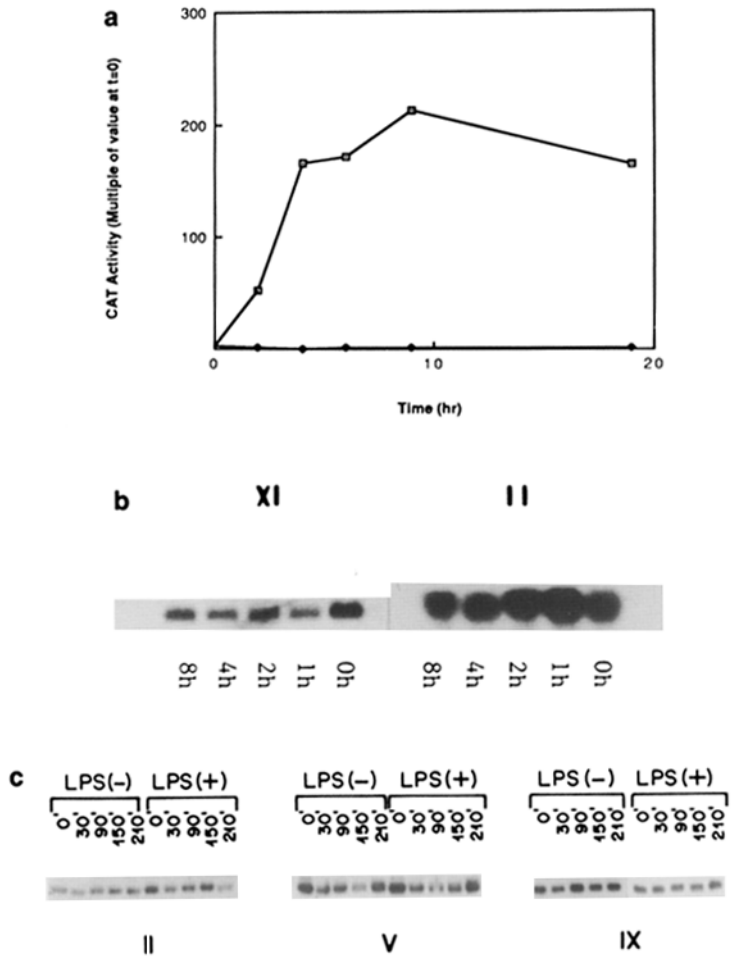


FIGURE 2. (*a*) CAT activity expressed by clones containing constructs II and IX as a function of time after cell activation by endotoxin. Cells containing each construct were plated at a density of $10^4$/ml in 24-well plates, and exposed to *E. coli* LPS (Difco Laboratories, strain 0127:B8) at a concentration of 1 μg/ml. At the times indicated, cells were scraped from the wells, lysed by sonication, and assayed for CAT activity. Activity is presented with reference to the initial (unstimulated) activity present in each cell type. (*b*) CAT mRNA levels expressed by clones containing constructs II and IX as a function of time after cell activation by endotoxin. Total cellular RNA obtained from matched cultures was harvested at the indicated times after cell activation by endotoxin, subjected to electrophoresis under denaturing conditions, transferred to nitrocellulose, and allowed to hybridize with a probe for the CAT coding sequence. Autoradiography was then performed (longer exposure for blots from clone II than for blots from clone IX). (*c*) Blot hybridization analysis of mRNA stability. RAW 264.7 cells containing each construct were maintained in the presence of actinomycin D (1 μg/ml), with or without added endotoxin (1 μg/ml), for the indicated time periods. Cytoplasmic RNA was harvested, and ~10 μg of each sample was electrophoresed in 1.2% agarose in the presence of formaldehyde and transferred to nitrocellulose. The RNA was probed with a $^{32}$P-labeled antisense transcript prepared from the CAT cDNA. LPS (+), cells incubated with endotoxin. LPS (−), cells incubated without endotoxin. Roman numerals refer to the constructs illustrated in Fig. 1.

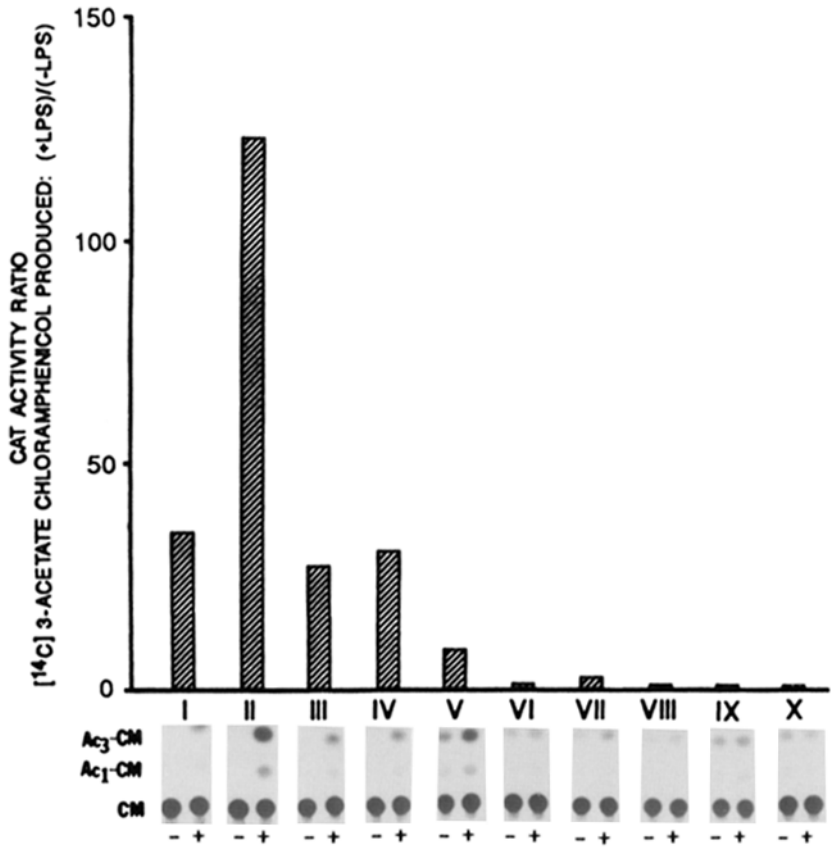


FIGURE 3. Induction of CAT activity by endotoxin in cells transfected with post-transcriptional reporter constructs. Cells containing each of the constructs shown in Fig. 1 were plated at a density of $10^4$/ml in 24-well plates, and incubated for 6 h in the presence (+) or absence (−) of endotoxin before lysis and assay of CAT activity at a dilution compatible with linear estimation. Dilutions were as follows: lysates I, II, and X were assayed undiluted; lysates III and IV were diluted 5-fold; lysate VI was diluted 10-fold; lysates V and VII were diluted 20-fold, and lysates VIII and IX were diluted 400-fold. The autoradiograms obtained, and the ratio of induced to noninduced counts present in the 3-acetylated form of chloramphenical in assays performed on lysates obtained from cells bearing each construct, are presented. Roman numerals refer to the constructs illustrated in Fig. 1.

the TTATTTAT element is represented is essential for mediation of the response to endotoxin.

We wished to exclude the possibility that endotoxin inducibility was independent of mRNA sequence, or simply a function of mRNA length. Therefore, we examined the inducibility of CAT activity in cells transfected with construct X. The distal coding and 3′-untranslated sequences of human ribonuclease inhibitor mRNA proved to be insensitive to the effects of endotoxin (0.9-fold induction), suggesting that the response is indeed sequence specific.

## Discussion

Both nuclear and cytoplasmic mechanisms contribute to the regulation of gene expression. On occasion, induction at different levels may act in a coordinate fashion, leading to amplified production of a protein product. Cachectin/TNF gene expression provides a case in point. Endotoxin causes transcriptional activation of cytokine biosynthesis (6, 17, 18). However, it is clear that the degree of activation observed at this level cannot account for the remarkable change observed at the protein level.

Our data sugget that regulation of cachectin/TNF biosynthesis may occur at the level of translation, and that induction by endotoxin is dependent upon the presence of sequences lying downstream from the coding region. This conclusion is based upon two observations.

First, while cells containing control constructs (lacking cachectin/TNF 3′-untranslated region sequences) were not induced to express CAT by endotoxin, constructs that contained 3′-untranslated region sequences were induced, most strongly so if the majority of the 3′-untranslated region was included in the transcript.

Second, no change in the level of CAT mRNAs was noted after activation, and the stability of CAT mRNAs over the period of induction was such that transient, selective stabilization of message could not account for the increase of CAT activity that was measured. This finding was somewhat surprising, since we had anticipated that the 3′-untranslated region of cachectin/TNF would confer instability. However, it is clear that UpA-rich sequence elements are not destabilizing in all situations, as in human T lymphoblasts treated with phorbol esters (10), or in *Xenopus* oocytes (12). We cannot exclude the possibility that selective stabilization of mRNA may contribute to the inducing effect of endotoxin under some circumstances, as in primary macrophage cultures.

Other instances of strictly translational regulation have been reported in eukaryotes (4, 5, 19–21). 5′-untranslated sequences, or structures separate from the mRNA molecule, were thought to govern translation in each instance. Here, we note that downstream sequences are also capable of responding to an inducing stimulus, and markedly influencing translational efficiency. Moreover, the magnitude of the effect that we have measured is comparatively large. We are presently uncertain as to the translational regulatory functions of the 5′-untranslated and coding regions of cachectin/TNF mRNA, and consider it possible that they, too, may help to confer the response to endotoxin. By itself, however, the 3′-untranslated region appears capable of mediating a substantial portion of the induction.

The functional significance of the 3′-untranslated region remains poorly understood. As a generalization, it may be said that each mRNA must encode its own stability, and its own translational efficiency, given that most mRNA molecules exist within a common cellular environment and are subject to the same external influences. Since coding sequences are largely constrained by structural requirements unrelated to regulation of stability or translational efficiency, it is likely that the untranslated regions serve as a repository of regulatory sequences. It has previously been demonstrated that antisense RNA directed against the 3′-untranslated region of a marker mRNA (encoding tissue plasminogen activator) blocks translational activation in mouse oocytes (21), and that the UpA-rich sequence present in IFN-$\beta$ mRNA, as well as granulocyte/macrophage-CSF mRNA, may impose translational blockade when inserted in a downstream position (22). The data that we have presented re-

veal that 3'-untranslated sequences may influence translation in a dynamic fashion. Conceivably, a variety of inducible genes may prove to be responsive at the same level, and indeed, the 3'-untranslated region may be retained largely for the purpose of regulating translation.

While a potent suppressor of translation (3, 12, 22), the TTATTTAT element is, by itself, only weakly responsive to endotoxin. Response to endotoxin is markedly enhanced by sequences that precede and follow TTATTTAT. True synergy appears to exist, insofar as separate expression of constructs V, VI, and VII leads to low inducibility, whereas the expression of construct II (approximately the sum of these parts) leads to inducibility that is superadditive.

As previously mentioned, expression of CAT activity was far lower in cells bearing constructs that included large stretches of the cachectin/TNF 3'-untranslated region. For example (Table I), before endotoxin activation, CAT activity expressed by cells containing construct IX was ~1,100 times higher than CAT activity expressed by cells containing construct II. A marked discrepancy was observed at the mRNA level. Cells containing construct IX expressed CAT mRNA at a level only about fourfold greater than that of the corresponding CAT mRNA expressed by cells containing construct II. Indeed, the "relative repression index" determined for each mRNA approximately reflects the inducibility of each mRNA: those species that are the most repressed in nonactivated cells are also the most inducible. It would seem probable that biosynthesis of CAT protein was repressed by sequences present in the 3'-untranslated region (including, but not limited to, the TTATTTAT element), and that activation by endotoxin led to "derepression" of translation.

Preliminary analysis of 3'-untranslated sequences derived from disparate endotoxin-responsive genes (cachectin/TNF, IL-1, and GM-CSF) has not revealed conservation at the level of primary structure, the TTATTTAT element notwithstanding (9, 10). Conceivably, secondary structural features of such mRNAs may be very important for recognition, or for presentation of the TTATTTAT element. The types of molecular interactions required for augmented translation remain to be determined.

Other studies (not shown) have revealed that activation of CAT mRNA translation may occur even in the presence of a transcriptional inhibitor (actinomycin D), but that a far weaker effect is observed under these conditions. This may suggest that de novo RNA and protein synthesis are required for transduction of the endotoxin effect.

## Summary

The biosynthesis of cachectin/TNF is largely regulated at a post-transcriptional level. Bacterial endotoxin, which strongly induces cachectin/TNF production, thus seems to elicit at least some of its effects by altering the macrophage cytoplasmic milieu. It has previously been shown that the 3'-untranslated TTATTTAT element present in numerous cytokines and proto-oncogenes is capable of repressing the translation of mRNA molecules in which it is represented. Using constructs in which the CAT coding sequence is followed by varying segments of the cachectin/TNF 3'-untranslated region, we now demonstrate that downstream sequences present in the cachectin/TNF mRNA are sufficient to mediate >200-fold induction of CAT synthesis in response to activation by endotoxin. Induction of CAT activity is not attributable to a change in cytoplasmic mRNA concentration, but to a marked en-

hancement of translational efficiency. The response to endotoxin represents "derepression," and is conferred chiefly by the translationally repressive TTATTTAT element, acting in concert with essential flanking sequences.

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## References

1. Vaidya, A. B., N. E. Taraschi, S. L. Tancin, and C. A. Long. 1983. Regulation of endogenous murine mammary tumor virus expression in C57BL mouse lactating mammary glands: transcription of functional mRNA with a block at the translational level. *J. Virol.* 46:818.
2. Miller, A. D., T. Curran, and I. M. Verma. 1984. c-fos protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. *Cell.* 36:51.
3. Kruys, V. I., M. G. Wathelet, and G. A. Huez. 1988. Identification of a translation inhibitory element (TIE) in the 3' untranslated region of the human interferon-beta mRNA. *Gene (Amst.).* 72:191.
4. White, K., and H. N. Munro. 1988. Induction of ferritin subunit synthesis by iron is regulated at both the transcriptional and translational levels. *J. Biol. Chem.* 263:8938.
5. Yen, T. J., P. S. Machlin, and D. W. Cleveland. 1988. Autoregulated instability of $\beta$-tubulin mRNAs by recognition of the nascent amino terminus of $\beta$-tubulin. *Nature (Lond.).* 334:580.
6. Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science (Wash. DC).* 232:977.
7. Beutler, B., J. Mahoney, N. Le Trang, P. Pekala, and A. Cerami. 1985. Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. *J. Exp. Med.* 161:984.
8. Mahoney, J. R. Jr., B. A. Beutler, N. Le Trang, W. Vine, Y. Ikeda, M. Kawakami, and A. Cerami. 1985. Lipopolysaccharide-treated RAW 264.7 cells produce a mediator which inhibits lipoprotein lipase in 3T3-L1 cells. *J. Immunol.* 134:1673.
9. Caput, D., B. Beutler, K. Hartog, S. Brown-Shimer, and A. Cerami. 1986. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc. Natl. Acad. Sci. USA.* 83:1670.
10. Shaw, G., and R. Kamen. 1986. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell.* 46:659.
11. Wilson, T., and R. Treisman. 1988. Removal of poly(A) and consequent degradation of c-fos mRNA facilitated by 3' AU-rich sequences. *Nature (Lond.).* 336:396.
12. Kruys, V., M. Wathelet, P. Poupart, R. Contreras, W. Fiers, J. Content, and G. Huez. 1987. The 3' untranslated region of the human interferon-beta mRNA has an inhibitory effect on translation. *Proc. Natl. Acad. Sci. USA.* 84:6030.
13. Crawford, D., K. Hagerty, and B. Beutler. 1989. Multiple splice forms of ribonuclease inhibitor mRNA differ only in the 5'-untranslated region. *Gene (Amst.).* In press.
14. Davis, L. G., M. D. Dibner, and J. F. Battey. 1986. Basic Methods in Molecular Biology. Elsevier Science Publishing Co., Inc., New York. 1–388.
15. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* 12:1044.
16. Shaw, W. V., and R. F. Brodsky. 1968. Characterization of chloramphenicol acetyltrans-

ferase from chloramphenicol-resistant Staphylococcus aureus. *J. Bacteriol.* 95:28.
17. Collart, M. A., D. Berlin, J. D. Vassalli, S. DeKossodo, and P. Vassalli. 1986. Gamma interferon enhances macrophage transcription of the tumor necrosis factor/cachectin, interleukin 1, and urokinase genes, which are controlled by short-lived repressors. *J. Exp. Med.* 164:2113.
18. Sariban, E., K. Imamura, R. Luebbers, and D. Kufe. 1988. Transcriptional and post-transcriptional regulation of tumor necrosis factor gene expression in human monocytes. *J. Clin. Invest.* 81:1506.
19. Walden, W. E., S. Daniels-McQueen, P. H. Brown, L. Gaffield, D. A. Russell, D. Bielser, L. C. Bailey, and R. E. Thach. 1988. Translational repression in eukaryotes: partial purification and characterization of a repressor of ferritin mRNA translation. *Proc. Natl. Acad. Sci. USA.* 85:9503.
20. Rouault, T. A., M. W. Hentze, S. W. Caughman, J. B. Harford, and R. D. Klausner. 1988. Binding of a cytosolic protein to the iron-responsive element of human ferritin messenger RNA. *Science (Wash. DC).* 241:1207.
21. Strickland, S., J. Huarte, D. Belin, A. Vassalli, R. J. Rickles, and J.-D. Vassalli. 1988. Antisense RNA directed against the 3′ noncoding region prevents dormant mRNA activation in mouse oocytes. *Science (Wash. DC).* 241:680.
22. Kruys, V., O. Marinx, G. Shaw, J. Deschamps, and G. Huez. 1989. Translational blockade imposed by cytokines-derived UA-rich sequences. *Science (Wash. DC).* 245:852.