Functionally Novel Tumor Necrosis Factor-α–Modulated CHR-Binding Protein Mediates Cyclin A Transcriptional Repression in Vascular Endothelial Cells

Raj Kishore, Ioakim Spyridopoulos, Corinne Luedemann, Douglas W. Losordo

Abstract—Local expression of tumor necrosis factor-α (TNF-α) at the sites of arterial injury after balloon angioplasty, suppresses endothelial cell (EC) proliferation and negatively affects reendothelialization of the injured vessel. We have previously reported that in vitro exposure of ECs to TNF-α induced EC growth arrest and apoptosis. These effects were mediated, at least in part, by downregulation of cell cycle regulatory proteins. In the present study, we report potential mechanism(s) for TNF-α–mediated suppression of cyclin A in ECs. TNF-α exposure to ECs completely abrogated cyclin A mRNA expression via mechanisms involving both transcriptional and posttranscriptional modifications. TNF-α inhibited de novo cyclin A mRNA synthesis and suppressed cyclin A promoter activity. Utilizing deletion mutants of human cyclin A promoter, we have identified CDE-CHR (Cell cycle–Dependent Elements–Cell cycle genes Homology Region) region of cyclin A promoter as a target for TNF-α–mediated increase in specific DNA binding activity to the CHR elements. This increase in binding activity by TNF-α was mediated via the induction of a functionally novel 84-kDa protein that binds specifically to CHR in Southwestern assays. UV cross-linking and SDS-PAGE analysis of proteins eluted from specific complex confirmed the presence of this 84-kDa protein. Moreover, induction of this protein by TNF-α was protein synthesis dependent. Additionally, exposure of ECs to TNF-α markedly reduced cyclin A mRNA stability. Targeted disruption of this protein could potentially be a therapeutic strategy to rescue EC proliferation in vivo. (Circ Res. 2002;91:307-314.)

Key Words: endothelial cells ■ tumor necrosis factor-α ■ cell cycle–dependent elements ■ cell cycle gene homology region

Tumor necrosis factor-α (TNF-α), a pleiotropic cytokine, exerts effects ranging from induction of immunomodulatory molecules to regulating cell proliferation and differentiation and apoptosis in various cell types, including endothelial cell (ECs).1–3 TNF-α is expressed in arteries during atherosclerosis and restenosis with increased expression after balloon injury in multiple animal models.4–7 We have previously shown that blockade of TNF-α improves reendothelialization after balloon angioplasty, suggesting that TNF-α acts to delay endothelial regrowth at sites of balloon injury.8 We also reported that exposure of primary endothelial cells to TNF-α in vitro inhibited proliferation, arresting the ECs in late G1 phase accompanied by enhanced apoptosis.9 TNF-α–mediated EC cycle arrest has been shown to involve the repression of cell cycle regulatory genes, including that of cyclin A.8–10 However, the molecular mechanisms underlying TNF-α–mediated cyclin A repression are poorly understood. Moreover, the cis-elements that may be the target of TNF-α and trans-factors interacting with them remain to be elucidated.

In the present study, we show that the inhibitory effect of TNF-α on cyclin A gene expression is mediated both by a transcriptional repression as well as by a reduction in mRNA stability. Moreover, we show for the first time that CHR elements within cyclin A promoter are the target of TNF-α. Furthermore, we report a functionally novel, TNF-α inducible, 84-kDa protein that binds specifically to CHR (Cell cycle genes Homology Region) elements and appears to be involved in transcriptional repression of cyclin A.

Materials and Methods

Cell Culture

Bovine arterial endothelial cells (BAECs) were isolated as previously described.11 Cells in passages 3 to 6 were maintained in MEM supplemented with 10% FBS, 100 U/mL streptomycin/penicillin, and 50 μg/mL gentamycin in a 5% CO2 atmosphere. For synchronization, cells were serum starved for 48 to 72 hours.

Constructs, Transient Transfection, and Reporter Assays

Cyclin A–Luc promoter reporter constructs containing wild-type (wt) and/or mutated fragments from mouse cyclin A promoter have been described before.12 BAECs were transiently transfected with luciferase reporter promoter constructs (pGL2-basic) containing...
various fragments representing either wt or mutated DNA binding sites from mouse cyclin A promoter using Lipofectamine (Gibco) following manufacturer’s instructions. Cells were trypsinized, pooled, and replated as per experimental requirement and were treated or not with 40 ng/mL recombinant human TNF-α for 24 hours. Cells were harvested and assayed for luciferase activity using Berthold Lumat LB9501 luminometer. Luciferase activity was normalized to the alkaline phosphatase activity produced by a cotransfected alkaline phosphatase plasmid (pSVAPAP), which served as transfection efficiency control.

RNA Isolation and Ribonuclease Protection Assay (RPA)
Synchronized BAECs were released from serum starvation and were further cultured in the presence or absence of TNF-α (40 ng/mL) for indicated time. Total cellular RNA was isolated using Trizol reagent (Life Technologies Inc). For mRNA stability experiments, cells were treated with actinomycin D (Sigma, 5 μg/mL), before RNA was isolated. Human cyclin multiprobe DNA template (Pharmingen) and [α-32P]UTP (NEN) was used to synthesize in vitro transcribed antisense riboprobe and RPA were performed using RPA III TM kit (Ambion) following manufacturer’s instructions. Briefly, 5 to 10 μg of RNA was hybridized with radiolabeled probe and was then digested with RNase A/T1. RNase protected transcripts were then precipitated and were run on a 5% sequencing gels, dried, and autoradiographed.

Nuclear Run-On
Nuclear run-on experiments to measure nascent cyclin A transcripts were essentially performed as described elsewhere.13,14

Figure 1. TNF-α suppresses cyclin A mRNA expression. Synchronized ECs were serum stimulated in the presence or absence of TNF-α for indicated times. Total cellular RNA was isolated and was analyzed by RPA for cyclin A, cyclin B, and cyclin D1 expression. Representative autoradiograph is shown. mRNA expression of cyclins was corrected to that of GAPDH and quantified by densitometric analysis. Data represent average of 3 similar experiments.

Southwestern Analysis
Nuclear proteins (50 μg) were resolved by 12% SDS-PAGE gels, electrotransferred to PVDF membrane, and rocked for 10 minutes at 4°C in binding buffer (25 mmol/L HEPES/KOH, 60 mmol/L KCl, 1 mmol/L DTT, 1 mmol/L EDTA, and 6 mol/L guanidinium chloride). The membrane was transferred to binding buffer containing 3 mol/L guanidinium chloride and incubated as in previous step. Renaturation was achieved by 8 successive washes in binding buffer, each with a 50% stepwise reduction of guanidinium chloride, and a final 1-hour incubation in the absence of guanidinium chloride plus 5% nonfat dry milk and 5 μg/mL sonicated salmon sperm DNA. The membrane was then incubated with 32P-labeled oligonucleotides in binding buffer plus 0.25% nonfat dry milk for 2 to 4 hours at room temperature. Membranes were washed, air dried, and autoradiographed.

UV Cross-Linking of DNA-Protein Complexes
The binding reactions were UV cross-linked by placing handheld UV lamp (254 nm) at a distance of 2 cm from reactions for 20 to 30 minutes. UV cross-linked DNA-bound proteins were size fractionated on a 12% SDS-PAGE gel. Alternatively, after EMSA, wet gel was wrapped in saran wrap and autoradiographed. The specific DNA-protein complex was excised from the gel UV cross-linked and incubated overnight with SDS sample buffer at room temperature. Eluted proteins were resolved by SDS-PAGE. Gel was dried and autoradiographed.

Statistical Analysis
Data are presented as mean±SEM. Student’s t test was used to evaluate the differences between groups, and statistical significance was assigned when P<0.05.

Results
EC Cyclin A mRNA Is Specifically Downregulated by TNF-α
We have previously shown that treatment of ECs with TNF-α suppressed cyclin A protein levels as well as cyclin-dependent kinase (cdk) 2 activity.15 To determine the specificity of this suppression, we analyzed mRNA expression of cyclins A, B, and D1 in ECs exposed to TNF-α. As shown in Figure 1, cyclin A mRNA was almost undetectable after

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8-hour TNF-α treatment. However, TNF-α exposure resulted in no discernible changes in the mRNA levels of cyclin B and cyclin D1. These data suggest that TNF-α specifically down-regulates cyclin A mRNA expression in ECs.

### TNF-α Suppresses De Novo mRNA Synthesis of Cyclin A

To investigate whether TNF-α–induced repression of cyclin A mRNA reflects a decreased transcriptional rate, nuclear run-on experiments were performed. Serum stimulation of synchronized BAEC stimulated de novo cyclin A mRNA synthesis (Figure 2). However, exposure of cells to TNF-α for 8 to 16 hours blunted the de novo cyclin A mRNA transcription 5-fold at 16 hours. There was no effect of TNF-α on control β-actin mRNA synthesis.

### Cyclin A mRNA Is Specifically Destabilized by TNF-α

Although TNF-α reduces the cyclin A mRNA transcription, decrease in the rate of de novo mRNA synthesis at 16 hours after TNF-α treatment (Figure 2) does not account for the total loss of cyclin A mRNA at the same time points as observed earlier (Figure 1). These data indicated the presence of additional TNF-α–mediated suppressive mechanism(s). Because TNF-α is known to influence mRNA stability and because cyclin A regulation also occurs at the level of mRNA stability, we investigated the influence of TNF-α on cyclin A mRNA stability. Cells were stimulated with serum, with or without TNF-α, and in the presence of actinomycin D (5 μg/mL) for 4 to 24 hours. RNA was isolated and analyzed by RPA for cyclin A, cyclin D1, and GAPDH mRNA levels. As shown in Figure 3, under basal condition cyclin A mRNA decayed with an approximate half-life of 5.5 hours. However, the half-life of cyclin A mRNA was reduced to approximately 3.0 hours in the presence of TNF-α. There was no significant change in the half-life of cyclin D1 mRNA in response to TNF-α, which decayed with an approximate half-life of 8 hours in the presence or absence of TNF-α. These results suggest that both transcriptional and posttranscriptional mechanisms mediate TNF-α suppressive action on cyclin A gene expression.

### CDE-CHR cis-Elements Are Essential for Efficient Cyclin A Promoter Activity

We focused our next series of experiments on understanding TNF-α–mediated transcriptional suppression of cyclin A. Utilizing reporter constructs containing various fragments of the cyclin A promoter (Figure 4) and its binding site deletion mutants linked to a luciferase reporter gene, we first analyzed the requirement of individual transcription factor binding sites for the transcription of cyclin A. ECs were transiently transfected with each of the reporter constructs and fold induction of reporter activity over control pGL2 vector was documented. As indicated in Figure 4, a reporter construct encompassing −924 to +100 region of cyclin A promoter and a shorter fragment containing regions −79 to +100 were equipotent in driving the transcription of the reporter gene. A further truncation of promoter (−54/+100) rendered the promoter ineffective, suggesting minimal requirement of at least −79/+100 region for optimal promoter activity. These data suggest that cis-elements within −79/+100 region of cyclin A promoter harbor elements that are both necessary and sufficient for effective cyclin A transcription. This conclusion was supported by the observation that deletion of CDE elements from both −924/+100 and −79/+100 frag-
ments repressed cyclin A promoter activity (P<0.01 and P<0.001, respectively). Furthermore, deletion of either AP1 or ATF binding sites from the −924/+100 fragment did not significantly suppress promoter activity. As expected removal of all 3 binding sites (AP1, ATF, and CDE-CHR) from −924/+100 promoter completely abolished the promoter activity. These data confirm the finding of other studies that CDE-CHR cis-elements in the cyclin A promoter are essential for efficient transcription of cyclin A.

**TNF-α–Mediated Suppression of Cyclin A Promoter Activity Requires CDE-CHR Elements**

To delineate the cis-elements that may be the target of TNF-α suppressive action, a subset of BAECs transiently transfected with reporter constructs, as shown in Figure 4, were treated with TNF-α before the analysis of reporter activity. As shown in Figure 5, reporter activity was significantly suppressed by TNF-α in cells transfected with those constructs that contained CDE-CHR elements. Deletion of either AP1 or ATF binding sites from −924/+100 construct had no effect on TNF-α–mediated repression of promoter activity. However, deletion of CDE-CHR significantly abrogated the suppressive action of TNF-α on cyclin A promoter activity (P<0.001). Taken together these results suggest that CDE-CHR elements are not only required for optimal promoter activity but are also the target of TNF-α–mediated suppression of cyclin A promoter activity.

**TNF-α Modulates CDE-CHR Binding Activity**

We next asked whether TNF-α could modulate the binding of transcription factors to the CDE-CHR elements in functional assays. To this end, EMSAs were performed using synthetic oligonucleotides spanning the CDE-CHR elements of the cyclin A promoter or their mutant forms wherein either CDE or the CHR sites were mutated. Nuclear extracts from synchronized ECs formed two complexes with CDE-CHR oligonucleotides (Figure 6). Serum stimulation of cells for 16 hours completely abrogated complex II and greatly reduced complex I. These data agree with other published reports that suggest that in growth-arrested cells, CDE-CHR repressor elements are occupied with trans-factors such as CDF-1, thereby inhibiting transcription of cyclin A. On growth stimulation, these factors are released, allowing the transcription to proceed. In ECs, TNF-α increased the binding activity of complex I both at 4 hours and more appreciably at 16 hours. Unlike that by serum-stimulated extracts, complex I formation by TNF-α–treated extracts, could not be competed by 25- to 50-fold and modestly competed by 100-fold excess of cold CDE-mCHR oligonucleotide. This implies that complex I represents binding activity specific to CHR elements and not to both CDE and CHR elements like CDF-1. As a control, 25-fold excess of self competition completely abrogated both complexes. These data suggest either a sustained binding of factors to CHR, thereby repressing...
transcription of cyclin A despite serum stimulation, or recruitment/induction of additional protein/factor in response to TNF-α, which keeps the CHR occupied and thereby represses promoter activity.

CHR and not the CDE Binding Activity Is Modulated by TNF-α
To gain insight into the individual contribution of the CDE and/or the CHR elements, bindings assays were performed utilizing an oligonucleotide in which CDE site was nucleotide substituted and by competing the binding with oligonucleotide, with substitution mutations of either CDE (self) or that of CHR sites. As depicted in Figure 7, nuclear proteins from asynchronously growing BAECs formed a single complex with mobility similar to that of complex I in Figure 6. This binding activity was increased in nuclear extracts from cells treated with TNF-α for 8 hours. Furthermore, a 25-fold excess of unlabeled mCDE-CHR oligonucleotide effectively inhibited this binding activity, whereas a 100-fold excess of unlabeled CDE-mCHR oligonucleotide did not. These data indicate that modulation of binding activity by TNF-α represents the binding of nuclear proteins to the CHR elements rather than to the CDE elements. EMSA using labeled CDE-mCHR probe also formed a single complex with no significant changes by TNF-α in the binding activity (data not shown).

TNF-α–Induced 84-kDa Protein Binds Specifically to Cyclin A CHR Elements
To identify the proteins, which formed complexes with CDE and CHR elements in EMSA, we performed Southwestern experiments. Nuclear proteins from asynchronously growing BAECs with or without TNF-α were probed with 32P-labeled CDE-mCHR or mCDE-CHR oligonucleotides. As shown in Figure 8A, two proteins of approximately 35 and 27 kDa were detected with CDE-mCHR probe. However, levels of neither protein changed in response to TNF-α. Similarly, two proteins of approximately 39 and 30 kDa were observed in extracts from control cells when probed with mCDE-CHR oligonucleotide. Interestingly, an additional protein of approximately 84 kDa was found to react with mCDE-CHR probe in the nuclear extracts from TNF-α–treated cells. These results further confirm that modulation in the CHR binding activity by TNF-α was specific and most likely due to the induction or modulation of a new protein.

CHR Binding Complex Contains TNF-α–Induced 84-kDa Protein
To confirm that 84-kDa protein in TNF-α–treated extracts identified in Figure 8A does bind to CHR elements, we performed UV cross-linking experiments. EMSA reactions
were assembled as before followed by UV cross-linking. The reactions were then boiled in SDS loading buffer and resolved on 12% SDS-PAGE. In reactions probed with CDE-mCHR, the 35-kDa protein seen before was observed again (Figure 8B). In reactions probed with mCDE-CHR probe, the previously identified (Figure 8A) 39-kDa protein was found cross-linked to oligonucleotide. Similarly, the 84-kDa protein from TNF-α-treated extract cross-linked with mCDE-CHR probe, further confirming the Southwestern results. Moreover, the induction of 84-kDa protein by TNF-α was protein synthesis–dependent because treatment of cells with cycloheximide (5 μg/mL) along with TNF-α failed to induce the protein.

To further confirm that this TNF-α–induced 84-kDa protein is a constituent of CHR binding activity in TNF-α–treated extracts, the specific band from mCDE-CHR EMSA was excised, UV cross-linked, and eluted DNA–protein mixture was subjected to SDS-PAGE. The results, shown in Figure 8C, confirm that TNF-α–induced 84-kDa protein is indeed a constituent of CHR binding activity.

Discussion

Cyclins control the transitions between the phases of the eukaryotic cell cycle as regulatory subunits of the cyclin-dependent kinases (cdks). Cyclin A mRNA levels increase at the G1–S boundary and reach peak levels in S phase. The short promoter fragment of the cyclin A gene has been shown to harbor most, if not all, regulatory elements required for cell cycle regulation and efficient transcription. These elements comprise two contiguous repressor binding sites: CDE and CHR. Modulations in the binding of trans-factors, such as CDF-1, to these repressor elements during various phases of cell cycle is thought to interfere with the transactivation function of factors upstream and regulate phase-specific expression of cyclin A. Apart from intrinsic regulation of cyclin A gene expression, exogenous stimuli including cytokines TGFβ, IFNγ, and TNF-α have also been shown to modulate cyclin A expression in various cell types.

The pleiotropic cellular effects of the TNF-α are thought to result from modulations in gene expression. However, because TNF-α modulates the expression of a wide variety of genes, many without known function, the changes in cellular activity mediated by TNF-α, including suppression of growth, are at best partially understood. In ECs, TNF-α activates inflammatory responses and influences angiogenesis, but also negatively affects EC proliferation and apoptosis. Inhibition of EC proliferation and enhanced apoptosis in response to TNF-α has significant bearing on endothelial recovery after balloon angioplasty, which may in turn contribute to the development of restenosis. Indeed, we have earlier reported that blockade of locally expressed TNF-α at the site of balloon injury improves the process of reendothelialization. We and others have also reported that in vitro exposure of primary ECs to TNF-α inhibits their proliferation and impairs their ability to progress through the cell cycle. This inhibitory effect of TNF-α, at least partially, depends on the downregulation of cell cycle regulatory genes, including that of cyclin A. Although TNF-α–mediated repression of cyclin A has been previously reported in ECs and other cell types, the molecular mechanisms for such repression are largely unknown. In the present study, we have investigated the molecular basis of TNF-α–mediated suppression of cyclin A in ECs. First, we found that TNF-α specifically downregulates cyclin A mRNA and that this downregulation is mediated both by a decreased transcription as well as via enhanced degradation of cyclin A mRNA. Secondly, we have identified CDE-CHR corepressor cis-elements in the cyclin A promoter as the target of TNF-α–mediated transcriptional repression. Finally, we have shown that TNF-α induces a functionally novel 84-kDa protein that binds specifically to CHR corepressor element in the cyclin A promoter.
Cyclin A promotes cell cycle progression by associating with and stimulating cyclin-dependent kinases cdc2 and cdk2 and is a critical regulator of the cell cycle at both G1-S junction and during G2-M transition.28 Like that of other cell cycle molecules, the level of cyclin A is controlled primarily by cyclical changes in mRNA expression.12,28,29 However, there is growing body of evidence suggesting that cytokines such as TGFβ1, INFγ, and TNF-α also inhibit the expression of cyclin A in various cell types.13,22,23 Transcriptional repression of cyclin A has been suggested in response to both TGFβ1 and INFγ.13,22 Inhibition of cyclin A promoter activity by TGFβ1 requires an intact ATF site, and this effect involves decreased phosphorylation of CREB and ATF-1.22,23 INFγ-mediated inhibition of cyclin A gene transcription is independent of individual cis-acting elements.13 However, neither the cis-elements that may be the target of TNF-α nor the molecular mechanisms for TNF-α-mediated cyclin A transcriptional repression are yet defined. We have not only provided the evidence that TNF-α suppress de novo cyclin A mRNA synthesis (Figure 2), but have also identified CDE-CHR elements in cyclin A promoter as the potential target of TNF-α. It is now well established that CDE-CHR elements, defined as corepressor elements in cyclin A promoter, control cell cycle–dependent expression of cyclin A.16,17,30 We have identified these same cis-elements as the target of TNF-α suppressive action. Enhanced destabilization of cyclin A mRNA in response to TNF-α (Figure 3) is yet another interesting observation. Although increased cyclin A mRNA stability at different stages of cell cycle has been reported before,29 we now demonstrate TNF-α-induced modulations in the half-life of cyclin A. It is noteworthy that the 3′ untranslated region of cyclin A mRNA contains A+U rich elements (AREs).29,31 AREs have long been established as an important determinant of mRNA stability/instability, depending on their interaction with either mRNA stabilizing proteins like HuR or mRNA destabilizing protein such as AUF1.29–33 HuR has been shown to interact with cyclin A AREs, resulting in marked stabilization of the cyclin A mRNA in late G1-S phase.29 Whether TNF-α acts by inhibiting HuR/ cyclin A-ARE binding or increases the affinity of cyclin A-AREs to bind to RNA destabilizing protein AUF1 remains to be elucidated. However, because the same ARE sequences bind to both proteins, depending on their ability to shuttle between nucleus and cytoplasm, it remains a possibility that TNF-α may modulate the affinity of cyclin A-AREs to the two functionally different ARE binding proteins.

We have shown that TNF-α specifically upregulates DNA binding activity to CHR elements. Although, changes in protein-DNA binding are by no means required for altered transcriptional activity, as evident by INFγ-mediated suppression of cyclin A promoter activity despite no changes in DNA binding activity to cis-elements,11 changes in EC cyclin A transcriptional activity induced by TNF-α seem to coincide with the altered DNA binding activity. Disruption of CDE-CHR site renders insensitivity to TNF-α both in terms of promoter activity as well as to DNA binding activity. Similar functional role for the ATF site in cyclin A promoter has been linked to the TGFβ1-mediated suppression of cyclin A.

In order to understand how CHR participates in TNF-α-mediated cyclin A downregulation, we tried to characterize cyclin A CHR binding activity. We show that binding to the CHR can occur in the presence of mutations within CDE element. The existence of a DNA-protein complex on the CHR seems to preclude binding to CDE, because only complexes corresponding to each individual element were visible by gel mobility shift assays (data not shown). This could either be due to the binding of mutually exclusive complex (Figure 6), or to the existence of hetero-oligomers with decreased affinity for the dual element. At least two protein/factors are known to bind to CDE-CHR corepressor elements. An in vitro binding activity termed CDF-1 has been extensively described, which, based on DNA footprinting studies, binds to both CDE and CHR elements and represses the transcription of cyclin A in quiescent cells.16,18 On stimulation with growth factors, binding of this factor to CDE-CHR is released, thereby activating the transcription factors upstream and derepressing the transcription of cyclin A.18 However, the protein constitution of this binding activity remains unknown. Philips et al34 described yet another 90 to 95 kDa protein, CHF, which binds specifically to cyclin A CHR and not to CDE elements. Whether these proteins/factors are modulated by cytokines is not yet elucidated. The capacity to renature the cyclin A CHR binding activity observed in our study, after electrophoresis through denaturing SDS-PAGE led to the identification of a 84-kDa protein. This size estimation was confirmed after UV cross-linking to a radioactive oligonucleotide in a standard band shift experiment (Figure 8).

The 84-kDa CHR binding protein described in the present study appears to be different from either CDF-1 or CHF. Unlike CDF-1, this protein does not seem to bind to CDE elements. Although protein described here resembles CHF in terms of specificity for CHR elements, lack of sequence information, dominant-negative CHF constructs, and anti-CHF antibodies preclude us from conclusively eliminating the possibility of this protein as CHF. However, it is noteworthy that a number of variables argue against it being CHF. Firstly, as shown in Figure 8B, unlike CHF, this is a sustained binding activity at 16 hours in TNF-α-treated protein extracts (Figures 6 and 7). Furthermore, absence of this protein in quiescent cells (Figure 8B) is in contrast to CHF, which is found in abundance in quiescent cells.34 Finally, protein described in this study differs from CHF in apparent molecular mass (80 to 84 kDa versus 90 to 95 kDa CHF).

Taken together these data suggest that the 84-kDa polypeptide is instrumental in establishing TNF-α–regulated repression of cyclin A transcription in ECs. Targeted disruption of this protein could potentially be a therapeutic strategy to rescue EC proliferation in vivo. At the moment, however, many questions are still open. First of all, we do not know whether other cell cycle–regulated genes like cdc2, B-myb, or cdc25C, which contain similar DNA regulatory elements, are the target of TNF-α and if yes, whether they are under control of a common or a family of factors. In the latter case, 84-kDa
protein described here would belong to a family of factors with specificity toward a selected subset of genes. Alternatively, binding selectivity of this 84 kDa protein could be affected either by the binding of additional cofactors or by posttranslational modifications, which would redirect it to other promoters. The mechanism of TNF-α–mediated inhibition itself is still ill defined. The cyclin A promoter is devoid of a TATA element and as such gives rise to many transcription initiation sites. Interestingly, the protein identified here binds downstream from the major initiation sites and could thus interfere with RNA polymerase progression, leading, for example, to transcriptional pausing. Additional experiments as well as identification of this TNF-α–induced protein would be required to answer some of these questions.

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References

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