Coordination of ATF6-mediated Transcription and ATF6 Degradation by a Domain That Is Shared with the Viral Transcription Factor, VP16*

Donna J. Thuerauf, Lisa E. Morrison, Holly Hoover, and Christopher C. Glembotski††

From the San Diego State University Heart Institute and the Department of Biology, San Diego State University, San Diego, California 92182

ATF6 is a 670-amino acid endoplasmic reticulum (ER) transmembrane protein that is cleaved in response to ER stress. The resulting N-terminal fragment of ~400 amino acids translocates to the nucleus and activates selected ER stress-inducible genes, such as GRP-78 and sarco/endoplasmic reticulum ATPase, which are required for cell survival. In studying the mechanism of ATF6-activated transcription, we found that when HeLa cells were transfected with a plasmid encoding ATF6-(1–373), ER stress-inducible reporter gene activation was high, but ATF6-(1–373) expression was low, unless a proteasome inhibitor was added. In contrast, transfection with a plasmid encoding ATF6-(94–373) resulted in low reporter activation and high expression of ATF6-(94–373), which was independent of the proteasome inhibitor. Thus, the information responsible for transcriptional activation and proteasomal degradation must lie within the N-terminal 93 amino acids of ATF6. This portion of ATF6 was found to be homologous to the herpes simplex viral protein, VP16. One 8-amino acid domain of particular interest in this region of ATF6 is 75% identical to the VN8 region in VP16. VN8 is required for VP16-mediated transcription, as well as rapid degradation of VP16 by proteasomes. Point mutations in the VN8-like region of ATF6 caused a loss of transcription, increased expression levels, and an increase in half-life. Thus, the potent transcriptional activities and rapid degradation of ATF6 and VP16 require the VN8 domains in each protein. Homology searches indicate that ATF6 is the only eukaryotic protein known that possesses an active VN8 domain, raising questions about how this domain evolved and the functional importance underlying its appearance in only these two transcription factors.

Stresses leading to alterations in the ER environment can result in the incorrect folding of nascent proteins in the ER (1). A number of genes induced under such conditions are targeted to the ER, where they aid in folding and/or counteract the stress (2–6). A regulatory element located in many of these genes is the ER stress-response element (ERSE), which is required for transcriptional induction of many ER stress-response genes (7). Activating Transcription Factor 6 (ATF6), a member of the ATF/cAMP-response element-binding protein family of transcription factors (8), is required for induction of numerous ER stress-response genes that possess the ERSE sequence (9), such as GRP-78 and SERCA2. Along with other proteins, ATF6 binds to the ERSE sequences in these genes, an event that is required for transcriptional induction.

ATF6 is composed of 670 amino acids and resides in the ER membrane, probably as a result of the hydrophobic sequence between amino acids 378 and 398 (Fig. 1A) (10). Upon ER stress, the cytosolic N-terminal portion of ATF6 is released as a result of regulated intramembrane proteolysis (10, 11). The proteases responsible for this cleavage are apparently the same as those required for sterol regulatory element-binding protein maturation and probably cleave ATF6 in and/or near the intra-ER membrane region (12). Following proteolytic cleavage, the N terminus of ATF6, which possesses several putative nuclear localization signals and a basic leucine zipper (b-Zip) domain between residues 308 and 369, translocates to the nucleus where it combines with several other proteins to form an ERSE-binding complex that is responsible for the induction of ER stress-responsive genes (7, 10).

Although the ATF6 transcriptional activation domain (TAD) is known to lie in the N-terminal half of the protein, which is released from the ER by regulated intramembrane proteolysis (13, 14), little is understood about how ATF6 activates transcription. During the course of experiments designed to address this problem, we found that many of the forms of ATF6 that displayed potent transcriptional activity were expressed at extremely low levels. This led us to the present study where we examined the hypothesis that transcriptionally active forms of ATF6 are susceptible to rapid degradation.

MATERIALS AND METHODS

Cell Culture

HeLa Cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. HeLa cells were resuspended at 5–9 × 10⁶ cells per 400 µl of cold Dulbecco’s phosphate-buffered saline and electroporated in a 0.4-cm gap electroporation cuvette at 250 V and 950 microfarads using a GenePulser II Electroporator (Bio-Rad). The cells were then plated at a density of 0.5 × 10⁶ per 24-mm well for luciferase assays and 1.5 × 10⁶ per 35-mm well for Western blots, or 3 × 10⁶ per 60-mm dish for metabolic labeling experiments.

Plasmids

The following plasmids were used as indicated in this study. CMV-Gal—CMV-β-gal, which codes for a galactosidase reporter driven by the CMV promoter, was used to normalize for transfection efficiency. Gal4-Luc—pG5E1b-Luc, which encodes a luciferase reporter driven by the Gal4 activation domain, was used to detect activation by ATF6. GRP-78—pBI121-GRP78, which encodes human GRP-78 driven by the CMV promoter, was used to normalize for expression levels. SERCA2—pBCE121-SERCA2, which encodes human SERCA2 driven by the CMV promoter, was used to normalize for expression levels.

Received for publication, February 20, 2002, and in revised form, March 19, 2002
Published, JBC Papers in Press, March 21, 2002, DOI 10.1074/jbc.M201749200

* This work was supported in part by National Institutes of Health Grants NS/HL-25073 and HL-56861 (to C. C. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† To whom correspondence should be addressed: San Diego State University Heart Institute and the Dept. of Biology, San Diego State University, San Diego, California 92182. Tel.: 619-594-2959; Fax: 619-594-5676; E-mail: cglembotski@sciences.sdsu.edu.
‡ The abbreviations used are: ER, endoplasmic reticulum; ERSE, ER stress-response element; b-Zip, basic leucine zipper; TAD, transcriptional activation domain; CMV, cytomegalovirus; dbd, DNA binding domain; ATF6, activating transcription factor 6.
by five GAL4-binding sites (15), was obtained from R. Davis (University of Massachusetts Medical School).

Gal4/ATF6-(1–273), -(1–93), -(1–38), and -(1–28)—Construction of Gal4/ATF6-(1–273) has been described previously (13). By using Gal4/ATF6-(1–273) as the template, a KpnI site was introduced into the Gal4/ATF6 junction and a termination codon and an AN site were introduced on the 3′ side of residues 93 (ATF6-(1–93)), 38 (ATF6-(1–38)), and 28 (ATF6-(1–28)). PCR products were then digested with KpnI and SacI and then ligated into the Gal4/dbd vector, pSG424.

FLAG-ATF6-(1–373), -(39–373), -(94–373), -(1–51/94–373), and -(39–51/94–373)—Construction of the parent vector, 3× FLAG-ATF6-(1–373), has been described previously (16). All references in this paper to the FLAG epitope use this 3× FLAG construct. By using FLAG-ATF6-(1–373) as the template, PCR was carried out using sense primers that introduced an XhoI site 5′ of amino acids 1, 39, or 94, in FLAG-ATF6-(1–373) and an antisense primer that introduced a termination codon and a KpnI site 3′ of residue 373. To create FLAG-ATF6-(1–51/94–373) and -(39–51/94–373), an antisense primer was designed to terminate at ATF6 amino acid 51 and to introduce an XhoI site. PCR products that encompassed ATF6-(1–51) or ATF6-(39–51) were generated and inserted 5′ of ATF6 amino acid 94 in the FLAG-ATF6-(94–373).

FLAG-ATF6-(1–373-dbd-M) and FLAG-ATF6-(1–373-VN8-M)—Site-directed mutagenesis was performed, using the QuickChange kit (Stratagene, Inc.), to substitute 3 amino acids in a portion of the putative DNA binding domain (dbd) of FLAG-ATF6-(1–373) (K315T, N316A, and R317A) using the following primers: sense, cag caa cgt atg ata caa get gca gaa tcc tct gtt cag; antisense, ctc cca agg taa caa atc c
tgc agc tgt aac gaa atg ata atc cat cag tgt cgt. The same procedure was used to substitute 2 amino acids in a portion of the VN8-like region (VN8) of FLAG-ATF6-(1–373) (F62A and L64A) using the following primers: sense, gat aat ctt gat aac aac gnc ttc tgc age agc tgt cat cat aag tgg cgt ctt ggt gat; antisense, ctc cca agg taa caa atc c
tgc agc tgt aac gaa atg ata atc cat cag tgt cgt. In all cases the nucleotides in bold were responsible for the desired amino acid substitutions.

GRP78-ERSE-luc—This construct encodes the active ERSE from the human GRP-78 gene driving SV-40/luciferase in the vector, pGL2-p (Promega), and has been described previously (16).

Reporters

β-Galactosidase—After the indicated times, cells were lysed in 500 μl of ice-cold lysis buffer (25 mM Gly-Gly, pH 7.8, 15 mM MgSO4, 4 mM EDTA, 0.25% Triton X-100, 1 mM dithiothreitol). Cell lysate was centrifuged, and 100 μl of supernatant was combined with 400 μl of galactosidase buffer (60 mM NaHPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, 1 mg/ml chlorophenol red-t-N-galactopyranoside, and 50 mM 2-mercaptoethanol). After incubation for 1 h, the absorbance was measured at 570 nm.

Luciferase—After cell lysis and centrifugation, as described above, 100-μl samples of cell lysate were combined with 100 μl of luciferase buffer (the above described lysis buffer containing 0.3 mM n-luciferin and 3 mM ATP). An Optcompt II luminometer (MGM Instruments, Inc.) was used to measure light emission for each sample for 10 s. Relative luciferase activities were determined by dividing luciferase values by β-galactosidase values. All values shown are the mean of three cultures ± S.E.

Western Analyses

Cells were extracted in 2× Laemmli buffer containing 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 15 μl of 2-mercaptoethanol, boiled for 5 min, fractionated by 10% SDS-PAGE, and then transferred to a polyvinylidene difluoride membrane. Membranes were then probed for a Gal4 ddb antibody (Santa Cruz Biotechnology, sc-510) or a FLAG antiserum, M2 antibody (Sigma, F-3165).

Pulse-Chase Experiments

HeLa cells were transfected with 30 μg of the test expression vector, as described above, and 3 × 105 cells were plated on 60-mm dishes. Following a 14–18-h incubation in serum-containing medium, the cells were rinsed three times with warm Hanks’ buffer (Invitrogen) and incubated for 2 h with 250 μCi of [3H]methionine/cysteine (Easytag Express Protein Labeling Mix, PerkinElmer Life Sciences) diluted in 2 ml of methionine/cysteine-free Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 5% dialyzed fetal calf serum (Invitrogen). Following a 2-h incubation, this medium was removed, and the plates were washed twice and then incubated with 3 ml of chase medium (Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 2 mM unlabeled methionine, and 2 mM unlabeled cysteine). At the indicated times, the cells were rinsed three times with phosphate-buffered saline and then scraped into 100 μl of lysis buffer (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 5 mM sodium pyrophosphate, 10 mM sodium glycerophosphate, 50 mM NaF, 150 mM NaCl) containing 0.1% SDS. The lysate was then diluted with 400 μl of lysis buffer containing no SDS so that the final concentration of SDS was 0.2%. The lysate was cleared by centrifugation, and the supernatant was incubated 12–18 h at 4 °C with 2 μg of FLAG antibody (Sigma, F-3165) followed by incubation with protein G-Sepharose beads and elution with SDS-PAGE sample buffer. Immunoprecipitated material was then resolved by SDS-PAGE (10% SDS gel), dried down, and exposed on a PhosphorImaging screen.

RESULTS

Previous studies (13, 14) demonstrated that the region responsible for transcriptional activation lies within the N-terminal 273 amino acids of ATF6. To resolve better the location of the ATF6 transcriptional activation domain (TAD), expression constructs were prepared that encode proteins composed of the yeast Gal4 DNA binding domain (Gal4 ddb; Gal4-(1–147) (17)) fused to the N terminus of ATF6-(1–273) and to other C-terminally truncated forms of ATF6 (Fig. 1A). When tested in HeLa cells, the transcriptional activities of the fusion proteins were apparently high, even after removal of all but the N-terminal 38 amino acids of ATF6 (Fig. 1B). Gal4/ATF6-(1–28) did not support significant transcriptional activity, indicating a minimal requirement for the N-terminal 38 amino acids.

When expression of the fusion proteins was assessed, Gal4/ATF6-(1–273) and Gal4/ATF6-(1–93) were undetectable (Fig 1C, lanes 1 and 3), unless the cells had been incubated with the proteasome inhibitor, ALLN (N-acetyl-Leu-Leu-Nle-CHO) (Fig. 1C, lanes 2 and 4). In contrast, the more truncated fusion proteins were expressed at high levels in a manner that was generally independent of whether ALLN was present (Fig. 1C, lanes 5–8). Thus, when the protein expression level was taken into account, the specific transcriptional activity of Gal4/ATF6-(1–93) was much greater than that of Gal4/ATF6-(1–38), indicating that a major transcriptional activation domain resides between amino acids 38 and 93 of ATF6. These results also indicated that the fusion proteins displaying the greatest transcriptional activities were expressed at the lowest levels. Because expression of transcriptionally active fusion proteins was enhanced considerably by ALLN, these forms of ATF6 must be rapidly degraded by proteasomes in a manner that coordinates with their transcriptional activities.

To evaluate these interesting properties in a context relevant to the ER stress response, the abilities of various forms of ATF6 (Fig. 2A) to activate a reporter gene flank an ERSE were assessed. ATF6-(1–373) maximally activated ERSE-inducible transcription; however, ATF6-(39–373) and - (94–373) displayed only 10% and 2% of maximal activity, respectively (Fig.

| Internal Notes |
|----------------|
| 1 In Fig. 1B, the observed luciferase levels for Gal4/ATF6-(1–93) and -(1–38) are fortuitously similar, which may give the appearance that they possess similar transcriptional potencies. However, as can be seen in Fig. 1C, the expression level of Gal4/ATF6-(1–38) is very high, while the expression level of 1–93 is very low. Thus, the ability of each molecule of Gal4/ATF6-(1–38) to stimulate transcription is a great deal lower than the transcriptional induction ability of each molecule of Gal4/ATF6-(1–93). This is due in part to the removal of the sequences in 52–93, which is addressed further in Fig. 2. |
| 2 One of the genes induced during the ER stress response is glucose response protein, GRP-78, which is targeted to the ER lumen and serves a chaperone function (4–6). A regulatory element in the GRP-78 gene, the ER stress response element (ERSE), is required for transcriptional induction during the ER stress response. This element has been found in other ER stress-responsive genes (7). Accordingly, it has been used in the present study as an indicator of ATF6-inducible transcription. |
| 3 Forms of ATF6 that include the b-Zip region are considerably more active than ATF6. In addition, there is a large body of evidence that ATF6 is a dimer or higher order complex. |
| 4 Forms of ATF6 that include the b-Zip region are considerably more active than ATF6. In addition, there is a large body of evidence that ATF6 is a dimer or higher order complex. |

20735
emphasizing the importance of the N-terminal 93 amino acids. To resolve better the location of critical domains in this region of ATF6, several constructs harboring an internal deletion were prepared. ATF6-(1–51/94–373) and (39–51/94–373) displayed only 12 and 3% of maximal activity, respectively (Fig. 2B). Thus, the rank order of transcriptional activity for these forms of ATF6 was 1–373 \gg 39–373 = 1–51/94–373 > 94–373 = 39–51/94–373, indicating that domains in both 1–38 and 52–93 were indispensable for full activity. In contrast to the relative activities, however, the rank order of expression and the dependence on ALLN was reversed: 39–51/94–373 = 94–373 > 1–51/94–373 = 39–373 \gg 1–373 (Fig. 2C, lanes 1–6), supporting the hypothesis that the forms of ATF6 with the greatest transcriptional activity are the most susceptible to degradation. Because removal of either 1–38 or 52–93 resulted in a dramatic 70-fold loss of specific transcriptional activity (Fig. 2D, compare column 1 to 2 and 4), it is apparent that domains within both of these regions of ATF6 are indispensable for maximal activity.

Given the results to this point, it appears that forms of ATF6 that are actively engaged in transcription are highly susceptible to rapid degradation. Moreover, forms of ATF6 harboring mutations that reduce transcriptional potency display coordi-
ATF6-mediated Transcription and Degradation

FIG. 3. Alignment of portions of human ATF6 and VP16. Human ATF6-(1–100), GenBank accession number P18850, was aligned with VP6-(356–490) GenBank accession number P06492, using ClustalW (20) (npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_clustalw.html). The identical strongly conserved and weakly conserved residues are indicated in red, green, and blue, respectively. Also shown (see box) is the VN8 region of VP16-(441–448) and the VN8-like region of ATF6-(61–68).

FIG. 4. Effect of mutations in the VN8-like region on ATF6 expression and transcriptional activity. A, diagram of ATF6 constructs. Shown are the approximate locations of the VN8-like region of ATF6-(61–67) and the substitutions of F62A and L64A that were used to create ATF6-(1–373)-VN8-M. B, expression of ATF6-related proteins. HeLa cells were co-transfected with the FLAG-ATF6 constructs shown as well as GRP78-ERSE-luciferase and CMV-β-gal. Cultures were incubated for various times (chase) in medium containing unlabeled methionine and cysteine. Cultures were extracted after the chase times shown; FLAG-related material was immunoprecipitated, and SDS-PAGE followed by radioimaging was carried out, as described under Materials and Methods. C, densitometry analysis. The image from a was digitized, and the densities of each band were averaged and plotted to obtain half-lives. Each value represents the average density and S.E. of three values obtained at each of the chase time shown.

FIG. 5. Effect of mutations in the VN8-like region on half-life. A, SDS-PAGE. HeLa cells were transfected with either FLAG-ATF6-(1–373) or FLAG-ATF6-(1–373)-VN8-M. Following a 2-h incubation with medium containing [35S]methionine/[35S]cysteine, the cultures were incubated for various times (chase) in medium containing unlabeled methionine and cysteine. Cultures were extracted after the chase times shown; FLAG-related material was immunoprecipitated, and SDS-PAGE followed by radioimaging was carried out, as described under Materials and Methods. Three identically treated cultures were used for each chase time. B, densitometry analysis. The image from A was digitized, and the densities of each band were averaged and plotted to obtain half-lives. Each value represents the average density and S.E. of three values obtained at each of the chase time shown.

through mutation in the DNA binding domain. For this purpose we used ATF6-(1–373)-dbd-M, a form of ATF6 known to be inactive as a result of mutations in the b-Zip domain (16, 18) (Fig. 2A). ATF6-(1–373)-dbd-M displayed very low activity (Fig. 2B), as expected, and it was expressed at relatively high levels, with little dependence on ALLN (Fig. 2C, lanes 11 and 12); the specific transcriptional activity was reduced by about 200-fold, compared with 1–373 (Fig. 2D, column 6). Thus, four different constructs with mutations within the N-terminal 93 amino acids, and one with a mutation in the b-Zip domain, displayed low levels of transcriptional activation and high levels of expression that were relatively insensitive to ALLN, consistent with the hypothesis that proteasomal degradation of ATF6 is linked to ATF6-mediated transcription.

To begin to understand the features of ATF6 that engender these properties, a search was carried out to identify ATF6-like proteins for which transcription and degradation are linked. Although a number of potent eukaryotic transcription factors (e.g. Myc, β-catenin, and Rel) exhibit such properties (19), only VP16, the virion protein 16 transcription factor from herpes simplex virus type I, possesses significant homology to ATF6. VP16 is a potent transcriptional activator of herpes simplex immediate early genes. Our search revealed that the C-terminal TAD of VP16 bears striking homology to the N-terminal TAD of ATF6 (Fig. 3). ClustalW alignment analysis (20) showed that 35% of the residues in ATF6-(1–100) were conserved or strongly conserved with those in VP16-(356–489). Of particular interest is a region of ATF6 between residues 61 and 68, which is 75% identical to a region of VP16 between residues 441 and 448 (Fig. 3, boxed sequence). In VP16, these 8 amino acids are known as VN8 (21) and are required for transcriptional activation and the rapid proteasomal degradation of VP16 (22). The relationship between transcriptional potency and degradation was demonstrated by Salghetti et al. (19), who showed that as the numbers of VN8 repeats fused to the Gal4 DNA binding domain were increased, transcriptional potency increased and fusion protein expression levels decreased. Additionally, Phe-442 and Leu-444 have been shown to be critical for VN8 function, such that substitution of alanine at these
positions abolishes transcriptional activation and stabilizes the protein (19, 21, 22).

Accordingly, a construct encoding ATF6-(1–373) harboring F62A and L64A substitutions in the VN8-like region was prepared (Fig. 4A, 1–373-VN8-M). When tested in HeLa cells, the expression level of 1–373-VN8-M was ~7-fold higher than 1–373 (Fig. 4B), supporting the hypothesis that Phe-62 and Leu-64 are important for low level expression of ATF6. In contrast to the effects on expression, compared with 1–373, the 1–373-VN8-M exhibited an approximate 5-fold reduction of transcription (Fig 4C), which translates to a 27-fold reduction of specific transcriptional activity. Thus, Phe-62 and Leu-64 are required for both efficient transcription and low level expression of ATF6.

Experiments were carried out to determine whether the low level of ATF6 expression is the result of a short half-life and, if so, whether the F62A and L64A substitutions increased ATF6 expression by increasing its half-life. Pulse-chase labeling analyses showed that ATF6-(1–373) was, indeed, relatively short lived, displaying a half-life of about 40 min (Fig. 5, 1–373). Moreover, mutating Phe-62 and Leu-64 to alanine resulted in an approximate 2-fold increase in the half-life (Fig. 5, 1–373-VN8-M). Accordingly, the increased levels of ATF6-(1–373)-VN8-M compared with 1–373 are partly the result of a decrease in the rate of degradation.5 The finding that two amino acid substitutions in a transcription factor nearly 400 amino acids in length can make a 2-fold difference in half-life and a 5-fold reduction of transcriptional activity is, to our knowledge, unprecedented. Taken together with the results shown in Fig. 4A, these findings indicate that in addition to being required for optimal transcription, Phe-62 and Leu-64 also contribute to establishing the rate at which ATF6 is degraded, constituting another demonstration that ATF6 transcriptional activity and ATF6 degradation are coupled.

**DISCUSSION**

ATF6 is not highly homologous to other potent unstable eukaryotic transcription factors. However, a region near the N terminus of ATF6 is homologous to the C-terminal TAD of the viral transcription factor, VP16 (Fig. 3 and Fig. 6A). Of particular interest is the area of VP16 between residues 441 and 448, the VN8 region. ATF6 is the only eukaryotic protein that we were able to find with a sequence that would be predicted to behave like VN8 does in VP16. The N terminus of G13, the closest known relative of ATF6 (23, 24), exhibits a great deal of homology to ATF6; however, it does not appear to possess an active VN8-like sequence (Fig. 6B). This is because there is an absolute requirement for phenylalanine at position 2 of the VP16 VN8, such that substitution of valine at this position, as naturally occurs in G13, results in a 90% loss of VP16 transcriptional activity (25). Accordingly, because G13 apparently lacks a functional VN8 sequence, compared with ATF6, it would be predicted to possess reduced transcriptional potency. Consistent with this view is a recent study showing that G13 displays reduced transcriptional activity compared with ATF6 and that it is expressed at higher levels (24). Accordingly, although G13 and ATF6 display many of the same structural and functional features, they most likely employ very different transcriptional activation mechanisms. Future studies of these mechanistic differences and how they contribute to the roles of ATF6 and G13 during the stress response will be of interest.

VP16 possesses several acid-rich regions between residues 380 and 425 (Fig. 3), which play important roles in transcriptional activation. ATF6 also possesses acid-rich domains in this region of the TAD, the most notable of which is between residues 25 and 35 (Fig. 6B). Evidence from the present study

---

5 In preliminary experiments we have found that transcriptionally active forms of ATF6 reduce the expression rates of many proteins, including those expressed from transgenes, mostly likely through a global translational inhibition, which is a hallmark of the ER stress response. Accordingly, it is most likely that ATF6-(1–373)-VN8-M is translated at a higher rate than 1–373 and that this, coupled with a 2-fold reduction of half-life, accounts for the 5-fold increase in the expression level of ATF6-(1–373)-VN8-M compared with 1–373.
supports a role for this region in ATF6-mediated transcription (see Fig. 1 and Fig. 2), and disruption of this 11 amino acid stretch reduces transcription.6 A similar acid-rich region can be found in G13 (Fig. 6B); however, it is naturally disrupted by a 3 amino acid insert, which is also consistent with the reduced transcriptional activity of G13, compared with ATF6 (24). Thus, based on sequence homology and on the results of the present study, this N-terminal acid-rich region is predicted to serve an important role in ATF6-mediated transcription, although it may be a minor role compared with the VN8-like sequence within ATF6-(61–68).

The processes governing the coordination of ATF6-mediated transcription and ATF6 degradation are not yet resolved. Several other potent transcription factors that are very unstable display similar properties, and recent studies have shown that in many of those cases the sequences that signal their ubiquitination, the degron, overlaps closely with the TAD (19, 22, 26). Additionally, recruitment of the unstable transcriptional activators to DNA is necessary for proficient degradation (22), and the 19 S proteasome subunit has been shown recently to play a critical role in transcriptional elongation (27). RNA polymerase II co-immunoprecipitates with ubiquitin-protein ligases, ubiquitin-hydrolases, and other proteasome proteins in the nucleus (19, 22, 28), supporting the hypothesis that proteasomal machinery actually composes a portion of the transcription complex (29). In fact, it is possible that ubiquitination itself, in addition to serving as a molecular tag for protein degradation, can also serve as a post-translational modification required for optimal activity of some proteins in the transcription complex. The demonstration that ubiquitination of RNA polymerase II is required for optimal activity supports this view (30). Also, it has been postulated that ubiquitination of transcription factors serves to recruit the 19 S proteasomal subunit to promoters where it serves a chaperone role to promote transcription (31).

In summary, ATF6 is a potent, unstable transcription factor that displays the property of coordinate transcriptional activation and degradation. Limiting the activities, locations, and quantities of strong, transiently active transcription factors is likely to be critical for proper cellular regulation. Such a mechanism might be required to enable the fine regulation of the activities of transcription factors that could, if left unchecked, have deleterious effects on long term cell survival. Certain genes induced by long term activation of ATF6, such as CHOP/GADD-153, could potentially lead to cell death, indicating a need for finely tuned transient induction of ATF6. Future studies oriented toward understanding more about the domains of ATF6 that are responsible for linking transcription and degradation will reveal more about the role of ATF6 in the ER stress response. It will also be interesting to determine whether G13, a close relative of ATF6, also displays coordinate transcription and degradation; if it does, then deciphering the mechanism should provide clues as to how and why two such similar transcription factors might have evolved so they target enhanced expression of the same ER-stress response genes but through different transcriptional/degradation regulatory mechanisms. Finally, the high degree of homology between the ATF6 and VP16 TADs implies some overlap in transcriptional mechanisms, suggesting that during viral infection, VP16 might in some ways mimic some of the actions of ATF6 during the ER stress response.

Acknowledgments—We thank Xuan Lam, Joshua Martindale, Julia Miller, Mylo Wagner, and Jason Wall for critical reading of the manuscript.

REFERENCES

1. Spear, K., and Ng, T. W. (2001) Traffic 2, 515–523
2. Kozutsumi, Y., Segal, M., Normington, K., Gething, M. J., and Sambrook, J. (1988) Nature 332, 462–464
3. Kaufman, R. J. (1999) Genes Dev. 13, 1211–1233
4. Munro, S., and Pelham, H. R. B. (1986) Cell 46, 291–300
5. Lee, A. S. (1987) Trends Biochem. Sci. 12, 20–24
6. Lee, A. S. (1992) Curr. Opin. Cell Biol. 4, 267–273
7. Li, W.-W., Sistonen, L., Morimoto, R. I., and Lee, A. S. (1994) Mol. Cell. Biol. 14, 5533–5546
8. Hai, T., and Hartman, M. G. (1991) Gene (Amst.) 101, 1–11
9. Yoshida, H., Haze, K., Yanagi, H., Yura, T., and Mori, K. (1998) J. Biol. Chem. 273, 33741–33749
10. Haze, K., Yoshida, H., Yanagi, H., Yura, T., and Mori, K. (1999) Mol. Biol. Cell 10, 3787–3799
11. Hoppe, T., Rape, M., and Jentsch, S. (2001) Curr. Opin. Cell Biol. 13, 344–348
12. Ye, J., Rawson, R. B., Komuro, R., Chen, X., Dave, U. P., Prywes, R., Brown, M. S., and Goldstein, J. L. (2000) Mol. Cell 6, 1555–1564
13. Thureauff, D. J., Arnold, N. D., Zachner, D., Hanford, D. S., DeMartini, K. M., McDonough, P. M., Prywes, R., and Glembotski, C. C. (1998) J. Biol. Chem. 273, 20636–20643
14. Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M., and Mori, K. (2000) Mol. Cell. Biol. 20, 6755–6767
15. Seth, A., Gonzalez, F. A., Gupta, S., Raden, D. L., and Davis, R. J. (1992) J. Biol. Chem. 267, 24796–24804
16. Thureauff, D. J., Hoover, H., Meller, J., Hernandez, J., Su, L., Andrews, C., Dillmann, W. H., McDonough, P. M., and Glembotski, C. C. (2001) J. Biol. Chem. 276, 48309–48317
17. Marmorstein, R., Carey, M., Ptaszke, M., and Harrison, S. (1992) Nature 356, 408–414
18. Wang, Y., Shen, J., Arenzana, N., Tirasophon, W., Kaufman, R. J., and Prywes, R. (2000) J. Biol. Chem. 275, 27013–27020
19. Salghetti, S. E., Muratani, M., Wijnen, H., Futcher, B., and Tansey, W. P. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3118–3123
20. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) Nucleic Acids Res. 22, 4673–4680
21. Tanaka, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4311–4315
22. Molinari, E., Gilman, M., and Natesan, S. (1999) EMBO J. 18, 6439–6447
23. Min, J., Shukla, H., Kozono, H., Bronson, S. K., Weissman, S. M., and Chaplin, D. D. (1995) Genomics 30, 149–156
24. Haze, K., Okada, T., Yoshida, H., Yanagi, H., Yura, T., Negishi, M., and Mori, K. (2001) Biochem. J. 355, 19–28
25. Regier, J. L., Shen, F., and Triezenberg, S. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 885–887
26. Desteforo, J. M. P., Rodriguez, M. S., and Hay, R. T. (2000) Cell. Mol. Life Sci. 57, 1207–1219
27. Fodorus, A., Gonzalez, F., Sun, L., Kodadek, T., and Johnston, S. A. (2001) Mol. Cell 7, 261–261
28. Wu, M., Hemesath, T. J., Takemoto, C. M., Horstmann, M. A., Wells, A. G., Price, E. R., Fisher, D. Z., and Fisher, D. E. (2000) Genes Dev. 14, 301–312
29. Thomas, D., and Tyers, M. (2000) Curr. Biol. 10, R341–R343
30. Mitsui, A., and Sharp, P. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6054–6059
31. Salghetti, S. E., Cady, A. A., Chenoweth, J. G., and Tansey, W. P. (2001) Science 293, 1651–1653