

Review

The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis

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Abstract

The mammalian ATF/CREB family of transcription factors represents a large group of basic region-leucine zipper (bZip) proteins which was originally defined in the late 1980s by their ability to bind to the consensus ATF/CRE site 'TGACGTCA'. Over the past decade, cDNA clones encoding identical or homologous proteins have been isolated by different laboratories and given different names. These proteins can be grouped into subgroups according to their amino acid similarity. In this review, we will briefly describe the classification of these proteins with a historical perspective of their nomenclature. We will then review three members of the ATF/CREB family of proteins: ATF3, ATF4 and ATF6. We will address four issues for each protein: (a) homologous proteins and alternative names, (b) dimer formation with other bZip proteins, (c) transcriptional activity, and (d) potential physiological functions. Although the name Activating Transcription Factor (ATF) implies that they are transcriptional activators, some of these proteins are transcriptional repressors. ATF3 homodimer is a transcriptional repressor and ATF4 has been reported to be either an activator or a repressor. We will review the reports on the transcriptional activities of ATF4, and propose potential explanations for the discrepancy. Although the physiological functions of these proteins are not well understood, some clues can be gained from studies with different approaches. When the data are available, we will address the following questions. (a) How is the expression (at the mRNA level or protein level) regulated? (b) How are the transcriptional activities regulated? (c) What are the interacting proteins (other than bZip partners)? (d) What are the consequences of ectopically expressing the gene (gain-of-function) or deleting the gene (loss-of-function)? Although answers to these questions are far from being complete, together they provide clues to the functions of these ATF proteins. Despite the diversity in the potential functions of these proteins, one common theme is their involvement in cellular responses to extracellular signals, indicating a role for these ATF proteins in homeostasis. © 2001 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction – a historical perspective and nomenclature

Activating Transcription Factor (ATF) was first named in 1987 to refer to proteins that bind to the adenovirus early promoters E2, E3 and E4 at sites with a common core sequence 'CGTCA' (Lee et al., 1987). cAMP responsive element binding protein (CREB) was named in 1987 to refer to proteins that bind to the cAMP responsive element

(CRE) on the somatostatin promoter (Montminy and Bilezikjian, 1987). The consensus binding site for ATF was later defined as TGACGT(C/A)(G/A) (Lin and Green, 1988), a sequence identical to the CRE consensus (TGACGTCA) (Deutsch et al., 1988 and references therein; for a review see Roesler et al., 1988). The identification of identical consensus sequences on two seemingly different sets of promoters – one on viral promoters and the other one on cellular promoters – generated much confusion in the early days and prompted many groups to purify the corresponding binding proteins. Over the years, identical or homologous cDNA clones encoding proteins that can bind to the ATF/CRE site have been isolated. More than 20 different mammalian cDNAs with the prefix ATF or CREB have been described. All these cDNAs encode proteins with the

Abbreviations: ATF, activating transcription factor; bZip, basic region leucine zipper; CREB, cAMP responsive element binding

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bZip DNA binding domain, and the proteins can be grouped into subgroups on the basis of their amino acid similarity: the CREB/CREM, CRE-BP1 (commonly known as ATF2), ATF3, ATF4, ATF6 and B-ATF subgroups. Proteins within each subgroup share significant similarity both inside and outside the bZip domain. Proteins between the subgroups, however, do not share much similarity other than the bZip 'motif'. Therefore, they should be considered as distinct proteins despite their common prefix (ATF or CREB). Table 1 lists the subgroups and their corresponding members (for the original references, see a previous review Hai et al., 1999).

The nomenclature in the literature for this family of proteins has been confusing. Not only are alternative names used to refer to the same proteins (see Sections 2–4 for specific ATF proteins reviewed in this article), but in some cases the same name is used to refer to different proteins. As an example, the term CREB2 has been used to refer to three different proteins: an alternatively spliced CREB (Yoshimura et al., 1990), CRE-BP1 (ATF2) (Flint and Jones, 1991; Yin et al., 1995) and ATF4 (Karpinski et al., 1992). Another example is ATF5. It is identical to Fos, but was mistaken as a new clone due to sequencing mistakes at the time of publication. Recently, a cDNA clone designated as hATF5 (human ATF5) was isolated (Pati et al., 1999). Although it is named as hATF5, it shares homology to ATF4 rather than fos (the original ATF5) (see Section 3.1 below on ATF4 homologous proteins). Therefore, the term ATF5 could refer to a cDNA identical to fos or a cDNA with homology to ATF4. The third example is the *Saccharomyces pombe* Atf1; it is most likely the 'functional homolog' of mammalian CRE-BP1 (ATF2), rather than a homolog of mammalian ATF1 (for more detail, see Hai et al., 1999). Therefore, to ensure the identity of a given protein, the best way is to inspect its amino acid sequence.

Overwhelming evidence indicates that the ATF/CREB proteins form selective heterodimers with each other, and

with other bZip proteins such as the AP-1 and C/EBP families of proteins (for examples, see reviews Chinenov and Kerppola, 2001; Hai et al., 1999). For each protein reviewed below, we will list some of the known heterodimeric partners. Because most of these results were derived from interaction studies in test tubes or transient transfection assays in cultured cells, the physiological significance of these heterodimers is not clear. The general view is that heterodimer formation can alter DNA binding specificity and transcriptional activities, thus expanding the ability of these bZip proteins to regulate gene expression. Although the lists of heterodimeric partners described below are merely compilations of data at this point, they may be useful as a reference source for certain studies.

Despite the different names for these bZip proteins, ATF/CREB, AP-1 (fos and jun proteins) and C/EBP, many lines of evidence indicate that the distinction between them is blurred. First, as shown by the heterodimeric partners described below, some proteins have been demonstrated to form heterodimers with members of another family more often than with members within their own family. Second, as described in a previous review, these bZip proteins can bind to each other's consensus sites and regulate transcription in a manner characteristic of the other family (for specific examples see Hai et al., 1999). Third, some proteins are more homologous to members of another family than to members within their own. As an example, Meyer and Habener (1993) pointed out that ATF3 is more homologous to the fos proteins (members of the AP-1 family) than to other subgroups of the ATF/CREB proteins as shown by the dendrogram analysis. Therefore, the names of these bZip proteins reflect the history of discovery, rather than the real distinction between them.

Below, we review three members of the ATF/CREB family of transcription factors: ATF3, ATF4 and ATF6 (for recent reviews on the CREB/CREM proteins, see Andrisani, 1999; Daniel et al., 1998; De Cesare et al., 1999; Montminy, 1997; Sassone-Corsi, 1998). For each ATF reviewed below, we will first describe the alternative names, the homologous proteins and heterodimeric partners. We will then describe their transcriptional activities and potential functions.

Table 1
The mammalian ATF/CREB family of transcription factors^a

Subgroup	Members	Alternative names
CREB	CREB CREM ATF1	ATF-47 TREB36, TCRATF1, ATF-43
CRE-BP1	CRE-BP1 ATFa CRE-BPa	ATF2, HB16, TREB, TCR-ATF2, mXBP
ATF3	ATF3 JDP-2	LRF-1, LRG-21, CRG-5, TI-241
ATF4	ATF4 ATFx	CREB2, TAXREB67, mATF4, C/ATF, mTR67 hATF5
ATF6	ATF6 CREB-RP	ATF6 α G13, ATF6 β
B-ATF	B-ATF JDP-1	

^a This table is not meant to be comprehensive; for details and the original references, see a previous review (Hai et al., 1999).

2. ATF3

ATF3 was originally isolated from a library derived from HeLa cells treated with tetradecanoylphorbol acetate (TPA) (Hai et al., 1989). Previously, we reviewed ATF3 and its potential roles in stress responses (Hai et al., 1999). Due to the space limit, we will briefly summarize the last review and discuss a few new findings since the last review.

2.1. Nomenclature, homologous proteins, dimer formation, and the transcriptional activity of ATF3 (for original references, see a previous review Hai et al., 1999)

Homologous cDNAs with about 95% identity to human

ATF3 at the amino acid level have been identified and referred to as LRF-1 (rat clone), or as LRG-21, CRG-5, or TI-241 (mouse clone). Most likely, they represent the same gene, and we will refer to them as ATF3 for the convenience of discussion. JDP-2 is a rat clone isolated by its ability to interact with c-Jun and has significant similarity to ATF3. It is about 65% similar to ATF3 throughout the entire protein, and over 80% similar to ATF3 within the bZip domain. Because of its similarity to ATF3, we classified it as a member of the ATF3 subfamily of proteins (Table 1). ATF3 homodimer is a transcriptional repressor; however, heterodimeric complex of ATF3 with c-Jun (or JunD) has been demonstrated to function as a transcriptional activator. Other bZip proteins that dimerize with ATF3 include ATF2, JunB, and gadd153/CHOP10.

2.2. Potential physiological functions of ATF3

2.2.1. Regulation of ATF3 expression – ATF3 as a stress-inducible gene

Thus far, the main clue for the physiological function of ATF3 has come from the studies of its expression pattern. Overwhelming evidence from different laboratories indicates that *ATF3* is a stress-inducible gene: its mRNA level greatly increases upon the exposure of cells to stress signals (see Hai et al., 1999 for original references). In animal models, *ATF3* is induced in the heart by myocardial ischemia and myocardial ischemia coupled with reperfusion (ischemia-reperfusion), in the liver by hepatic ischemia, partial hepatectomy, and chemicals such as alcohol, carbon tetrachloride or acetaminophen, in the brain by seizure, in the kidney by renal ischemia-reperfusion, in the skin by wounding, in the peripheral nerves by axotomy, and in the thymocytes by CD3 ϵ activation. In cultured cells, *ATF3* is induced by a variety of signals, including (a) cytokines, (b) genotoxic agents such as ultraviolet light and ionizing radiation, and (c) agents known to induce cell death or the JNK/SAPK signaling pathway such as anisomycin, cyclohexamide and doxorubicin. Since the last review, several reports added more signals to this long list of stresses that can induce *ATF3*: (a) pancreatic stresses such as partial pancreatectomy, ischemia-reperfusion to the pancreas, and streptozotocin treatment (Allen-Jennings et al., 2001), (b) microtubule binding agents such as taxol and colchicine (Shtil et al., 1999), (c) proteasome inhibitors such as lactacystin and MG132 (Zimmermann et al., 2000), (d) homocysteine (Cai et al., 2000), (e) hydrogen peroxide (Allen-Jennings et al., 2001), (f) endoplasmic reticulum (ER) stress (Cai et al., 2000), (g) hepatitis B virus X protein (Tarn et al., 1999), (h) nerve growth factor depletion (Mayumi-Matsuda et al., 1999), (i) the carcinogen benzo[a]pyrene diol epoxide (Gu et al., 2000), and (j) peroxisome proliferator-activator receptor (PPAR) activators (Nawa et al., 2000). In the study using hydrogen peroxide as a stress signal (Allen-Jennings et al., 2001), the induction of *ATF3* was almost completely inhibited by *N*-acetyl-L-cysteine (NAC), an

oxidant scavenger, suggesting that *ATF3* is induced by oxidative stress. In contrast, in the study using homocysteine as a stress signal (Cai et al., 2000), the induction was not affected by NAC. The apparent discrepancy between these two studies could be due to the differences in the stress signals, the cell types, and the concentrations of NAC. Despite this discrepancy, there is no doubt that *ATF3* is a stress-inducible gene. However, many questions remain. What are the signal transduction pathways that induce *ATF3*? What is the significance of this induction? What roles does *ATF3* play in these diverse responses? What are the target genes, and are they different in different tissues and responses? Currently, our understanding in these areas is limited. Below, we discuss three points relevant to the above questions: the kinetics of *ATF3* induction, the potential signaling pathways, and the correlation between *ATF3* induction and cellular injury.

2.2.1.1. The kinetics of ATF3 induction. The kinetics of *ATF3* induction are immediate and transient. In most of the systems examined thus far, *ATF3* mRNA increases shortly (usually within 2 h) after the exposure of cells to the signals. However, in the proteasome inhibitor experiment and all neuronal models, the kinetics of induction are much slower: The induction peaks at more than 8 h after exposure. The significance of this difference is not clear. The early and transient nature of *ATF3* induction suggests two things. First, a mechanism exists to turn off *ATF3* gene expression after its induction. Interestingly, *ATF3*, a transcriptional repressor, can repress the activity of its own promoter (Wolfgang et al., 2000), providing at least one potential mechanism for the transient nature of *ATF3* induction. Second, *ATF3*, in conjunction with other immediate early gene products, sets things in motion during early stress response. The combination of these events eventually leads to outcomes manifested by cells treated with the stress signals. It is possible that many of the target genes of *ATF3* are themselves transcription factors. Clearly, in order to understand the significance of *ATF3* induction by stress signals, it is important to identify the target genes. Several genes have been implicated to be the targets; they include *gadd153/Chop10* (Wolfgang et al., 1997; Fawcett et al., 1999), *E-selectin* (Chen et al., 1994; Nawa et al., 2000), and *phosphoenolpyruvate carboxykinase* (Allen-Jennings et al., 2001). However, much more work is required to elucidate the target genes of *ATF3*.

2.2.1.2. The signal transduction pathways involved in the induction of ATF3 by stress signals. Although information in this area has just begun to emerge, it is almost certain that stress signals induce *ATF3* through complex mechanisms involving multiple pathways in a signal- and cell type-dependent manner. Previously, we reviewed observations suggesting the involvement of several pathways in the induction of *ATF3* by stress signals: the JNK/SAPK

pathway, a p53-dependent pathway, and a p53-independent pathway (Hai et al., 1999). Since the last review, Kitajima and colleagues have provided evidence substantiating the notion that the JNK/SAPK pathway plays an important role in inducing ATF3. They showed that, in endothelial cells, inhibition of the JNK/SAPK pathway by dominant negative MKK4 or MKK7 reduced the ability of homocysteine to induce ATF3 (Cai et al., 2000).

2.2.1.3. Correlation of ATF3 induction with cellular injury.

As described above, the *ATF3* gene is induced in a variety of cell types by many stress signals, suggesting that ATF3 is a key regulator in cellular stress responses. Although the induction is neither tissue-specific nor stimulus-specific, one common theme of all the signals that induce *ATF3* is that they also induce cellular damage (Chen et al., 1996). Recently, Noguchi and colleagues provided further evidence to support this notion (Tsujino et al., 2000). They demonstrated that intraplantar injection of formalin, which induces neuronal injuries in dorsal root ganglia and spinal cord, induces *ATF3* at the injury sites. However, intraplantar injection of Freund's adjuvant, which induces inflammation but not injury, does not induce ATF3. Furthermore, transsynaptic stimulation (without damaging the neurons) does not induce ATF3. Therefore, the induction of *ATF3* appears to correlate with cellular injury.

2.2.2. Gain-of-function analyses of ATF3

The correlation between the induction of ATF3 and cellular injury raises an important question. Is the induction of *ATF3* a protective response for the cells to cope with stress, or a part of the cellular response that leads to detrimental outcomes? Our work using a gain-of-function approach suggests that expression of *ATF3* leads to detrimental outcomes: transgenic mice expressing *ATF3* in selective tissues have malfunction in the target tissues. As an example, transgenic mice expressing *ATF3* in the liver results in liver dysfunction and reduced expression of gluconeogenic genes (Allen-Jennings et al., 2001). Transgenic mice expressing *ATF3* in the pancreas have defects in endocrine pancreas development, reduced serum insulin levels and symptoms characteristics of insulin-dependent diabetes mellitus (Allen-Jennings et al., 2001). Furthermore, transgenic mice expressing *ATF3* in the heart have conduction abnormality and contractile dysfunction (Okamoto et al., 2001). Therefore, ATF3 appears to be a part of the cellular response that leads to detrimental outcomes. However, due to the intrinsic limitations of the gain-of-function approach, work using different approaches is required to substantiate this notion.

2.2.3. Interacting proteins with ATF3 and other studies

As described in the previous review (Hai et al., 1999), ATF3 has been demonstrated to interact with the HTLV-1 Tax protein and the hepatitis B virus (HBV) X protein (pX). The interaction of pX with ATF3 was demonstrated to

increase the transcriptional repression activity of ATF3 (Barnabas et al., 1997). Recently, Andrisani and colleagues delineated the region of pX that interacts with ATF3 to be amino acids 49–115 (Barnabas and Andrisani, 2000). Interestingly, they also demonstrated that pX induces the expression of *ATF3* (Tam et al., 1999). Because pX is implicated in many HBV-mediated processes including hepatocarcinogenesis and apoptosis, their work indicates that ATF3 may play a role in HBV-mediated processes. In addition to stress responses and HBV-mediated processes, ATF3 has also been reported to play a role in metastatic processes (Ishiguro et al., 1996, 2000). The first clue came from the observation that ATF3 (referred to as TI-241 in the report) is differentially expressed in the metastatic sublines of B16 melanoma but not in the parental B16 cells (Ishiguro et al., 1996). Furthermore, introduction of ATF3 into the low metastatic B16 cells can convert it into high metastatic cells (Ishiguro et al., 1996). Conversely, ATF3 antisense oligonucleotide has a tumorigenicity-inhibiting effect on the HT29 colon cancer cells (Ishiguro et al., 2000). In summary, ATF3 has been implicated to play a role in several processes: stress response, HBV-mediated processes and tumorigenicity. Much work is required to elucidate what roles ATF3 plays in these processes, and whether they are related.

3. ATF4

3.1. Nomenclature, homologous proteins, and dimer formation of ATF4

A partial cDNA encoding the human ATF4 was isolated on the basis of its ability to bind to the consensus ATF/CRE site (Hai et al., 1989). Subsequently, full-length cDNA clones encoding identical or homologous proteins were isolated by a variety of methods. These include the human clones TAXCREB 67 (Tsujimoto et al., 1991) and CREB-2 (Karpinski et al., 1992), and the mouse clones mATF4 (Mielnicki and Pruitt, 1991, mapped to mouse chromosome 15, see Mielnicki et al., 1993), mTR67 (Chevray and Nathans, 1992) and C/ATF (Vallejo et al., 1993). The human clones are virtually identical to each other and are more than 85% homologous to the mouse clones at the amino acid levels. For the discussion below, we will refer to these proteins as ATF4, although the results cited may be derived from studies using different names. ATF_x is a mouse protein with 55% identity to mATF4; therefore, we classified it as a member of the ATF4 subgroup of proteins (Table 1). Recently, a human cDNA encoding a protein with about 97% homology to ATF_x was isolated in a yeast two-hybrid screen using hCdc34, a human ubiquitin-conjugating enzyme, as a bait (Pati et al., 1999, see more below). Although it was named as hATF5, we consider it as a member of the ATF4 subfamily of proteins because of its homology to ATF_x. In this context, we note that genes with homology to the mammalian ATF4 have been identified in

other species, such as *Drosophila* (Hewes et al., 2001), *Aplysia* (Bartsch et al., 1995) and *C. elegans* (GenBank Accession number: CAA93757). The homology is mostly restricted to the bZip region, and due to the space limit, we do not review them here.

The mammalian ATF4 proteins have been demonstrated to form dimers with a variety of bZip proteins, including Fos (Hai and Curran, 1991), Jun (Hai and Curran, 1991; Chevray and Nathans, 1992; Kato et al., 1999), JunD (Shimizu et al., 1998), and several C/EBP proteins, C/EBP α , C/EBP β /CRP2, C/EBP γ (IgEBP or GPE-BP), and C/EBP ϵ /CRP1 (Nishizawa and Nagata, 1992; Vallejo et al., 1993; Vinson et al., 1993; Gombart et al., 1997). Therefore, ATF4 has been demonstrated to form heterodimers with members of the AP-1 and C/EBP family of proteins (rather than the ATF/CREB family of proteins). For some of these heterodimers, the binding preferences have been demonstrated to be different from that of the parental homodimers (Hai and Curran, 1991; Vinson et al., 1993; Vallejo et al., 1993).

3.2. Transcriptional activity of ATF4: is it an activator or a repressor?

ATF4 has been reported to be either a transcriptional activator (Vallejo et al., 1993; Liang and Hai, 1997; Reddy et al., 1997; Gachon et al., 1998; Butscher et al., 1998; Fawcett et al., 1999; Talukder et al., 2000) or a repressor (Karpinski et al., 1992; Jüngling et al., 1994; Shimizu et al., 1998; Mielnicki et al., 1996; Pati et al., 1999; Lassot et al., 2001). Most results were derived from transient transfection assays examining the effects of ATF4 on reporter genes driven by promoters with the ATF/CRE binding sites. Although the experiments were carried out in various cell lines on different promoters, five out of the six reports describing ATF4 as a repressor were carried out with a simultaneous expression of cAMP-dependent protein kinase (PKA) or with a stimulation of the endogenous PKA by forskolin. In contrast, none of the reports describing ATF4 as an activator included PKA in the studies. Therefore, it is possible that post-translational modification of ATF4 by PKA changes it from an activator to a repressor. It is also possible that ATF4 is less active than phosphorylated CREB (p-CREB), the active form of CREB that exists in the presence of PKA. However, ATF4 binds to the ATF/CRE site on the promoter, thereby replacing p-CREB and resulting in 'apparent' repression. Another possible explanation for the discrepancy is 'squenching', a term referring to transcriptional repression by an activator (Gill and Ptashne, 1988). ATF4 was demonstrated to interact with the co-activator CREB binding protein (CBP) and components of the general transcription machinery, such as TATA binding protein (TBP), TFIIB, and the RAP30 subunit of TFIIF (Liang and Hai, 1997). It is possible that overexpression of ATF4 sequesters these factors away from the promoter, resulting in transcriptional repression. Consistent with this interpretation, titration experiments in transient transfection

assay showed reduced reporter activity when a large amount of DNA expressing ATF4 was used; in addition, in vitro transcription assay indicated that purified ATF4 had a lower activity at a high concentration than at a low concentration (Liang and Hai, 1997). Therefore, it is important to carry out titration experiments to rule out the possibility of squenching before concluding whether ATF4 acts as an activator or a repressor under a specific condition. It is also important to find out whether any post-translational modification or interaction with other proteins alters the transcriptional activity of ATF4.

3.3. Potential physiological functions of ATF4

3.3.1. Regulation of ATF4 expression

ATF4 mRNA is present in all tissues examined thus far (for examples, see Karpinski et al., 1992; Vallejo et al., 1993; Kato et al., 1999); however, its level can be upregulated by a variety of extracellular signals in different cell types: by anoxia in fibroblasts (Estes et al., 1995), by calcium ionophore in lymphocytes (Tsujimoto et al., 1991), by homocysteine and lysophosphatidylcholine in endothelial cells (Kokame et al., 1996; Sato et al., 1998), and by heregulin (a ligand for epidermal growth factor receptor 3 and 4) in breast cancer cells (Talukder et al., 2000). In addition, the steady-state mRNA level of ATF4 increases by about four fold in fibrosarcoma cells that have become resistant to many DNA-interactive drugs (Levenson et al., 2000). Therefore, the ATF4 gene is upregulated by many extracellular signals. However, the significance of these upregulations is not clear. We note that ATF4 protein has a very short half-life of about 30–60 min (see below). Many of the upregulation results cited above were derived from DNA array or differential display, which detect alterations in mRNA levels. It is not clear whether those extracellular signals also affect the stability of ATF4 protein.

3.3.2. Gain-of-function and loss-of-function analyses of ATF4

Both gain-of-function and loss-of-function approaches have been used to investigate the functions of ATF4. Overexpression of ATF4 in NIH3T3 fibroblasts was demonstrated to reduce the ability of the ectopically expressed ras(V₁₂ → G₁₂) oncogene to transform cells as judged by morphological examination and foci formation (Mielnicki et al., 1996). Because the level of ras mRNA is lower when ATF4 is co-transfected than that when a vector control is co-transfected, it is proposed that ATF4 represses the ras promoter and thereby reduces its ability to transform cells (Mielnicki et al., 1996). One caveat of interpreting these results is that ATF4 may repress transcription via 'squenching', as discussed above. Therefore, it is not clear whether the observed repression is due to a non-specific squenching or a specific inhibition of ras promoter by ATF4. Further work is required to clarify this point.

Knockout mice deficient in the ATF4 gene have been

generated independently by two groups, and both reported abnormal lens formation in the mice (Hettmann et al., 2000; Tanaka et al., 1998). The abnormal lens formation is, at least partially, due to p53-mediated apoptosis, since deletion of the *p53* gene in the *ATF4* knockout background reduced the apoptotic phenotype, resulting in lens formation (Hettmann et al., 2000). Furthermore, the knockout phenotype can be rescued by lens-specific expression of *ATF4* (Tanaka et al., 1998). It is interesting to note that the *Drosophila* homolog of *ATF4*, *cryptocephal* (*crc*), was demonstrated to play an important role in fly development during molting and metamorphosis (Hewes et al., 2001). In light of the results from these *ATF4* knockout mice, one function of *ATF4* appears to regulate development. Because *ATF4* expression (at the mRNA level and possibly at the protein level too) is regulated by many extracellular signals (see Section 3.3.1), it is tempting to speculate that one function of *ATF4* is to regulate development in responding to extracellular cues. This speculation is consistent with the fact that molting and metamorphosis in insects are regulated by the steroid hormone ecdysone (Thummel, 1996). It would be interesting to find out whether *ATF4* responds to any developmental cues in the eyes to regulate lens formation.

3.3.3. *ATF4*-interacting proteins

Yeast two-hybrid screens using different baits have revealed some unexpected *ATF4*-interacting proteins. Here, we review three of them: (a) Tax, a human T cell leukemia virus type I (HTLV I) transactivator, (b) Zip kinase, and (c) components of the proteasome system.

3.3.3.1. Tax. Tax transactivates the HTLV-1 promoter via the Tax-responsive elements that contain the consensus ATF/CRE core sequence (TGACG). Because Tax does not bind to DNA directly, it has long been postulated to activate the HTLV-1 promoter by interacting with cellular proteins. The presence of the functionally important ATF/CRE core sequence in the Tax-responsive element suggests that members of the ATF/CREB family of proteins may be the cellular proteins targeted by Tax. Consistent with this supposition, cDNAs encoding *ATF4* were isolated from lymphocyte libraries in two-hybrid screens using Tax as a bait (Reddy et al., 1997; Gachon et al., 1998). These studies further showed three lines of evidence to support an interaction between *ATF4* and Tax: (1) *ATF4* directly interacts with Tax by *in vitro* binding assays, (2) both *ATF4* and Tax localize in the nucleus by immunofluorescence assay, and (3) *ATF4* enhances the ability of Tax to transactivate the HTLV-1 promoter in transient transfection assays. Interestingly, a cDNA encoding *ATF4* was also isolated from a lymphocyte library in a 'southwestern' screen using the Tax-responsive element as a probe (Tsujiimoto et al., 1991). Therefore, these results suggest that *ATF4* may play a role in Tax-regulated HTLV-1 gene expression. We note that other ATF/CREB proteins have also been reported to play

a role in Tax-mediated gene regulation. In electromobility shift assay (EMSA), Tax has been demonstrated to enhance the DNA binding affinity of CREB, ATF2 and ATF3 (Zhao and Giam, 1992; Franklin et al., 1993; Wagner and Green, 1993; Low et al., 1994; Kwok et al., 1996). Therefore, multiple members of the ATF/CREB family of proteins have been implicated in Tax-mediated gene regulation.

3.3.3.2. Zip kinase. In an effort to find the *ATF4*-interacting proteins, Akira and colleagues screened libraries by yeast two-hybrid using *ATF4* as a bait. In addition to cDNAs encoding bZip proteins, they isolated cDNAs encoding a kinase with a leucine zipper motif, the Zip kinase (Kawai et al., 1998). This kinase can autophosphorylate itself *in vitro* and when overexpressed in fibroblasts it leads to morphological changes characteristic of apoptosis (Kawai et al., 1998). However, despite its interaction with *ATF4*, it is not clear whether *ATF4* is a substrate for this kinase, or whether the interaction between them affects the activity of either partner. Therefore, further studies are needed to elucidate the significance of the interaction between *ATF4* and Zip kinase.

3.3.3.3. Components of the proteasome system. A cDNA encoding *ATF4* was isolated in a two-hybrid screen using human β TrCP, an F-box protein of the E3 ubiquitin ligase complex, as a probe (Lassot et al., 2001). In addition, a cDNA encoding the human ATFx, another member of the *ATF4* subgroup of proteins (referred to as hATF5 in the report), was isolated in a two-hybrid screen using human Cdc34, an E2 ubiquitin conjugating enzyme, as a probe (Pati et al., 1999). Therefore, the *ATF4* subgroup of proteins (*ATF4* and ATFx) interacts with components of the proteasome system. Current evidence is consistent with the model that phosphorylation of *ATF4* targets *ATF4* to the proteasome system via its interaction with β TrCP; this in turn results in the ubiquitination and subsequent degradation of *ATF4* (Lassot et al., 2001). Consistent with this model, the half-life of *ATF4* is short (between 30 and 60 min), and inhibitors of the proteasome system increase its stability (Lassot et al., 2001 and our unpublished data). As pointed out by Lassot et al. (2001), *ATF4* contains the sequence DSGXXXS (residues 218–224), which is similar to the DSGXXS motif found in other β TrCP substrates, β -catenin, $\text{I}\kappa\text{B}\alpha$, and HIV-1 Vpu (see Lassot et al., 2001 for references). Significantly, phosphorylation of $\text{I}\kappa\text{B}\alpha$ at this motif by $\text{IKK}\alpha$ and $\text{IKK}\beta$ has been demonstrated to target $\text{I}\kappa\text{B}\alpha$ for degradation by the proteasome system (for a review see Karin, 1999). Therefore, the stability of *ATF4* may be regulated in a manner similar to that regulating the stability of $\text{I}\kappa\text{B}$. Clearly, identification of the kinase(s) and the signals that regulate this process will shed light on the functions of *ATF4*. It would be interesting to find out whether the Zip kinase phosphorylates *ATF4* at the DSGXXXS site and whether the extracellular signals that upregulate *ATF4* mRNA levels also stabilize *ATF4* protein.

4. ATF6

4.1. Nomenclature, homologous proteins, and dimer formation of ATF6

A partial cDNA encoding the human ATF6 was isolated on the basis of its ability to bind to the consensus ATF/CRE site (Hai et al., 1989). A human clone with significant similarity to ATF6 was isolated in an attempt to identify genes located in the HLA complex (Min et al., 1995). It was originally named CREB-related protein (CRB-RP), because it shares a small degree of homology to CREB: at the N' terminal region over a stretch of about 300 residues (from 83 to 375), it is 23% identical to CREB. Despite the implication of its name, however, CREB-RP is more similar to ATF6 than to CREB. It is similar to ATF6 in both the overall structure and amino acid sequences. CREB-BP and ATF6 have their bZip DNA binding domains in the middle of the protein, a structural arrangement different from most of the other ATF/CREB proteins, which have their DNA binding domains near the C' terminal. At the amino acid level, CREB-RP is homologous to ATF6 in multiple regions as described previously (Zhu et al., 1997). The homology within the basic region is striking: 21 out of 23 residues are identical and the other two residues are similar (basic). Therefore, CREB-RP is more similar to ATF6 than to CREB, and we classified it as a member of the ATF6 subfamily of proteins in Table 1. In a recent report, ATF6 is referred to as ATF α and CREB-RP as ATF6 β (Haze et al., 2001). Another gene G13, also located near the HLA complex, was isolated and found to be almost identical to CREB-RP (Khanna and Campbell, 1996). Therefore, all three names CREB-RP, ATF6 β and G13 refer to the same gene. In the rest of this review, we will use the ATF6 α and ATF6 β nomenclature. Currently, not much is known about the heterodimeric partners for ATF6, except that it does not form dimers with ATF1 or CRE-BP1 (ATF2) (Wang et al., 2000).

4.2. Transcriptional activity of ATF6

Thus far, all available data from transient transfection assays indicate that ATF6 α is a transcriptional activator (Yoshida et al., 1998, 2000; Wang et al., 2000). The transcriptional activation domain has been mapped to the N' terminal region of the protein using the GAL4 fusion approach (Yoshida et al., 2000; Thuerlauf et al., 1998). ATF6 β is also a transcriptional activator; however, transcriptional repression has been observed under certain conditions. As described below, ATF6 proteins are membrane-bound and a truncated version of these proteins translocates to the nucleus and regulates gene expression. Interestingly, overexpression of the truncated ATF6 β (p60ATF6 β) results in transcriptional activation (Haze et al., 2001), but overexpression of the full-length ATF6 β , the membrane-bound form, results in transcriptional repres-

sion (Yoshida et al., 1998). The reason for this negative effect of the full-length ATF6 β is not clear.

4.3. Potential physiological functions of ATF6

Although cDNA encoding ATF6 α was first isolated on the basis of its ability to bind to the ATF/CRE consensus site TGACGTCA (Hai et al., 1989), emerging data indicate that ATF6 α is involved in gene regulation through two binding sites distinct from the ATF/CRE site. The first is the ER stress response element (ERSE), which is involved in gene regulation in ER stress response (also known as the unfolded protein response, UPR); the second is the serum response element (SRE), which is involved in gene regulation in serum response. Below, we briefly review the roles of ATF6 α in each response.

4.3.1. ER stress response

Using ERSE, a sequence with little resemblance to the ATF/CRE consensus sequence, as a probe in a yeast 'one-hybrid screen', Mori and colleagues isolated cDNA encoding ATF6 α (Yoshida et al., 1998). This seminal discovery led to a series of important studies that demonstrate a role for ATF6 α in ER stress response (Haze et al., 1999; Li et al., 2000; Wang et al., 2000; Yoshida et al., 1998, 2000). By immunofluorescence assay and cellular fractionation analysis, ATF6 α was demonstrated to be predominantly an ER membrane protein; upon ER stress, it is cleaved and the N' terminal portion of the protein (p50) translocates into the nucleus and activates transcription (Zhu et al., 1997; Haze et al., 1999; Li et al., 2000). The cleavage of ATF6 α is achieved by a two-step process involving site-1 protease (S1P) and site-2 protease (S2P), the same two proteases that process SREBP in response to low cholesterol (Ye et al., 2001). Although cDNA encoding ATF6 α was isolated on its basis to bind to ERSE, ATF6 α by itself does not bind to ERSE, a 19 nt motif CCAATN9CCACG, in vitro by EMSA. Instead, it interacts with NF-Y or YY-1 which binds to the CCAAT half site of ERSE. Formation of this ternary complex requires the recognition of the CCACG half site on ERSE by ATF6 α , and the proper spacing between CCAAT and CCACG on the DNA. This ternary complex has a slower mobility than that of the binary complex between DNA and NF-Y (or YY-1) (Yoshida et al., 2000, 2001; Li et al., 2000). In this context, we note that a new ERSE, called ERSE-II, was identified recently (Kokame et al., 2001). ERSE-II has an inverted CCAT box and a 1 bp spacing between the two half sites. Although direct binding of ATF6 to the site has not been demonstrated, this site has been shown to mediate ATF6 α -dependent ER stress response (Kokame et al., 2001).

Because the CCAAT half site on ERSE is always occupied as evidenced by genomic footprinting (Li et al., 1994), ATF6 α , which binds to the CCACG half site, is thought to be the inducible component of the ERSE-mediated gene regulation. This supposition is consistent with the observa-

tion that ATF6 α is cleaved and translocated to the nucleus upon ER stress (see above). The functional significance of ATF6 α in ER stress response is further substantiated by a few observations. First, ectopic expression of p50ATF6 α (the cleaved form of ATF6 α) results in high expression of the endogenous ER stress-induced genes, such as *GRP78*, *CHOP*, and *XBP-1* (Yoshida et al., 2000, 2001; Haze et al., 2001). Second, dominant negative ATF6 α blocks ER stress-induced gene expression (Yoshida et al., 2000; Wang et al., 2000). Third, the transcriptional activity of ATF6 α can be further increased by ER stress (Yoshida et al., 1998; Wang et al., 2000). Therefore, all these results indicate that one physiological function of ATF6 α is to regulate gene expression during ER stress response. Recently, Mori and colleagues demonstrated that ATF6 β also participates in the mammalian ER stress response and has many properties similar to those of ATF6 α (Haze et al., 2001; Yoshida et al., 2001).

4.3.2. Serum response

In an effort to identify the interacting proteins of serum response factor (SRF), Prywes and colleagues isolated ATF6 α cDNA by a modified yeast two-hybrid screen (Zhu et al., 1997). They demonstrated that ATF6 α interacts with the transcriptional activation domain of SRF. Furthermore, this interaction may play an important role in SRF-mediated gene regulation, because antisense ATF6 α strongly reduced serum induction of an appropriate reporter gene (Zhu et al., 1997). Although SRF had long been recognized to activate transcription by interacting with a group of proteins called ternary complex factors (TCFs), this important finding demonstrated that SRF can also activate transcription by a TCF-independent manner. This finding led to the demonstration that ATF6 α also plays a role in SRF-mediated activation of the cardiac specific gene atrial natriuretic factor (*ANF*) (Thuerauf et al., 1998). Furthermore, ATF6 α was demonstrated to be phosphorylated by p38 and antisense ATF6 α inhibited the ability of p38 to upregulate *ANF* gene expression (Thuerauf et al., 1998).

In summary, recent evidence implicated ATF6 α in two cellular responses – ER stress response and serum response – via different DNA elements. However, it is not clear at present how the cells integrate these different signals to proper gene regulation. We note that these two responses are mediated by DNA elements with little resemblance to the ATF/CRE consensus sequence, raising the question whether ATF6 α indeed binds to the ATF/CRE site. Recently, using the site selection approach, Prywes and colleagues identified a binding site for ATF6 α as ‘TGACGTG(G/A)’ (Wang et al., 2000). This sequence closely resembles the ATF/CRE consensus TGACGTCA except at the underlined residue. The underlined G residue dramatically increases the affinity of the DNA to ATF6 α . It is possible that ATF6 α regulates a set of genes via this binding site. If this is the case, ATF6 α would be able to regulate genes via at least three distinct binding sites: ERSE

(and ERSE-II), SRE and an ATF/CRE-like site. Although binding to ERSE and SRE requires the interaction with other proteins (NF-Y or YY-1 for ERSE and SRF for SRE), binding to the ATF/CRE-like site can be achieved by ATF6 α alone, at least in vitro. This versatility allows one gene product to be involved in multiple processes, and highlights the complexity of gene regulation in mammalian systems.

5. Conclusions and future perspectives

In conclusion, all three ATF proteins reviewed above (ATF3, ATF4 and ATF6) are modulated by extracellular signals (at the level of their expression or activity). These observations indicate that one common role for the ATF/CREB family of proteins is their involvement in homeostasis. Consistent with this notion, the CREB/CREM and CREBP1 (ATF2) subfamilies of proteins are also modulated by extracellular signals. CREB is expressed in most cell types, but the protein is phosphorylated after stimulation (for reviews see Andrisani, 1999, #1854; Daniel et al., 1998, #1861; De Cesare et al., 1999, #1859; Montminy, 1997, #1312; Sassone-Corsi, 1998, #1314). CREBP1 (ATF2) is post-translationally modulated by stress kinases (Gupta et al., 1995; Raingeaud et al., 1995) and ubiquitination (Firestein and Feuerstein, 1998; Fuchs et al., 1997). Taken together, the ATF/CREB proteins are the receivers of signaling pathways and play a role in homeostasis. We note that AP-1 (Fos/Jun) and C/EBP bZip proteins have also been suggested to play a role in homeostasis (for reviews see Darlington et al., 1995; Yeh and McKnight, 1995; Karin et al., 1997; Xanthoudakis and Curran, 1996), indicating a common function for all these bZip proteins.

To understand the physiological function of these ATF/CREB proteins, it is important to elucidate how these factors contribute to homeostasis, how they are induced by extracellular signals and what target genes they regulate. It is almost certain that each ATF/CREB protein regulates a variety of target genes in a context-dependent manner via complex mechanisms. It is important to select specific systems or paradigms to elucidate their function. Information engendered from each paradigm would provide a glimpse to the multifaceted roles of these proteins in cellular response to extracellular signals. It is reasonable to expect that integration of the information from different paradigms would then provide a more complete picture for the physiological function of these proteins.

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