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ATF

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Abstract

The mammalian activating transcription factor (ATF)/cAMP responsive element binding protein (CREB) family of transcription factors represents a group of basic region-leucine zipper (bZip) proteins that were originally defined in the late 1980s by two criteria: (1) they bind to the consensus ATF/CRE site 'TGACGTCA' *in vitro*; and (2) they form homo- or heterodimers. Over the years, more cDNAs encoding proteins that fulfill the above criteria have been isolated. The names of these cDNAs vary depending on the researchers who made the isolation and on the history of discovery, rather than the function of these proteins. Thus far, more than 50 bZip proteins have been identified. In this article, the classification of the ATF proteins is described and then they are compared with other bZip proteins. This is followed by a review of four ATF proteins: ATF2, ATF3, ATF4, and ATF6. Despite the diversity in the functions of these proteins, one common theme is their involvement in stress responses; their potential role in respiratory diseases is discussed.

Introduction

The term activating transcription factor (ATF) was first used in 1987 to refer to an activity that binds to the ATF consensus site TGACGT(C/A) (G/A) on the adenovirus early promoters E2, E3, and E4. cAMP responsive element binding protein (CREB) was named in the same year to refer to an activity that binds to the cAMP responsive element (CRE) 'TGACGTCA' on the somatostatin promoter. The fact that both these consensus binding sites had the same sequence but for two seemingly different promoter elements – one on viral promoters and the other on cellular promoters – generated much confusion initially. Later, the names ATF and CREB were used to refer to a group of bZip proteins that conform to the following criteria: (1) they bind to the consensus ATF/CRE sequence 'TGACGTCA' *in vitro*; and (2) they form homo- or heterodimers. Over the years, more than 10 different mammalian ATF/CREB cDNAs have been isolated and these can be divided into subgroups based on their amino acid similarity: the CREB/CREM, CREBP1 (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 from different 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 members. Because these cDNAs were isolated by a number of researchers with

Table 1 The mammalian ATF/CREB family of transcription factors

Subgroup	Members	Alternative names
CREB	CREB CREM ^a ATF1	CREB-1, CREB-327, ATF47
CRE-BP1	CRE-BP1 ATFa CREBPA	ATF-43, TREB, TREB36, TCRATF1
ATF3	ATF3 JDP2	ATF2, CREB-2, TCR-ATF2, mXBP, TREB, HB16
ATF4	ATF4	ATF7
ATF6	ATF4L1 ATF6 ATF6 α	LRF-1, LRG-21, CRG-5, TI-241
B-ATF	CREB-RP B-ATF JDP1	CREB2, TAXREB67, mATF4, C/ATF, mTR67
		hATF5
		ATF6 β , CREBL1, G13
		p21SNFT, DNAJC12

^aThe CREM gene also encodes a protein product ICER from an alternative intronic promoter.

different perspectives, the nomenclature in the literature for these proteins has been confusing. Not only are alternative names used to refer to the same protein, but also in some cases the same name is used to refer to different proteins. For example, ATF3, LRF-1, LRG-21, CRG-5, and TI-241 all refer to the same protein. However, CREB2 has been used to refer to three different proteins: an alternatively spliced CREB, CRE-BP1 (ATF2), and ATF4. Therefore, the only way to be sure of the identity of a given protein is to establish its amino acid sequence and not rely on its name alone.

Although the term 'activating transcription factor' implies that these proteins activate transcription, some ATFs (such as ATF3) are transcriptional repressors. This misnomer reflects the prevailing concept in the mid-1980s that a given DNA site is recognized by a single protein. Since deletion of the ATF/CRE site from the corresponding promoters reduced their activity, it was deduced that the binding protein must be an activator. Now, it is clear that binding sites are recognized by multiple proteins. It is not surprising that some binding proteins are transcriptional activators whereas others are suppressors, allowing both positive and negative modulation of the promoters.

In the following sections, ATF/CREB is compared with other bZip proteins and four ATF proteins (ATF2, ATF3, ATF4, and ATF6) are described with a focus on one aspect of their function – stress response.

ATF versus Other bZip Proteins

Although the ATF/CREB proteins were originally defined by their ability to form heterodimers and to bind to the same consensus sequence, three observations indicate that these are arbitrary criteria and the distinction between ATF/CREB and other bZip proteins is blurred. First, overwhelming evidence, including a recent comprehensive analysis of bZip association by coil-coil array, indicates that members of the ATF/CREB family form selective heterodimers with other bZip proteins such as the AP-1, C/EBP, and Maf families of proteins. Second, the AP-1 and Maf consensus binding sites are identical (TGACTCA), and only differ by one nucleotide from the ATF/CREB consensus binding site (TGACGTCA). Third, dendrogram analysis of the bZip region indicates that some ATF proteins are more closely related to bZip proteins without the ATF prefix than those with the prefix. One reason for this apparent discrepancy is that the original ATF clones (ATF1–ATF8) were isolated based on their ability to bind to the sequence TGACGTCA, not on their amino acid sequence

similarity. Therefore, the name ATF reflects the history of discovery, rather than the similarity between them. With the bZip DNA binding domain, these proteins have evolved to bind to a subset of sequences on the genome. However, by having other functional domains, they can interact with different proteins and exert a diversity of functions. The formation of heterodimers with other bZip proteins further expands their flexibility and versatility to regulate gene expression. One common theme of most bZip proteins identified thus far is their involvement in responding to extra- and/or intracellular stimulation. Four ATF proteins, ATF2, ATF3, ATF4, and ATF6, are reviewed below with a focus on their function in stress response.

ATFs in Stress Response

ATF4 has been well documented to play a role in endoplasmic reticulum (ER) stress response. The ER is the organelle for proper folding and processing of proteins destined for plasma membrane or secretion. When unfolded or misfolded proteins accumulate in the ER, the cell initiates a complex and well-choreographed response – the ER stress response or unfolded protein response (UPR) – to transmit the signals from the ER to the nucleus and cytoplasm. One result of the ER stress response is activation of the eIF2 α kinase PEK (also called PERK or EIF2AK3), which phosphorylates the translational initiation factor eIF2 α . eIF2 α phosphorylation attenuates the translation of most mRNAs to reduce the burden on the ER but at the same time increases the translation of specific mRNAs, including the ATF4 mRNA. ATF4, in turn, turns on the expression of many downstream genes, presumably to help the cell cope with the stress. The preferential upregulation of ATF4 protein synthesis by eIF2 α phosphorylation puts ATF4 in a critical position in cellular stress response. Because other stress pathways, such as viral infection and nutrient deficiency, also lead to eIF2 α phosphorylation, ATF4 serves as an integration point for various stress responses.

ATF6 has also been well documented to play a role in ER stress response. It is synthesized as a precursor protein, which binds to the ER chaperone Bip/grp78 and localizes on the ER membrane. During ER stress, ATF6 dissociates from Bip/grp78 and translocates to the Golgi where it is cleaved by proteases to liberate its active N'-terminal domain (ATF6N or p50). ATF6N is then translocated to the nucleus and upregulates its target genes, including those encoding ER chaperone proteins. Significantly, ATF6N can also downregulate gene expression: it binds to the sterol regulatory element-binding protein 2 (SREBP2) and

represses the target genes of SREBP2, resulting in the attenuation of the lipogenic effects of SREBP2. This action has significant physiological implications, since prolonged nutrient deprivation, such as amino acid or glucose deficiency, induces ER stress. By activating ATF6, the cells reduce the lipogenic effect of SREBP2, and thus save energy sources to withstand the stress. Therefore, ATF6 plays an important role in ER stress and in coordinating stress response with energy homeostasis.

ATF2 is a ubiquitously expressed protein with the highest levels found in the brain. Upon exposure to a variety of stress signals, the mitogen-activated protein-kinase (MAPK) cascades including the p38 and JNK/SAPK pathways are activated, resulting in the dual phosphorylation of ATF2 at Thr69 and Thr71. The consequence of this dual phosphorylation is an increase in the half-life and transactivation activities of ATF2. Since p38 and JNK/SAPK are activated by many stress signals (such as genotoxic agents, inflammatory cytokines, UV irradiation, and osmotic stress), ATF2 is well recognized to play an important role in cellular stress response. Another recognized function of ATF2 is its involvement in oncogenesis. ATF2 is induced by growth-stimulating factors via a Ras- and p38-dependent pathway, and is thought to affect the function of oncoproteins, such as c-Jun, c-Myc, E1A, and Tax. As an example, ATF2 dimerizes with c-Jun and may affect Jun-dependent cell-cycle progression, cell survival, and apoptosis.

ATF3 is also a stress-inducible gene. Overwhelming evidence from many laboratories indicates that

ATF3 is induced by a variety of stress signals. However, it differs from the above three ATFs in its mode of induction: ATF2 is induced by posttranslational modification – phosphorylation by stress kinases; ATF4 is induced by translational regulation – increased translation of the ATF4 mRNA upon eIF2 α phosphorylation; ATF6 is induced by posttranslational modification – proteolytic cleavage that allows subcellular translocation. ATF3, on the other hand, is induced at the mRNA level; its steady-state mRNA level is usually not detectable but greatly increases upon exposure of the cells to stress signals. Because of this feature, ATF3 has been identified in many screens using DNA microarray, a technique comparing the steady-state mRNA levels of cells under different conditions.

One dramatic feature of ATF3 induction is that it is neither tissue specific nor stimulus specific. This ‘non-specificity’ is not unique to ATF3. Other genes such as Fos, Jun, and Erg-1 are also induced by a variety of signals in many different tissues. Therefore, the initial genome response to various seemingly unrelated signals appears to be the turning on of a set of common genes, irrespective of the nature of the signals or the type of cells. It suggests that the context of the cells would determine how cells ‘interpret’ the signals to give rise to a diversity of outcomes. Despite its well-documented induction, the functional consequence of ATF3 expression is not clear. Based on the current literature, we hypothesize the following. ATF3 modulates the cell cycle and cell death machinery. Under certain cellular contexts, it

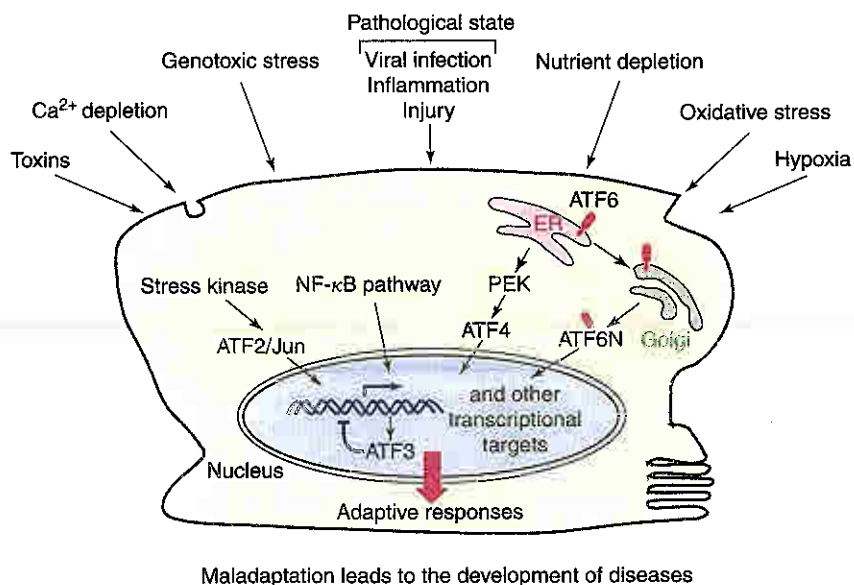


Figure 1 A schematic diagram for ATF proteins in stress response and adaptation. Two features in the figure are not described in the text: the involvement of the NF- κ B pathway in the induction of ATF3 by stress signals and the regulation of ATF3 gene expression by itself.

leads to tissue dysfunction and the development of diseases. In the context of already transformed cancerous cells, however, ATF3 promotes metastasis.

Potential Roles of ATFs in Respiratory Diseases

In conclusion, all four ATF proteins reviewed above are involved in the adaptive responses to cope with stress conditions. Interestingly, ATF4 has been demonstrated to be required for the induction of ATF3 by ER stress and ATF2 has been shown to transactivate the ATF3 promoter, indicating interplays among these ATF proteins. Figure 1 shows a schematic diagram for ATF proteins in stress response. Since proper adaptation to stress conditions is critical to the function of cells, it is conceivable that these ATF proteins play an important role in the pathophysiology of many diseases. Indeed, some circumstantial evidence suggests a potential role for this family of proteins in pulmonary diseases. The surfactant protein A and B promoters contain ATF/CRE sites. Specifically, ATF2 and CREB were demonstrated to be induced in the intact lung tissue by metallic particulates, and ATF2 was shown to regulate the surfactant protein B promoter. Furthermore, knockout mice deficient in ATF2 display severe newborn respiratory distress syndrome, the major cause of neonatal morbidity and mortality in developed countries. Therefore, members of the ATF/CREB family of proteins are involved in various stress responses and may be key players in the pathogenesis of stress-associated lung diseases.

See also: **Oncogenes and Proto-Oncogenes: Overview**; *jun* Oncogenes; MYC; RAS. **Transcription Factors: Overview.**

Further Reading

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Fox

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Abstract

The Forkhead box (Fox) proteins are an extensive family of transcription factors, which share homology in the winged helix/forkhead DNA-binding domain. Mutations of the Fox genes elicit pronounced defects of cellular proliferation, differentiation, and metabolic homeostasis, leading to developmental abnormalities and human disease. In this review we will focus on Fox family members that contribute to lung development and disease. Deletion of the *Foxa2* (*HNF-3β*) gene from respiratory epithelial cells is associated with airspace enlargement, goblet cell hyperplasia, and neutrophilic infiltration. Likewise, increased expression of *Foxa2* in the distal respiratory epithelium caused a striking inhibition in branching morphogenesis and vasculogenesis of the lung. *Foxj1* (*HFH-4*) deficient (*-/-*) mice display perinatal lethality due to defective differentiation of ciliated epithelial cells lining the pulmonary bronchioles and cerebral ventricles, leading to defects in lung function and hydrocephalus. Haploinsufficiency of the *Foxf1* (*HFH-8*) gene causes fusion of the lung lobes and abnormal development of peripheral lung capillaries leading to perinatal pulmonary hemorrhage in 50% of the newborn *Foxf1*^{+/-} mice that displayed an 80% reduction in lung expression levels of *Foxf1*. Earlier expression of the *Foxm1b* (*HFH-11b*) protein in transgenic mice following lung injury accelerates the onset of proliferation of different lung cell types. This partial list of genetic studies examining Fox proteins underscores the importance of this family in regulating genes involved in lung morphogenesis, respiratory diseases, and lung repair.