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ATF3 and Stress Responses

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The purpose of this review is to discuss ATF3, a member of the ATF/CREB family of transcription factors, and its roles in stress responses. In the introduction, we briefly describe the ATF/CREB family, which contains more than 10 proteins with the basic region-leucine zipper (bZip) DNA binding domain. We summarize their DNA binding and heterodimer formation with other bZip proteins, and discuss the nomenclature of these proteins. Over the years, identical or homologous cDNA clones have been isolated by different laboratories and given different names. We group these proteins into subgroups according to their amino acid similarity; we also list the alternative names for each member, and clarify some potential confusion in the nomenclature of this family of proteins. We then focus on ATF3 and its potential roles in stress responses. We review the evidence that the mRNA level of ATF3 greatly increases when the cells are exposed to stress signals. In animal experiments, the signals include ischemia, ischemia coupled with reperfusion, wounding, axotomy, toxicity, and seizure; in cultured cells, the signals include serum factors, cytokines, genotoxic agents, cell death-inducing agents, and the adenoviral protein E1A. Despite the overwhelming evidence for its induction by stress signals, not much else is known about ATF3. Preliminary results suggest that the JNK/SAPK pathway is involved in the induction of ATF3 by stress signals; in addition, IL-6 and p53 have been demonstrated to be required for the induction of ATF3 under certain conditions. The consequences of inducing ATF3 during stress responses are not clear. Transient transfection and in vitro transcription assays indicate that ATF3 represses transcription as a homodimer; however, ATF3 can activate transcription when coexpressed with its heterodimeric partners or other proteins. Therefore, it is possible that, when induced during stress responses, ATF3 activates some target genes but represses others, depending on the promoter context and cellular context. Even less is understood about the physiological significance of inducing ATF3. We will discuss our preliminary results and some reports by other investigators in this regard.

ATF3 Stress responses Transcription factors ATF/CREB

INTRODUCTION

The Mammalian ATF/CREB Family of Transcription Factors

Activating transcription factor (ATF) was first named in 1987 to refer to an activity that binds to the adenovirus early promoters E2, E3, and E4 at sites with a common core sequence "CGTCA" (71). cAMP responsive element binding protein (CREB) was named in 1987 to refer to an activity that binds to the cAMP responsive element (CRE) on the somatostatin promoter (96). The consensus binding site for ATF was later defined as TGACGT(C/A)(G/A) (76), a sequence identical to the CRE consensus (TGACGTCA) [(25) and references therein; for a review see (108)]. The identity of the binding sites for two seemingly different promoter elements—one on viral promoters and the other one on cellular promoters—generated much confusion and prompted many groups to purify the corresponding binding proteins. Some reports indicated that a single polypeptide binds to the consensus (55,76,96), whereas others indicated multiple polypeptides (20,48,61,89,105). In the past decade, many cDNAs encoding proteins that can bind to the ATF/CRE site have been isolated. Table 1 lists some of the mammalian clones. All listed clones are derived from one of the following species: human, mouse, or rat. Over the years, identi-

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TABLE 1
THE MAMMALIAN ATF/CREB FAMILY OF TRANSCRIPTION FACTORS

Subgroup	Members	Spliced Variants	Alternative Names
CREB*	CREB (42,50)	reviews (44,90,111)	ATF-47† (56)
	CREM (35)	(70), reviews (36,44,111)	
	ATF1 (49)	ATF1 (49)	TREB36 (140), TCRATF1 (72), ATF-43† (56,57)
CRE-BP1*	CRE-BP1 (85)	CRE-BP1 (41,85)	ATF2 (49), HB16 (62), TREB7 (140), TCR-ATF2 (72)
		CRE-BP2 (41)	mXBP‡ (78)
		CRE-BP3 (41)	
	ATFa (40)	ATFa (40)	ATFa2 (14)
		ATFaΔ (40)	ATFa1 (14)
		ATFa3 (14)	
		ATFa0 (103)	
	CRE-BPa (101)	CRE-BPaα (101,144)	
		CRE-BPaβ (144)	
		CRE-BPaγ (144)	
		CRE-BPaδ (144)	
ATF3§	ATF3 (49)	ATF3 (49)	LRF-1§ (52), LRG-21§ (28), CRG-5§ (31), TI-241§ (58)
		ATF3ΔZip (16)	
	JDP-2 (5)		
ATF4#	ATF4 (49)	ATF4 (49)	CREB2# (64), TAXREB67# (126), mATF4# (91), C/ATF# (130), mTR67# (18)
	ATFx (100)	ATFx (100)	
ATF6	ATF6 (49)	ATF6 (49)	
	CREB-RP (92)	CREB-RP (92)	G13 (66)
B-ATF¶	B-ATF (27)		
	JDP1 (5)	JDP1 (5)	

*The Nomenclature Committee of the Human Genome Project refers to CREB as CREB1 and CRE-BP1 as CREB2. CREB1 is mapped to human chromosome 2q32.3-q34 (124), and CREB2 to human chromosome 2q24.1-q32 (26). Therefore, CREB2 has been used to refer to three proteins: CRE-BP1, ATF4, and an alternatively spliced form of CREB (see text).

†The terms, ATF-43 and ATF-47, listed in the table refer to polypeptides: ATF-43 for 43 kDa and ATF-47 for 47 kD. ATF-47 is encoded by CREB (56) and ATF-43 encoded by ATF1 (57).

‡mxBP (a mouse clone) and hxBP (a human clone) (77) were both identified by their ability to bind to the X box of the MHC promoter. hxBP is not homologous to mxBP, but is identical to TREB5, a bZip protein that does not share significant similarity (except in the bZip motif) to any of the proteins listed in Table 1.

§ATF3 (a human clone) and LRF-1 (a rat clone) are over 95% similar at the amino acid level. LRG-21 (also referred to as CRG-5 or TI-241) is a mouse clone highly homologous to ATF3 or LRF-1.

#ATF4, CREB2, and TAXREB67 are human clones with the same amino acid sequences. mATF4, C/ATF and mTR67 are mouse clones with virtually identical amino acid sequences, except that mATF4 has extra 31 amino acids at the N′-terminal. The homology between the human and mouse clones is about 85%.

¶B-ATF (a human clone) shares 60% similarity to ATF3 (also a human clone) in the bZip region. However, it has no significant similarity to ATF3 outside of the bZip region; therefore, we classified it in a different subgroup.

ATF5, not listed in the table, is identical to Fos, a fact not recognized at the time of publication due to sequencing mistakes. Because the cDNA was isolated by screening the expression library with triplicated ATF/CRE consensus site (49), it indicates that, under some conditions, c-Fos can bind to DNA as a homodimer. This does not contradict the general observation that c-Fos does not bind to DNA as a homodimer; it simply indicates that, at high concentrations of proteins and DNA, a weak interaction can be detected.

ATF7 and ATF8 described in (49) were not sequenced; it is not clear whether they correspond to any other cDNAs.

cal or homologous cDNA clones have been isolated by different groups and given different names. Some cDNAs share substantial similarity (over 85%) at the amino acid level, but are derived from different species; we consider them homologues and refer to them using the first published name. The similarity of the homologues at the DNA level is usually lower than that at the amino acid level due to codon degeneracy. All these proteins have the basic region-leucine zipper (bZip) DNA binding domain, and can be grouped into subgroups on the basis of their amino acid similarity. Proteins within each subgroup share significant similarity both inside and outside of the bZip domain; proteins between the subgroups, however, do not share much similarity outside of the bZip "motif." It is important to note that, in most cases, the similarity is limited to the "motif," because even within the bZip domain, the sequences other than the basic resi-

dues or the leucine residues are not similar. Table 1 refers to the subgroups by the name of the first published cDNA within that subgroup, and lists the alternative names. For some members of this family, alternatively spliced variants have been identified and are indicated in the table.

We emphasize that the list is not comprehensive. It only includes some of the mammalian cDNAs, and cDNAs in the following categories are not listed. First, mammalian cDNAs encoding proteins that can bind to the ATF/CRE consensus site, but have no significant homology to any cDNAs with the prefix of "ATF" or "CREB" are not listed. One such example is E4BP4 (21), which has no significant similarity to any ATF/CREB proteins. Another example is Luman (82), a human clone similar to the mouse LZIP (11). Although Luman/LZIP binds to the ATF/CRE sites and shares some similarity to ATFa in the bZip region, it does not share significant similarity to ATFa outside of the bZip region, and does not have the prefix ATF or CREB. Therefore, we did not include them in the table. Third, cDNAs from nonmammalian species encoding bZip proteins that bind to the ATF/CRE site are not listed; examples are apCREB2 from aplysia (8), dCREB-A from *Drosophila* (118), and ACR1(131) and SKO1 (97) from yeast.

We also note that the nomenclatures in the literature for this family of proteins have been confusing. In addition to the alternative names (see Table 1), other sources of confusion include the following. First, CREB2 has been used to refer to three different proteins: an alternatively spliced CREB (140), CRE-BP1 (34,138), and ATF4 (64). (See Table 1 legend for the nomenclature of CREB1 versus CREB2 according to the Nomenclature Committee of the Human Genome Project.) Second, Atf1 from *Saccharomyces pombe* is most likely the "functional homologue" of mammalian CRE-BP1 (commonly referred to as ATF2) but not mammalian ATF1. This is because Atf1 is most similar to CRE-BP1 in the bZip region (116), and can be phosphorylated by the stress kinases (24,116), a feature similar to the mammalian CRE-BP1 (43,104). Therefore, to ensure the identity of a given cDNA, the best way is to inspect the amino acid sequences.

Cross Talk Between ATF/CREB, AP-1, and C/EBP Proteins

Besides ATF/CREB, other proteins also contain the bZip DNA binding domains [review in (54)]; they include the AP-1 [for a recent review, see (63)] and C/EBP families of proteins [(13,134); review in (128)]. These three families of proteins (ATF/CREB, AP-1, and C/EBP) were identified during investigations of different promoters in different contexts. Initially, they were thought to be "unrelated" protein families. However, growing evidence indicates that the distinction between these proteins is blurred. First, they can cross bind to each other's consensus sites (Table 2). Second, specific members of one family can form heterodimers with specific members of another family (Table 2). Third, members of one family can regulate transcription in a manner characteristic of the other family. As an example, JunB and JunD can regulate the proenkephalin promoter in a cAMP-dependent manner (67). Another example is that C/EBP proteins can activate certain promoters via a CRE site, instead of a C/EBP site (102,129). Fourth, naturally occurring composite sites have been observed; one example is the C/EBP-ATF composite site "TTGCATCA" (half C/EBP site TTGCGCAAT and half ATF/CRE-like site "TGACATCA") on the gadd153/Chop10 promoter (135). Therefore, the distinction between these proteins is blurred; the names reflect the history of discovery, but not necessarily the differences between them.

ATF/CREB Proteins and Homeostasis

Are there unifying themes for the functions of this diverse family of proteins? Perhaps, one common feature these proteins share is their involvement in cellular responses to extracellular signals. All these proteins or corresponding genes can be regulated by extracellular signals, either at the level of gene expression or at the level of posttranscriptional modifications. As an example, CREB is expressed in most cell types, but the protein is phosphorylated after stimulation [review in (44,95,111,112)]. CRE-BP1 (ATF2) is posttranslationally modulated by the viral protein E1A (79,84), stress kinases (43,104), and ubi-

TABLE 2
EXAMPLES FOR CROSS TALKS BETWEEN ATF/CREB, AP-1, AND C/EBP

	References
Cross Family Binding	
ATF/CREB proteins bind to the AP-1 consensus*	47,87
AP-1 proteins bind to the ATF/CRE consensus†	47,110
C/EBP proteins bind to the ATF/CRE consensus†	6,102
Cross Family Heterodimer Formation	
ATF/CREB and C/EBP	109,117,132
ATF/CREB and AP-1	9,15,18,19,27,32, 47,51,59,83,99
AP-1 and C/EBP	53

*AP-1 consensus: TGACTCA.
†ATF/CRE consensus: TGACGTCA.

quitination (33,38). ATF3, in contrast, is expressed at relatively low levels in most cell types, but its expression greatly increases upon the exposure of cells to many extracellular signals (see below and Table 4). ATF4 mRNA has been demonstrated to be present in many cells, but can be upregulated by extracellular signals such as anoxia (29), homocysteine (68), and lysophosphatidylcholine (114). Intriguingly, Akira and colleagues isolated a cDNA encoding a kinase—named Zip kinase—that can interact with ATF4 (65). Because of the prevailing involvement of kinases in signaling pathways, this result implies a potential link between signaling pathways and ATF4. However, it is not clear whether this interaction has any significance, and whether this kinase can phosphorylate ATF4. ATF6 has also been demonstrated to be regulated by extracellular signals. In an attempt to identify serum response factor (SRF)-interacting proteins, Prywes and colleagues demonstrated that ATF6 interacts with SRF (143). Therefore, ATF6 may play a role in the responses of cells to serum stimulation. In addition, ATF6 can be phosphorylated by p38 mitogen-activated protein kinase (MAPK) in vitro and its transcriptional activity can be enhanced by coexpressing p38 in transient transfection assay (125). Taken together, the ATF/CREB proteins appear to be the receivers of signaling pathways and may play a role in maintaining homeostasis—the tendency of the cells to respond to changes in an attempt to maintain a stable state. Table 3 summarizes the results described above, with additional known regulations for these proteins/genes. The table is not meant to be comprehensive, but to show a common feature that the ATF/CREB proteins/genes share—their ability to be regulated by extracellular signals. We note that AP-1 and C/EBP proteins have also been suggested to play a role in homeostasis (75,88).

ATF3 AND STRESS RESPONSES

Induction of ATF3 by Extracellular Signals

In an attempt to understand the physiological functions of ATF3, we examined the expression of ATF3 by in situ hybridization. Our rationale was that, if ATF3 is expressed in a specific manner (in specific tissues or at specific time), we may obtain a clue to its functions by this approach. At that time, a few observations prompted us to hypothesize that ATF3 is an immediate-early gene that responds to extracellular signals. First, the mRNA level of ATF3 is relatively low in most cell types examined, but greatly increases upon serum induction (16,93) or during liver regeneration (52). Second, the 3′ untranslated region of ATF3 mRNA contains several AUUUA sequences (16,52), a characteristic of the mRNAs of many immediate-early genes. Third, ATF3 is similar to c-Fos (a well-characterized immediate-early gene) in the bZip region, indicating that they may have evolved from a common ancestral gene (90). When we examined the expression of ATF3 by in situ hybridization in different tissues after a variety of treatments, we noticed the following correlation: signals that presumably induce tissue injury increase the levels of ATF3 mRNA, but signals that presumably do not induce tissue injury fail to do so [(17,139); Fig. 1, and our unpublished results]. Table 4 summarizes the results: (a) myocardial ischemia and myocardial ischemia coupled with reperfusion (ischemia–reperfusion) induce ATF3 in the heart; (b) chemical toxic-

TABLE 3
SOME KNOWN REGULATIONS OF ATF/CREB PROTEINS/GENES

Member	Mode of Regulation	Some Known Regulators
CREB	posttranslational	cAMP/PKA, calcium ionophore [review (44,95,111,112) and references therein], Tax (120,138,142)
CREM	posttranslational	cAMP/PKA, calcium ionophore [review (44,111,112) and references therein]
ATF1	posttranslational	cAMP/PKA (80,107), calcium ionophore/CaM kinase (80,119)
CRE-BP1	posttranslational	E1A (79,84), TPA (144), stress kinases (43,104)
	protein stability	ubiquitination (33,38)
	RNA level	partial hepatectomy (121)
ATF3	posttranslational	hepatitis B virus X protein (7), HTLV-1 Tax (81), PKA (19)
	RNA level	see Table 4
ATF4	posttranslational	Tax (39,106)
	RNA level	anoxia (29), homocysteine (68), lysophosphatidylcholine (114)
ATF6	posttranslational	p38 MAPK (125), SRF (143)

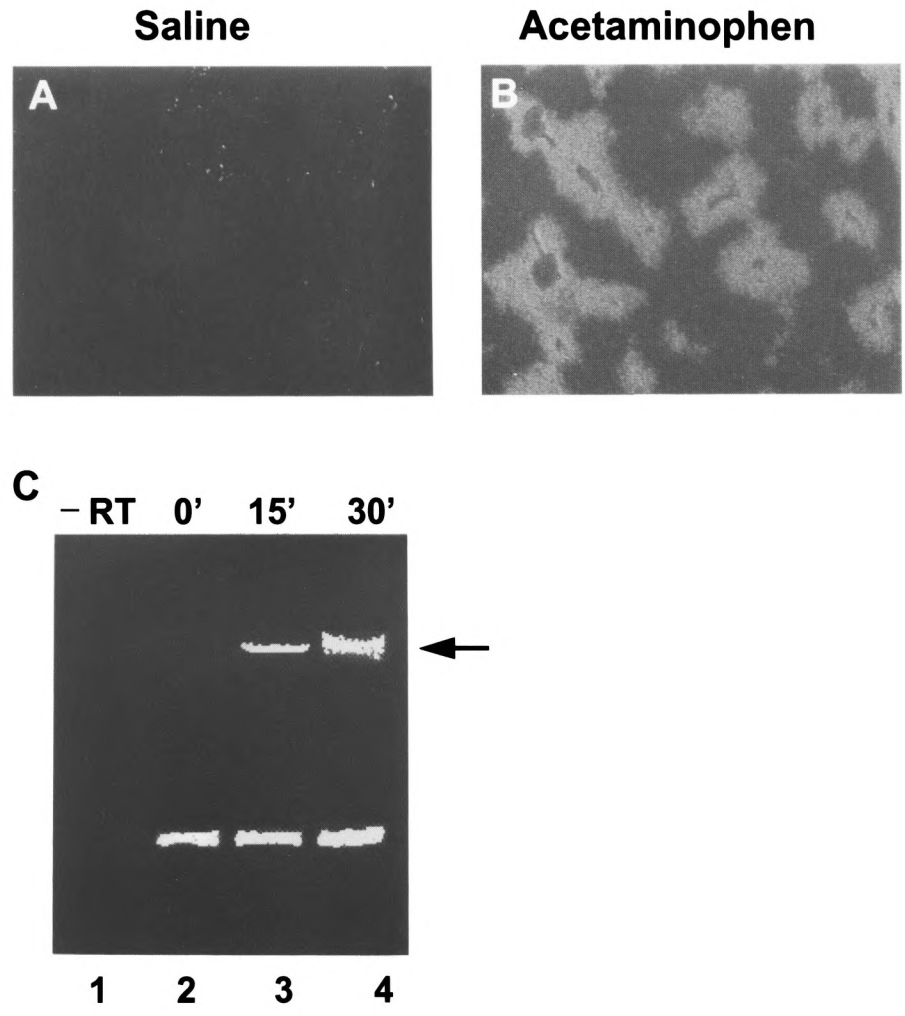

FIG. 1. Induction of ATF3 by acetaminophen and Fas antibody. (A, B) In situ hybridization assay showing induction of ATF3 in the liver by acetaminophen. Four-week-old male Sprague-Dawley rats were fasted for 42 h and IP injected with saline (A) or 500 mg/kg acetaminophen (B). Two hours later, the animals were sacrificed and the livers were analyzed by in situ hybridization using antisense ATF3 RNA as a probe as described previously (17). The pictures were produced by dark-field photography after radiographic emulsion for 10 days. (C) Reverse transcription coupled with polymerase chain reaction (RT-PCR) assay showing induction of ATF3 by Fas antibody. HL-60 cells (2×10^7) treated with Fas antibody for the indicated time periods (0, 15, or 30 min) were kindly provided by Drs. K. M. Coggeshall and K. Schlottmann. Total RNA was isolated using the Trizol method according to the manufacturer's instructions (Life Technologies), and 5 μg of RNA was analyzed by RT-PCR to amplify ATF3 mRNA (top band, indicated by arrow) and glyceraldehyde-3-phosphate dehydrogenase mRNA (GAPDH, bottom band). Twenty percent of the PCR products were resolved on a 2% agarose gel and visualized under UV after ethidium bromide staining. The primers are as follows: ATF3 N′-terminal (5′-GCTCTAGAAAAAAAGAGAAGACRGAGTCG-3′), ATF3 C′-terminal (5′-TCTCCAATGCGTTCAGGGTT-3′), GAPDH N′-terminal (5′-CATTGACCTCAACTACATGG-3′), and GAPDH C′-terminal (5′-ACCACCCTGTTGCTGTAGCC-3′). "-RT" indicates a RT-PCR reaction without reverse transcriptase.

ity of the liver by alcohol, carbon tetrachloride (CCl_4), or acetaminophen induces ATF3; (c) seizure induces ATF3 in the brain; (d) renal ischemia–reperfusion injury induces ATF3 in the kidney; (e) wounding induces ATF3 in the skin. However, light stimulation during the dark cycle of the circadian cycle, which presumably does not induce tissue injury, does not induce ATF3 in the suprachiasmatic nuclei where the circadian clock resides. The correlation between ATF3 expression and treatments that induce tissue injury led us to hypothesize that induction of ATF3 is a part of the cellular stress responses. Our results are consistent with other investigators' observations that ATF3 is induced after stress induction (also summarized in Table 4): (a) in the liver after partial hepatectomy (52) or hepatic ischemia (45); (b) in the dorsal root ganglia, spinal cord, and sciatic nerves after axotomy (127); and (c) in the thymic stroma after thymocyte activation by CD3ε cross-linking (73).

In addition to animal model systems, ATF3 has also been demonstrated to be induced in cultured cells by many treatments as summarized in Table 4:

TABLE 4
EXAMPLES OF TREATMENTS THAT INDUCE THE EXPRESSION OF ATF3

	Treatments	References
In whole organism (tissues)		
Liver	partial hepatectomy	17,52
	alcohol	17
	carbon tetrachloride	17
	acetaminophen	Fig. 1
	cycloheximide	our unpublished results
	hepatic ischemia	45
Heart	ischemia	17
	ischemia–reperfusion	17
Kidney	ischemia–reperfusion	139
Brain	seizure	17
Peripheral nerves	axotomy	127
Skin	wounding	our unpublished results
Thymus	anti-CD3ε	73
In cultured cells*		
Hepatocytes	cycloheximide	133
	EGF	133
	HGF	133
Leukemia cells	doxorubicin	141
Macrophages	cytokines†	28,31
	LPS, BCG	28,31
	PMA	31
Myeloid cells	Fas antibody	Fig. 1
Neuroblastoma	forskolin	19
	FGF	122
Neuroblastoma, macrophages	A23187	2,28,31
Various cell types	serum‡	3,16,60,93
	anisomycin§	74
	E1A#	46
	genotoxic agents¶ (ionizing radiation, UV, MMS)	4
Fibroblasts	other stimuli**	our unpublished results

All treatments listed in the table (except the EGF treatment) were demonstrated to increase the steady-state levels of ATF3 mRNA (see text). EGF was demonstrated to increase the steady-state levels of ATF3 protein in SK-N-MC neuroblastoma cells.

*Cell types for each treatment reported in the references are listed in the table. The list is not meant to imply that the induction is limited to the indicated cell types only.

†ATF3 is induced in macrophages by cytokines such as IL-4, IFN-α, IFN-β, IFN-γ, but not by IL-1α, IL-1β, IL-2, IL-6, TNF-α, or GM-CSF (28,31).

‡Serum can induce ATF3 in various cell types: HeLa (16), fibroblasts (3,60,93) and hepatocytes (93). Mouse clone number U56 described by Bravo and colleagues in 1988 (3) was later identified to be ATF3 [referred to as LRF-1 in (93)].

§Anisomycin induces ATF3 in NIH3T6 (74), HeLa, and 293 cells (our unpublished results).

#E1A induces ATF3 in various cells: human embryonic retinoblast (HER), normal rat kidney (NRK), and mouse P19 embryonal carcinoma (EC) cells (46).

¶Genotoxic agents such as UV, MMS, and ionizing radiation induce ATF3 in various cells including myeloid-lymphoid, lung cancer, breast carcinoma, and colon cancer lines (4). Our unpublished results indicate that UV induces ATF3 in HeLa, 293, and NIH3T6 cells.

**Our preliminary data indicate that cell death-inducing agents such as ATP, puromycin, and H_2O_2 also induce the expression of ATF3 in NIH 3T6 fibroblast (unpublished results).

(a) growth-stimulating factors such as serum (3,16,60,93), fibroblast growth factor (FGF) (122), epidermal growth factor (EGF) (133), hepatocyte growth factor (HGF) (133), and phorbol ester 12-myristate 13-acetate (PMA, also known as TPA) (31); (b) cytokines such as interferons (IFNs) and interleukin-4 (IL-4) (28,31); (c) genotoxic agents such as ionizing radiation, methyl methanesulfonate (MMS), and ultraviolet (UV) light (4); (d) signaling molecules A23187 [calcium ionophore (2,28,31)], and forskolin

(19); (e) bacterial products such as lipopolysaccharide (LPS) and Bacillus Calmette Guerin (BCG) (28,31); (f) viral protein adenovirus 12S-E1A (46); and (g) agents known to induce cell death or the JNK/SAPK signaling pathway such as doxorubicin (141), Fas antibody (Fig. 1), anisomycin (74), cycloheximide (133), and other agents described in the Table 4 legend. In most cases, ATF3 induction is immediate (within 2 h) and transient. However, the induction can be delayed and sustained (for at least 12 h) such as in the case of IFN-γ (31). Furthermore, the induction can be biphasic—two sequential peaks of expression—such as in the case of partial hepatectomy (45): Taub and colleagues showed that ATF3 is induced in two peaks at 2 and 48 h after partial hepatectomy. Because these time points correspond to the peak times of DNA replication after partial hepatectomy, they suggested that ATF3 (referred to as LRF-1) is a cell cycle-regulated gene [(45); and review in (123)]. Because this is the only study that examined ATF3 expression at 48 h after stimulation, this result indicates that it would be interesting to examine whether ATF3 is also induced by other signals at later stages (in addition to the early peak).

In summary, ATF3 is induced by many extracellular signals. The majority of them can be classified as stress signals; however, some of them (such as serum, FGF, and EGF) do not have an apparent link to stresses. In this context, the following report is intriguing and may provide some insights. Using DNA microarray representing about 8,600 human genes, Brown and colleagues studied the temporal program of transcription during the response of human fibroblasts to serum (60). They found that many features of the transcription program appear to be related to the physiology of wound repair, rather than simple cell proliferation: in addition to genes involved in cell proliferation, genes involved in tissue remodeling, cytoskeletal reorganization, angiogenesis, inflammation, and reepithelialization are induced. They pointed out that normal cells do not encounter serum (the soluble fraction of clotted blood), but become exposed to serum in the context of a wound. Therefore, the induction of ATF3 by serum in cultured cells may reflect a part of the complex cellular stress response to wounding. EGF (in serum) and FGF (in extracellular matrix) are also factors that the cells will encounter during wounding, thus their use in cultured cells may also represent a paradigm for studying wound repair. It is tempting to speculate that the induction of ATF3 by E1A may represent a part of the cellular "stress response" to viral infection, in addition to the cellular proliferating response to E1A.

Therefore, ATF3 is induced during the cellular responses to many stress signals, and can be viewed as a stress-inducible gene. We emphasize that this is probably an oversimplified view; however, it provides a handle to investigate the physiological functions of ATF3. In this context, we would like to note the following points. First, all induction experiments (except the FGF experiment) described above examined the steady-state levels of ATF3 mRNA. In most cases, it is not clear whether the increase in steady-state level is due to an increase in transcription or an increase in RNA stability, or both. One study using anisomycin suggested that the increase is due to an increase in both transcription and RNA stability (74). Second, although most reports cited above described low basal levels of ATF3 mRNA in untreated cells, some reports described detectable (or even relatively high) levels of ATF3 mRNA in certain cells. As an example, Northern blot showed clear expression of ATF3 in skeletal muscle, intestine, and stomach (52). Intriguingly, ATF3 was demonstrated to be expressed in the one cell stage by an expressed sequence tag (EST) sequencing project (1). In addition, using a sensitive reverse transcription coupled with polymerase chain reaction (RT-PCR), Freeman and colleagues demonstrated that ATF3 is expressed in all tissues they examined (37). Because of the sensitivity of the assays, the EST and RT-PCR results do not necessarily mean a high expression level of ATF3. However, they do indicate that ATF3 is expressed at a basal level in many cells. It is possible that its functions under normal condition are different from those under induced conditions. Third, the activity of ATF3 has been demonstrated to be regulated by protein–protein interactions. Interaction of hepatitis B virus X protein or HTLV-1 Tax protein with ATF3 enhanced its DNA binding activity (7,81). In addition, ATF3 was reported to activate the proenkephalin promoter, in the presence of protein kinase A (PKA) but not in the absence of PKA, in a transient transfection experiment (19). Therefore, the gene function of ATF3 can be regulated by multiple ways: the level of expression and the level of activity.

Signaling Pathways Involved in the Induction of ATF3 by Extracellular Signals

Preliminary results from our work suggest that the JNK/SAPK signaling pathway may be involved in the induction of ATF3 by stress signals. First, some of the signals that induce ATF3 also induce the JNK/SAPK pathway, such as UV, anisomycin, and cycloheximide (12,23,69,86). Second, overexpressing

MEKK, a component of the JNK/SAPK pathway, increases the CAT report driven by an ATF3 promoter fragment (Liang and Hai, unpublished results). Third, the ATF3 promoter contains potential binding sites for ATF2 and c-Jun (74), two transcription factors phosphorylated and activated by the pathway. Consistently, overexpressing ATF2 and c-Jun together increases the ATF3 promoter activity by a transient transfection assay (74). These correlative results are consistent with the notion that the JNK/SAPK pathway plays a role in the induction of ATF3 by stress signals. However, further analyses are necessary to prove this point. Results from other investigators indicate that IL-6 and p53 are involved in the induction of ATF3 by extracellular signals. Taub and colleagues demonstrated that the induction of ATF3 by partial hepatectomy is IL-6 dependent (22): in IL-6-deficient mice, the induction is attenuated, but can be rescued by IL-6 injection. In addition, Fornace and colleagues demonstrated that the induction of ATF3 by genotoxic agents is p53-dependent in some cells (4): the induction of ATF3 by ionizing radiation is attenuated in RKO/E6 cells in which p53 function is abrogated; furthermore, the induction is absent in the thymus from p53-deficient mice. However, this p53 dependence is not absolute, because ATF3 can be induced by ionizing radiation in certain cells carrying p53 mutations (4). Therefore, ATF3 can be induced in both p53-dependent and p53-independent manners. In light of the varieties of signals that can induce ATF3, it is almost certain that induction of ATF3 is regulated by complex mechanisms, involving multiple signaling pathways in signal- and cell type-dependent manners.

Transcriptional Activities of ATF3

ATF3 has different activities as a homodimer or heterodimer. By transient transfection and in vitro transcription experiments, we demonstrated that the ATF3 homodimer represses transcription from promoters containing its binding sites (16,135). The repression activity of ATF3 can be further enhanced by the hepatitis B virus X protein, at least in part, due to an increased DNA binding affinity (7). Consistent with the notion that ATF3 is a repressor, Towler and colleagues demonstrated that cotransfection of ATF3 attenuates the activation of the osteocalcin promoter by FGF and forskolin (10). In addition, Taub and colleagues demonstrated that cotransfection of ATF3 represses the activation of an artificial promoter by c-Fos and JunB (51). They further mapped the repression domain of ATF3 to a region between amino acids 40 and 84. Therefore, ATF3 has been demonstrated to repress promoters containing its binding sites. In this context, it is interesting to note that we observed activation of promoters by ATF3, if the promoters do not have the binding sites for ATF3 (16). Although the activation is usually low (less than five-fold), it is consistent. We proposed that this is due to the "sequestering of co-repressors" from the promoters by ATF3. Consistent with this co-repressor model, ATF3ΔZip, an ATF3 variant that lacks the functional DNA binding domain, does not inhibit transcription; instead, it activates transcription from promoters, presumably by sequestering negative factors away from the promoters. We note that the repression activity of ATF3 is inconsistent with the implication of its name: Activating Transcription Factor 3. This apparent discrepancy is due to a previous inaccurate assumption. When ATF was named in 1987 (71), on the basis of the information available at that time, it was assumed that there was only one ATF and that it was an activator. In retrospect, because ATF represents a large gene family, it is not surprising that some of its members are activators whereas others are repressors.

In addition to repressing transcription, ATF3 has also been demonstrated to activate transcription when coexpressed with other proteins such as HTLV-1 Tax (81), Ras (98), and PKA (19). In the case of Tax, ATF3 was demonstrated to interact directly with Tax; in the cases of Ras and PKA, it is not clear whether ATF3 interacts with any proteins or is modified covalently under those conditions. As described above, ATF/CREB proteins can form selective heterodimers. ATF3 forms heterodimers with ATF2 (49), c-Jun (47,51,52), JunB (51,52), and JunD (19,32,99). These heterodimers can act as activators or repressors depending on the promoter context. ATF3/c-Jun and ATF3/JunD have been demonstrated to activate promoters containing ATF/CRE or related sites (19,51), whereas ATF3/JunB can either activate or repress depending on the promoter context (51). In summary, the ATF3 homodimer has been demonstrated to be a transcriptional repressor, and thus far no evidence indicates that ATF3 by itself is an activator (on promoters with its binding sites). However, ATF3 can activate transcripiton when coexpressed with its heterodimeric partners, or other proteins. We emphasize that all the above observations were made from transient transfection or in vitro transcription experiments. Because of the limitations of these assays, the data should be interpreted with the caveat that they may not reflect the "true" in vivo situation.

One important question for studying any transcription factor is "What are the target promoters?" Currently, two promoters have been identified to be "potential" targets for ATF3: gadd153 (135) and ATF3 itself (137). The fact that ATF3 can repress its own

promoter explains, at least in part, the transient nature of its induction. Autorepression of gene expression has been reported previously for other transcription factors, such as c-Fos (113) and ICER (94). Therefore, it is not surprising that ATF3 may repress its own promoter. However, because the results were obtained by transient transfection assays, the same caveat discussed above should be applied. Currently, biologically important target promoters for ATF3 during stress responses have not been identified. As described above, ATF3 has been demonstrated to either activate or repress transcription. It is possible that, when induced during stress responses, ATF3 activates some target genes but represses others, depending on the promoter context and cellular context.

The Physiological Significance of Inducing ATF3 During Stress Responses

As discussed above, induction of ATF3 is a part of the cellular stress responses. However, it is not clear whether it is a beneficial stress response (such as heat shock response) or a detrimental stress response (such as inflammatory response). As a first step toward addressing this issue, we took an ectopic expression approach and made stable cell lines expressing ATF3 under the tetracycline regulatable promoter (136). Our results indicate that expression of ATF3 leads to defects in nuclear export (136), implying a detrimental effect. We also generated transgenic flies (in collaboration with Dr. H. Vaessin) and transgenic mice to ectopically express ATF3; our preliminary results are consistent with the notion that expression of ATF3 is detrimental. One intrinsic problem of the ectopic expression approach is that the gene in question is expressed out of the context. In the case of stress responses, many genes, in addition to ATF3, are induced. In the presence of other gene products, expression of ATF3 may have different effects. Genes with context-dependent functions have been demonstrated. As an example, ectopic expression of oncogene E1A leads to apoptosis, a phenomenon referred to as "oncogenic killing" [review in (30)]; however, if the function of p53 is abrogated by ectopically coexpressing E1B, E1A can transform cells [review in (30,115)]. Similar dichotomous functions have been demonstrated for oncogenes E2F and Myc [review in (30,115)].

One lesson these studies revealed is that, despite its intrinsic problem, the ectopic expression approach is valuable: it can reveal important (albeit incomplete) pictures for the gene function under examination. The detrimental effects we observed in the cell lines and transgenic animals ectopically expressing ATF3 most likely only reflect one facet of ATF3 function; a more complete picture needs further investigation from different approaches. In this context, we note the following observations. First, ATF3/c-Jun dimer has been suggested to play a role in cell cycle progression (51,123) and E1A-mediated transformation (46), a notion based on the observation that ATF3/c-Jun expression correlates with cell cycle progression and E1A-mediated transformation. Second, ATF3 was isolated from B16 mouse melanoma cells as a gene differentially expressed in the high-metastatic sublines. Significantly, introducing ATF3 into low-metastatic sublines convert them to high-metastatic cells, implying "positive" effects of ATF3 on metastasis (58). Sequence analyses indicate that the cDNA isolated from the high-metastatic sublines encodes the wild-type ATF3 protein, excluding the possibility that the observed effect is due to a mutant version of ATF3. Therefore, ATF3 may play a role in regulating cell cycle machinery. These observations combined with our results (which suggest a detrimental effect of ATF3) are reminiscent of the "oncogenic killing" phenomenon described above—ectopic expression of a gene that triggers the cell cycle machinery leads to detrimental consequences.

CONCLUSIONS AND PERSPECTIVES

Since the first isolation of ATF3 cDNA a decade ago, overwhelming evidence indicates that ATF3 is induced by many stress signals. However, not much else is known about ATF3. Because of the varieties of signals that can induce ATF3, it is important to elucidate the signaling pathways involved in the induction. In light of the multiplicity and redundancy in many induction processes, it is almost certain that induction of ATF3 is regulated by complex mechanisms, involving multiple signaling pathways in signal- and cell type-dependent manners. Clearly, our understanding of the physiological significance of inducing ATF3 during stress responses is rudimentary. Because ATF3 is a transcription factor, in order to understand the molecular mechanisms and "sequences of events" for its actions, it is necessary to elucidate the target promoters of ATF3. However, even with some target genes in hand, it may still be difficult to sort out whether induction of ATF3 is a beneficial or detrimental stress response. The complexity of genes induced during stress responses makes it a challenge to address the effects/consequences of inducing ATF3 during stress responses. However, understanding these effects/consequences will be a significant step toward our understanding of gene regulation during stress responses.

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