ATF2 – at the crossroad of nuclear and cytosolic functions

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Journal of Cell Science 125, 1–10
© 2012. Published by The Company of Biologists Ltd
doi: 10.1242/jcs.095000

Summary
An increasing number of transcription factors have been shown to elicit oncogenic and tumor suppressor activities, depending on the tissue and cell context. Activating transcription factor 2 (ATF2; also known as cAMP-dependent transcription factor ATF-2) has oncogenic activities in melanoma and tumor suppressor activities in non-malignant skin tumors and breast cancer. Recent work has shown that the opposing functions of ATF2 are associated with its subcellular localization. In the nucleus, ATF2 contributes to global transcription and the DNA damage response, in addition to specific transcriptional activities that are related to cell development, proliferation and death. ATF2 can also translocate to the cytosol, primarily following exposure to severe genotoxic stress, where it impairs mitochondrial membrane potential and promotes mitochondrial-based cell death. Notably, phosphorylation of ATF2 by the epsilon isoform of protein kinase C (PKCe) is the master switch that controls its subcellular localization and function. Here, we summarize our current understanding of the regulation and function of ATF2 in both subcellular compartments. This mechanism of control of a non-genetically modified transcription factor represents a novel paradigm for ‘oncogene addiction’.

Key words: ATF2, Transcription, DNA damage, Mitochondria, Melanoma, Skin cancer, JNK, p38 MAPK, PKC

Introduction
The activating transcription factor (ATF; also known as cAMP-dependent transcription factor) and cAMP-response-element-binding (CREB) families of transcription factors comprise 16 members of the activator protein 1 (AP1) transcription factor superfamily. ATF and CREB proteins can homodimerize or heterodimerize with members of the Jun, Fos or Maf transcription factor families to form complexes that regulate diverse cellular functions, such as stress responses, embryonic development, disease development and cell death (Lopez-Bergami et al., 2010). ATF2 requires phosphorylation by Jun N-terminal kinase (JNK), p38 (MAPK14), or extracellular-signal-regulated kinase 1 (ERK1) in order to be transcriptionally active. After its activation following stress and cytokine stimuli, ATF2 contributes to the cellular responses to hypoxic or osmotic stress, DNA damage, viral infection and cell death (Bhoumik et al., 2007; Choi et al., 2009; Merika et al., 1998; Wang et al., 2011). The precise transcriptional output of ATF2 is dictated by its dimerization partners, which are predominantly members of the AP-1 family. The importance of the transcriptional activity of ATF2 has been demonstrated in organismal development of several genetic models (see Box 1).

Although there are no reports of genetic changes in ATF2, altered ATF2 expression and/or activity have been implicated in several pathological conditions, including neurological diseases and cancer (Chen et al., 2008; Pearson et al., 2005; Reimold et al., 1996; Yamada et al., 1997). Intriguingly, ATF2 can elicit oncogenic or tumor suppressor activities depending on the tissue or cell type; earlier work has associated these functions with its nuclear or cytoplasmic localization (Berger et al., 2003; Bhoumik et al., 2008a). Although the mechanisms underlying these opposing activities are being elucidated, recent studies reveal that ATF2 also has transcription-independent functions in the DNA damage response, chromatin remodeling and mitochondrial membrane organization, thereby highlighting the diverse location-dependent functions of this protein (Bhoumik et al., 2008b; Bhoumik et al., 2005; Cho et al., 2001; Lau et al., 2012). In this Commentary, we summarize our current understanding of the functions of ATF2 and the link between its subcellular localization and oncogenic or tumor suppressor activities.

Regulation of ATF2
The ATF2 gene is located on chromosome 2q32 and encodes a 505-amino-acid protein, which is ubiquitously expressed, with more abundant expression in the brain (Kara et al., 1990; Takeda et al., 1991). Although the existence of numerous truncated ATF2 isoforms is predicted on the basis of splice variation (Box 2), only full-length ATF2 has been extensively studied to date. Similar to other AP-1 transcription factors, the ATF2 protein contains a basic leucine zipper (bZIP) domain within its C-terminus (amino acids 350–414) that enables homo- or hetero-dimerization. The bZIP domain contains nuclear localization and export sequences that facilitate trafficking of ATF2 to and from the nucleus; the latter function is regulated by exportin-1 (Liu et al., 2006). ATF2 also contains an N-terminal zinc finger region and a transactivation domain, which regulates its transcriptional activity (Nagadoi et al., 1999) through an intramolecular autoinhibitory interaction (Li and Green, 1996). Phosphorylation of the N-terminal residues Thr69 and Thr71 in response to mitogenic or stress signals is required to relieve this intramolecular interaction, which enables ATF2 dimerization and subsequent transcriptional activity (Gupta et al., 1995; Li and Green, 1996; Livingstone et al., 1995).
Box 1. Loss of ATF2 function in murine models

A number of somatic and tissue-specific knockout mouse models have been used to examine the effects of loss of ATF2 function. The targeted disruption of the Atf2 gene, inducing complete somatic loss of ATF2, results in postnatal lethality that is associated with severe respiratory defects and meconium aspiration syndrome (Ackermann et al., 2011; Maekawa et al., 1999). In this murine model, the reduced levels of PDSFp2 protein and cytrophoblast cell populations that are detected in the knockout placenta are attributed to neonatal respiratory distress and lethality. Another murine model, which targets the disruption of the Atf2 gene by the introduction of a mutation at position +826 relative to the transcription start site, produces similar postnatal lethality. Detailed characterization of mutant pups found that they exhibited severe neurological and skeletal defects, including a 50% loss of Perkinje cell populations in the cerebellar molecular-granular layer, overt skeletal dwarfism and hypochondroplasia (Reimold et al., 1996). These findings demonstrate the crucial role that ATF2 plays in development and highlight its particular functional involvement in pleural, neuronal and skeletal tissues. These phenotypes appear to be specific for ATF2, despite the fact that ATF2 requires dimerization with other AP1 transcription factors, such as Jun, for transcriptional activities [note: somatic loss of Jun in knockout mice results in distinctly different consequences, including midgestational lethality and specific impaired hepatogenesis (Hilberg et al., 1993; Johnson et al., 1993)]. A transgenic mouse expressing ATF2 where Thr69 or Thr71 (the required phosphorylation sites for transcriptional activation) is replaced by an alanine residue exhibits a similar lethality and cyanotic phenotype to the somatic knockout model (Maekawa et al., 1999), indicating that phosphorylation and transcriptional activation of ATF2 is also crucial for viability and development (Breitwieser et al., 2007).

Tissue-specific ablation of Atf2 has illustrated the requirements for ATF2 in individual tissue types. Neuronal-specific deletion of Atf2, mediated by nestin-driven Cre, results in embryonic cranial motor neuron degeneration, specifically in the hypoglossal, abducens and facial nuclei regions (Ackermann et al., 2011). In terms of disease models, work from our laboratory has demonstrated that the melanocyte-specific expression of a transcriptionally inactive ATF2, mediated by tyrosinase-driven Cre, is sufficient to block melanoma development in the NrasG12D; Ink4A−/− model (Ink4A is also known as Cdkn2a) murine melanoma model (Shah et al., 2010). By contrast, expression of transcriptionally inactive ATF2 in the keratinocytes of the DMBA-TPA-induced [7,12-dimethylbenz(a)anthracene and 12-O-tetradecanoylphorbol-13-acetate, respectively] murine skin cancer model increases the number and size of skin papilloma, as well as their formation rate (Bhoumik et al., 2008a). Expression of transcriptionally inactive ATF2 in mammary tissues bearing a mutant p53 also accelerates tumor development (Maekawa et al., 2007).

Post-translational regulation of ATF2

A number of upstream kinases activate ATF2 by direct phosphorylation, including mitogen-activated protein kinases (MAPks), such as ERK1, and stress-activated protein kinases (SAPks) (Fig. 1). Correspondingly, the two major kinases that phosphorylate ATF2 on Thr69 and Thr71 are the SAPks JNK and p38 (Gupta et al., 1995; Livingstone et al., 1995; van Dam et al., 1995). A role for the MAPK ERK in Thr71 phosphorylation of ATF2 has also been proposed (Ouweens et al., 2002). Phosphorylation of ATF2 by these kinases occurs within minutes of the stress stimulus, rendering ATF2 an ‘early response’ protein. Later on in the stress response, ATF2 can be phosphorylated on Ser121 by several protein kinase C (PKC) isoforms (including α, βI, βIII and γ) (Yamasaki et al., 2009), which promotes cooperation between ATF2 and Jun, activating transcription. Recently, our laboratory reported that PKCε phosphorylates ATF2 on Thr52, which promotes its nuclear retention and transcriptional activity (Lau et al., 2012). ATF2 is also phosphorylated by ataxia-telangiectasia mutated (ATM) kinase (Fig. 1), which mediates its transcription-independent role in the DNA damage response (Bhoumik et al., 2005).

In addition to being phosphorylated, ATF2 is also acetylated on Lys357 and Lys374 by p300/CREB-binding protein (CBP, also known as CREBBP), which contributes to its transcriptional activity (Karanam et al., 2007). Binding of ATF2 suppresses the acetyltransferase activity of the transcriptional coactivator p300/CBP. Notably, the relationship between acetylation and phosphorylation of ATF2, in the context of its transcriptional activities, has yet to be elucidated.

Regulation of ATF2 transcription

Little is known of the transcriptional control of ATF2. The putative minimal ATF2 promoter is composed of one cyclic AMP response element (CRE) and three Sp1 elements located between positions –50 and +90 relative to the transcription start site (Nagase et al., 1990). To date, these elements have been associated with the activities of E2F4 transcription factor and biliverdin IXα reductase (Cam et al., 2004; Kравets et al., 2004). Further scanning of the promoter using the ECR Browser (http://ecrbrowser.dcode.org/) reveals the presence of conserved regulatory elements that are potentially recognized by transcription factors, such as AP2α, AP4, the Sox family members 1, 5 and 9, androgen receptor, and transcriptional coactivator CCAT/enhancer-binding protein (C/EBP), which suggests that ATF2 transcription is likely to be regulated by diverse signaling pathways and to be dependent on cellular context. ATF2 protein expression increases as cells transit from G1 through the S phase of the cell cycle (Shimizu et al., 1998). Dynamic temporal and spatial regulation of ATF2 expression has been demonstrated during blastulation and gastrulation of Xenopus laevis (Villareal and Richter, 1995). Transcription of ATF2 is also induced by viral proteins, as has been shown for the Epstein–Barr viral nuclear antigen 1 (EBNA1) (O’Neil et al., 2008). EBNA1 binds to the ATF2 promoter and induces transcription as part of a pro-angiogenic transcription program in nasopharyngeal carcinoma cells. The stability of ATF2 mRNA transcripts is also regulated. Binding of the cytoplasmic RNA-binding protein HuR to the 3′-untranslated region (UTR) stabilizes ATF2 mRNA, whereas intracellular polyamines destabilize ATF2 mRNA (Xiao et al., 2007). ATF2 transcript levels are also negatively regulated by the microRNA miR-26b in lung cancer cells, and this suppression is relieved by ionizing radiation (Arora et al., 2011). Alternative splicing has also been predicted to result in over a dozen of ATF2 splice forms (Box 2).

Regulation of ATF2 protein stability

The stability of ATF2 protein is regulated by ubiquitylation and proteasomal degradation. Previous work from our laboratory has demonstrated that N-terminal phosphorylation and heterodimerization of ATF2 reduces its transcriptional activity by promoting ubiquitylation-dependent degradation (Fuchs and Ronai, 1999). Binding of JNK to ATF2 under non-stressed conditions serves to limit the availability of ATF2 by promoting its degradation under conditions in which it is not required (Fuchs et al., 1997). However, the E3 ubiquitin ligases that are involved in
the ubiquitylation and degradation of ATF2 have not yet been identified. The SUMO-conjugating enzyme Ubc9 has been shown to interact with ATF2 and to affect ATF2 stability (Firestein and Feuerstein, 1998), although ATF2 SUMOylation has not been formally shown.

**Nucleolar functions of ATF2**

**Transcriptional roles of ATF2**

ATF2 homodimers display poor transcriptional activity, and thus heterodimerization of ATF2 is essential for its transcriptional function. Depending on the heterodimeric partner, ATF2 binds to different response elements on target genes and elicits distinct transcriptional programs. For example, ATF2–CREB or ATF2–Jun complexes predominantly exhibit DNA-binding specificity for the eight-base CRE 5′-TGACGTCA-3′ (Hai et al., 1989). ATF2 also binds to other promoter sequences on its target genes, including the interferon (IFN)-γ promoter (i.e. 5′-AAAACTTGTGAAATTAGCTAATCCTT-3′), the stress-response elements (StREs; 5′-CCGGTGGAGTGCA-3′) and UV response elements (URE; 5′-TGACACAAC-3′) (Gong et al., 2002; Lopez-Bergami et al., 2010; Ronai et al., 1998; van Dam and Castellazzi, 2001). Binding of ATF2–AP-1 dimers to DNA alters the local structure of DNA and facilitates the recruitment and directional orientation of other regulatory transcriptional complexes, which either enhance (i.e. enhanceosomes) or repress (i.e. repressosomes) transcription. Such a coordinated transcriptional regulation is illustrated by the ATF2–Jun-mediated enhanceosome assembly at the promoter of IFN-β (IFNB1) (Falvo et al., 2000; Falvo et al., 1995). Conversely, binding of ATF2 to the histone acetyltransferase TIP49b effectively suppresses its transcriptional activity (Cho et al., 2001). ATF2–JunB dimers bind to the SOX10 promoter and suppress its transcription, with concomitant silencing of microphthalmia-associated transcription factor (MITF) transcription in melanocytes and melanoma cells (Shah et al., 2010). In Table 1, we have categorized the known transcriptional targets of ATF2 into functional groups, along with the respective AP1 family binding partner, case-specific stimuli and cell types (see also the UCSD Signaling Gateway Molecule Pages for ATF2; http://www.signaling-gateway.org/molecule/query?afcsid=A000347).

ATF2 can also affect transcription of target genes in trans through its interaction with other transcription factors. In hypoxia, for example, ATF2 binds and stabilizes hypoxia-inducible factor 1α (HIF1α), thereby promoting its transcriptional activity (Choi et al., 2009). ATF2–MaFA dimers provide another example of transactivation; together they contribute to the induction of insulin transcription in concert with the binding of Pdx1 and B cell E box transactivator 2 (Beta2, also known as TCF3) to adjacent A-box elements on the insulin promoter (Han et al., 2011).

The transcriptional function of ATF2 is also modulated by its interaction with transcriptional coactivators or corepressors. For example, p300/ CBP and C/EBPβ bind to the bZIP domain of ATF2, disrupting its intrinsic autoinhibition and augmenting its transcriptional activity (Duyndam et al., 1999; Shuman et al., 1997). This mechanism is exploited by pathogens as a means to activate transcription. For example, the viral proteins E1A and Epstein–Barr viral nuclear antigen-2 (EBNA2) enhance ATF2 transcriptional output by promoting its heterodimerization with either CREB or Jun (Abdel-Hafiz et al., 1993; Hagmeyer et al., 1995). Other viral transactivators, such as Epstein–Barr BZLF1 and BRLF1, enhance ATF2 transcriptional activities indirectly through their effect on the ATF2-phosphorylating kinases JNK or p38, whereas human vaccinia-related kinase 1 (VRK1) activates and stabilizes ATF2 through direct phosphorylation of Ser62 and Thr73 (Sevilla et al., 2004).

ATF2 also affects more global transcriptional programs through its association with histone modifying enzymes. For instance, the fission yeast ATF2 homologs, Atfl and Per1, are essential for accurate histone H3 or H4 deacetylation and Swi6-mediated heterochromatin assembly in *Schizosaccharomyces pombe*, and loss of Atfl or Per1 results in loss of heterochromatin silencing (Jia et al., 2004; Kim et al., 2004). During amino acid deprivation, the recruitment of ATF2 to the amino acid response element (AARE; 5′-ATTGCATCA-3′) is required for subsequent acetylation of histones H4 and H2B (Bruhat et al., 2007). ATF2 recruitment of the repressive
macroH2A histone variant to the IL8 promoter silences IL8 transcription in B cells (Agelopoulos and Thanos, 2006). Interestingly, ATF2 has been shown to affect global heterochromatin organization in Drosophila, and disruption of the ATF2–chromatin interaction in response to stress results in heritable heterochromatin defects that are maintained in subsequent generations (Seong et al., 2011). Thus, ATF2 not only controls the transcription of its specific target genes through direct binding to DNA promoter elements, but also has a key regulatory role in chromatin restructuring through its interaction with chromatin-modifying proteins.

**Non-transcriptional functions in the nucleus**

ATF2 also exhibits nuclear functions that are distinct from its transcriptional activity, including its involvement in chromatin restructuring and the DNA damage response. Our earlier studies showed that ATM phosphorylates ATF2 on Ser490 and Ser498 in response to DNA damage, which promotes its colocalization with components of the MRE11–RAD50–NBS1 (MRN) complex in ionizing radiation-induced foci (IRIF) (Bhoumik et al., 2005). Mutation of human ATF2 Ser490 or 498 to alanine abrogates this phosphorylation event and perturbs IRIF formation and subsequent DNA repair. ATF2 phosphorylation by ATM also contributes to the intra-S phase checkpoint, which enables proper repair of damaged DNA (Bhoumik et al., 2005). ATF2 knock-in mice, in which the sites in mouse ATF2 that correspond to amino acids 490 and 498 were mutated to alanines, exhibit genomic instability reflected in greater susceptibility to develop tumors and greater sensitivity to ionizing radiation (Li et al., 2010). In this context, ATF2 interaction with the histone acetyltransferase Tip60 constitutes a positive feed-back loop mechanism through which ATF2 promotes ATM activities. Genotoxic stress attenuates the interaction between Tip60 and ATF2, which stabilizes Tip60 and promotes the subsequent acetylation and activation of ATM (Bhoumik et al., 2008b).

**Functions of ATF2 in the cytoplasm**

**Cytoplasmic accumulation of ATF2**

Although it has been known for some time that ATF2 can also be found in the cytoplasm (Berger et al., 2003), its specific function there remains unclear. Exportin-1 facilitates the nuclear export of ATF2, although the precise mechanisms that control the nucleocytoplasmic trafficking of ATF2 are unknown (Liu et al., 2006). Notably, a cytoplasmic splice isoform of ATF7, ATF7-4, was recently found to inhibit the phosphorylation and activation of both ATF7 and ATF2 (Diring et al., 2011).

Localization of ATF2 to the cytoplasm has been observed under conditions of cellular stress and in disease states (Berger et al., 2003; Deng et al., 2011). Cytoplasmic accumulation of ATF2 has been detected in the degenerating hippocampal regions and cortical neurons of patients with neurodegenerative diseases such as Alzheimer’s, Parkinson’s and Huntington’s disease (Pearson et al., 2005; Yamada et al., 1997). Consistent with these observations, in vivo and in vitro studies have shown that neuronal injury by nerve fiber transection or doxorubicin treatment is accompanied with loss of nuclear ATF2 (Martin-Villalba et al., 1998). ATF2 also accumulates in the cytoplasm of prostate cancer cells after treatment with ionizing irradiation (Deng et al., 2008). Furthermore, melanoma cell death is observed after forced expression of N-terminal ATF2 peptides that induce cytoplasmic accumulation and thereby reduce the transcriptional activity of endogenous ATF2 (Bhoumik et al., 2004). Collectively, these observations point to stress- or damage-induced cytosolic localization of ATF2, which is associated with cell death.

Analyses of tumor microarrays have revealed the principal differences between melanoma and non-malignant skin cancers. Whereas the nuclear enrichment of ATF2 correlates with poor prognosis in melanoma, cytoplasmic ATF2 is associated with a more favorable clinical outcome (Berger et al., 2003). Notably, cytosolic localization of ATF2 is also seen in non-malignant skin tumors, e.g. squamous and basal cell carcinomas (SCCs and BCCs, respectively) (Bhoumik et al., 2008a). Hence, the nuclear accumulation of ATF2 appears to be associated with its oncogenic activities, because this localization is observed in melanoma (Shah et al., 2010), whereas the cytosolic localization, as are observed in non-malignant skin tumors (Bhoumik et al., 2008a), is associated with its tumor suppressor activities. Consistent with this, the cytosolic accumulation of ATF2 that is observed in prostate cancer cells following ionizing irradiation has been associated with a transient latency of tumor proliferation and a more ‘differentiated’ state (Deng et al., 2008).
<table>
<thead>
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<th>API binding partner</th>
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<th>Transcriptional regulation</th>
<th>Target gene (encoded protein)</th>
<th>References</th>
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<td><strong>Cell cycle</strong></td>
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<tr>
<td>ATF2, JunD</td>
<td>Rat chondrosarcoma cells, MEFs</td>
<td>Serum</td>
<td>T1</td>
<td>Cdc2 (cyclin A1)</td>
<td>(Shimizu et al., 1998)</td>
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<td>BRCA1, Oct-1, Neurofibromin-1</td>
<td>MEFs</td>
<td>Anisomycin, hypoxia</td>
<td>T1</td>
<td>Gadd45b, Gadd45a</td>
<td>(Maková et al., 2008; Makova et al., 2007)</td>
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<tr>
<td>BRCA1, Oct-1, Neurofibromin-1</td>
<td>MEFs</td>
<td>Anisomycin, hypoxia</td>
<td>T1</td>
<td>Senp3b (maspin)</td>
<td>(Makova et al., 2008; Makova et al., 2007)</td>
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<td>CREB1</td>
<td>Murine chondrocytes</td>
<td>TGFβ, PD98059</td>
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<td>Ccnd1 (cyclin D1)</td>
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<td>JunD</td>
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<td>TR</td>
<td>Cdk4 (Cdk4)</td>
<td>( Xiao et al., 2010)</td>
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<td>n/a</td>
<td>Murine chondrocytes</td>
<td>n/a</td>
<td>T1</td>
<td>Jh1 (RbE)</td>
<td>(Vitez-Cruz et al., 2008)</td>
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<tr>
<td><strong>Immune and inflammatory</strong></td>
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<td>Jun</td>
<td>Endothelial (HUVEC)</td>
<td>UV</td>
<td>T1</td>
<td>SEL3 (Slam1)</td>
<td>(Baud et al., 1997)</td>
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<td>CREB1</td>
<td>Myeloid leukemia (K562)</td>
<td>Sodium butyrate, troglitazone A</td>
<td>T1</td>
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<td>Jun</td>
<td>T-cells (Jurkat)</td>
<td>Ionomycin, PMA</td>
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<td>IFNg (interferon-γ)</td>
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<td>Jun</td>
<td>Murine macrophages (RAW264.7)</td>
<td>LPS</td>
<td>T1</td>
<td>Il1α, Il1β (interleukin-1α, β)</td>
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<td>Jun</td>
<td>Human primary lung and foreskin fibroblasts</td>
<td>Interferon-β</td>
<td>T1</td>
<td>Il6 (interleukin-6)</td>
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<td>PDGF</td>
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<td>Il6 (interleukin-6)</td>
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<td><strong>Cell death</strong></td>
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<td>Transformed human embryonic kidney (293T)</td>
<td>Oxidative stress (H2O2)</td>
<td>T1</td>
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<td>Jun</td>
<td>Endothelial (HUVECs)</td>
<td>Growth factors (VEGF, EGF)</td>
<td>T1</td>
<td>BCL2L1 (Bcl-XL)</td>
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<td>Rat condylar cell granule neurons (CGN), murine immortalized ganglion cell line (oT3-1)</td>
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<td>T1</td>
<td>Htrk (DP)</td>
<td>(Ma et al., 2007; Torres et al., 2009)</td>
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<td>JDP2</td>
<td>HeLa, HEK293, MEFs</td>
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<td>TR</td>
<td>DNT13 (CHOP)</td>
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<td><strong>DUSPs</strong></td>
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<td>Jun</td>
<td>Embryonal carcinoma (F9), rat condylar cell granule neurons, monkey kidney epithelial (COS1), human cervical carcinoma (HeLa, SKOV), NIH3T3, hepatocellular carcinoma (HepG2, HuH7)</td>
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<td>T1</td>
<td>JUN (Jun)</td>
<td>(Fu et al., 2001; Kawasaki et al., 1998; Yamashita et al., 2009)</td>
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<td>JDP2</td>
<td>Embryonal carcinoma (F9)</td>
<td>RA-induced differentiation</td>
<td>TR</td>
<td>JUN (Jun)</td>
<td>(Jin et al., 2002)</td>
</tr>
<tr>
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<td>Normal fibroblasts, HeLa, HeK293, MEFs</td>
<td>IR, amino acids, potassium, genistein-refractory hormone (GmRH), tetracycline acid (TA)</td>
<td>T1</td>
<td>ATP4 (ATF3)</td>
<td>(Chavereon et al., 2009; Fu et al., 2011; Kool et al., 2003; Lee et al., 2010; Mayer et al., 2004)</td>
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<td><strong>Lmx1a, Pdx1, Bet2</strong></td>
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<td>Jun</td>
<td>Embryonic liver, sympathetic neurons</td>
<td>p30 feedback signaling, NGF withdrawal</td>
<td>T1</td>
<td>Dusp5 (MKP1)</td>
<td>(Breitwieser et al., 2007; Kristiansen et al., 2010)</td>
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<td>n/a</td>
<td>Murine fetal hepatocytes</td>
<td>Antisense</td>
<td>T1</td>
<td>Dusp4 (BBS)</td>
<td>(Breitwieser et al., 2007)</td>
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<td>T1</td>
<td>Dusp5 (MKB)</td>
<td>(Breitwieser et al., 2007)</td>
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<td>Jun</td>
<td>Murine embryonic stem cells</td>
<td>FGF2</td>
<td>T1</td>
<td>Hes1 (HES1)</td>
<td>(Sanillaarn et al., 2010)</td>
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<td>Jun</td>
<td>Rat aortic endothelial cells</td>
<td>Thrombin</td>
<td>T1</td>
<td>Arg1 (arginase)</td>
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<td>Rat osteosarcoma (ROS17/2.8, ROS252), primary murine calvarial osteoblast (MCO)</td>
<td></td>
<td>T1</td>
<td>Col2A1 (COL2A1)</td>
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<td>Murine lymphocytic leukemia cells (L1210)</td>
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<td>T1</td>
<td>Pykd (PKCδ)</td>
<td>(Min et al., 2008)</td>
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<td>Jun, JunD, ATF2</td>
<td>NB1373, HEK293</td>
<td>TPA, FGF2</td>
<td>T1</td>
<td>FLAV (flavin)</td>
<td>(Cirillo et al., 1999; D’Onadio et al., 1997)</td>
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<td>Murine endothelial and endothelial-derived cells</td>
<td>CoCl2, hypoxia</td>
<td>T1</td>
<td>Cbfβ (Cbfβ)</td>
<td>(Lich et al., 2006)</td>
</tr>
<tr>
<td>n/a</td>
<td>Murine macrophages (RAW264.7)</td>
<td>LPS</td>
<td>T1</td>
<td>Socs3 (SOCS3)</td>
<td>(Hirao et al., 2009)</td>
</tr>
<tr>
<td>MaF/A, Pbx1, Bet2</td>
<td>Forkolin, UV</td>
<td></td>
<td>T1</td>
<td>Bsl1 (fusulin)</td>
<td>(Han et al., 2011; Hay et al., 2007)</td>
</tr>
<tr>
<td>n/a</td>
<td>Choriocarcinoma cells (Jar, CHO)</td>
<td>Hypoxia</td>
<td>T1</td>
<td>Pdgfr (PDGFRα)</td>
<td>(Makova et al., 1999)</td>
</tr>
<tr>
<td>n/a</td>
<td>Long epithelial cells</td>
<td>Rb expression</td>
<td>T1</td>
<td>Fgf2 (FGF2)</td>
<td>(Kim et al., 1992)</td>
</tr>
<tr>
<td>n/a</td>
<td>Mucoblasts (C2C12)</td>
<td>Exosome</td>
<td>T1</td>
<td>Lgr6 (LGFRα)</td>
<td>(Altschuler et al., 2005)</td>
</tr>
<tr>
<td>n/a</td>
<td>Vascular smooth muscle cells</td>
<td>TGFB</td>
<td>T1</td>
<td>Ctgf (CTGF)</td>
<td>(Lin et al., 2008)</td>
</tr>
<tr>
<td>n/a</td>
<td>Retinal pigment epithelial cells</td>
<td>Valproic acid</td>
<td>T1</td>
<td>Sglul (SGLUL)</td>
<td>(Song et al., 2011)</td>
</tr>
<tr>
<td>NF-YA</td>
<td>Jurkat cells</td>
<td>UV</td>
<td>T1</td>
<td>Hhob (RhoB)</td>
<td>(Ahn et al., 2011; Fritz and Kaina, 2003)</td>
</tr>
<tr>
<td>n/a</td>
<td>Brown adipocytes</td>
<td>Nicotinic receptor</td>
<td>T1</td>
<td>Fgf2 (FGF2)</td>
<td>(Hondares et al., 2011)</td>
</tr>
<tr>
<td>n/a</td>
<td>Rat adrenal medulla-derived cells (PC12)</td>
<td>Nicotinic</td>
<td>T1</td>
<td>Tr (tyrosine hydroxylase)</td>
<td>(Guergiuev et al., 2006; Suzuki et al., 2003)</td>
</tr>
<tr>
<td>NFAT, Jun</td>
<td>Dendritic cells, monocytic leukemia cells (THP1)</td>
<td>TLR2 ligation</td>
<td>T1</td>
<td>TNFα, NFκB</td>
<td>(Altunay et al., 2010; Kusmato et al., 2010; Lawrence et al., 2011)</td>
</tr>
<tr>
<td>n/a</td>
<td>Adrenocortical carcinoma cells (H295R)</td>
<td>Angiotensin II, K+</td>
<td>T1</td>
<td>CTGF (CTGF)</td>
<td>(Nagae and Rane, 2010)</td>
</tr>
<tr>
<td>n/a</td>
<td>Marine macrophages (RAW)</td>
<td>cAMP</td>
<td>T1</td>
<td>Tgase1/2 (thiols acid)</td>
<td>(Liao et al., 2010)</td>
</tr>
<tr>
<td>n/a</td>
<td>Mouse A91 cells</td>
<td>TNFα, MG132</td>
<td>TR</td>
<td>Socs3 (SOCS3)</td>
<td>(Desh et al., 2011)</td>
</tr>
</tbody>
</table>

Known transcriptional targets of ATF2 are displayed by functional groups as follows: cell cycle, immune and inflammatory, cell death, API1, dual-specificity phosphatases (DUSPs) and others. Each target gene is listed with associated API1 partner, cell type and stimulus, if known. The nature of transcriptional regulation by ATF2 for each of these targets is indicated as transcriptional induction (T1, green), or transcriptional repression (TR, pink).
**ATF2 localization at the mitochondria following genotoxic stress**

The finding that ATF2 is localized in the cytoplasm in non-malignant skin tumors prompted us to investigate the possibility that ATF2 harbors a cytosolic function. In SCC cells, genotoxic stress induces a fraction of nuclear ATF2 to translocate to the cytoplasm within ~8–24 hours, where it localizes at the mitochondrial outer membrane (Lau et al., 2012). This nuclear export coincides with reduced transcriptional activity of ATF2. We used mass spectrometry to analyze ATF2-associated proteins within the cytoplasm, and identified a cluster of mitochondrial proteins that included hexokinase 1 (HK1) and voltage-dependent anion channel 1 (VDAC1) (Lau et al., 2012). Complexes of HK1 and VDAC1 have been associated with mitochondrial membrane pore permeability, and disruption of these complexes is often observed in response to cellular stress that induces apoptosis, including genotoxic stimuli (Abu-Hamad et al., 2008; Shoshan-Barmatz et al., 2009). These disruptions result in impaired mitochondrial membrane potential with concomitant leakage from the mitochondria – hallmarks of mitochondrial-dependent cell death. We have shown that ATF2 is part of the HK1–VDAC1 complex both by immunostaining and biochemical analysis, and mobilization of ATF2 to mitochondria results in decreased HK1 binding to VDAC1 (Lau et al., 2012). ATF2 recruitment to the mitochondria is also associated with reduced membrane potential, activation of the pro-apoptotic Bcl-2 family protein BAX, leakage of cytochrome c and sensitization of cells to genotoxic-stress-induced cell death. Collectively, our recent studies reveal a new function for cytoplasmic ATF2 in promoting mitochondrial-based cell death following exposure to genotoxic stress (Lau et al., 2012).

The nuclear localization and mitochondrial function of ATF2 are dependent on PKCε. The nuclear export of ATF2, which enables its localization and function at the mitochondria, has been observed following genotoxic stimuli in both non-malignant (keratinocytes, melanocytes and fibroblasts) and malignant (BCC and early-phase melanoma) cells (Lau et al., 2012). Significantly, an exclusion of ATF2 from the nucleus is not observed in the more aggressive melanoma cells, which prevents ATF2 from functioning at the mitochondria in these cells (Lau et al., 2012). The control of ATF2 nuclear export is lost in progressively malignant melanoma cells. In particular, we found that PKCε-mediated phosphorylation of ATF2 on Thr52 is required for its nuclear localization and must be attenuated to allow its nuclear export and localization to the mitochondria. Phosphorylation of ATF2 on Thr52 is reduced following genotoxic stress in most cells tested. However, this is not the case in melanomas, in which the expression and activity of PKCε are markedly higher and ATF2 phosphorylation on Thr52 is thus maintained (Lau et al., 2012). It is plausible that the mechanism identified in melanoma will be of relevance to a number of other tumors that have a high level of PKCε.

**Interplay between cytosolic and nuclear ATF2 – a paradigm for oncogenic addiction**

‘Oncogene addiction’ is a phenomenon whereby key cancer cell phenotypes are driven by an activated oncogene. Turning off the oncogenic signal often results in cell death, illustrating the addiction. To date, oncogenic addiction has been largely associated with genomic mutations that commit signaling pathways towards maintaining or further promoting transformed phenotypes (i.e. metastasis). Once the oncogenic pressure is relieved (by targeted inhibition of the gene or pathway), the transformed phenotype is partially or fully alleviated. Although the mechanism underlying the upregulation of PKCε in melanoma is not yet known, it is probable that it is linked to mutation(s) currently being discovered as part of the effort to map the melanoma genome (Dutton-Regester and Hayward, 2012; Walia et al., 2012). In the case of ATF2, PKCε functions as the addicting signal to maintain nuclear localization of ATF2 and thus prevents its pro-apoptotic function at the mitochondria. Thus, as long as PKCε activity is sustained, ATF2 will exhibit oncogenic functions within the nucleus. ATF2 addiction to PKCε therefore establishes a new paradigm for oncogenic addiction, which is likely to be relevant to other transcription factors that are able to elicit both oncogenic and tumor suppressor activities.

**Future perspectives**

A growing number of transcription-dependent and -independent functions have been described for ATF2, while attesting to its capacity to regulate diverse and often opposing functions, a number of questions remain to be addressed.

Understanding how post-translational regulation of ATF2 influences its choice of transcriptional partners will provide great insight into the cell-type-specific transcriptional activities of ATF2. There is growing evidence of a role for ATF2 in global transcription through its effect on histone modifications and imprinted transcriptional signatures, which points to the importance of ATF2 in establishing general transcriptional programs in addition to its specific activity in concert with its AP1 family binding partners. The proportion of nuclear ATF2 that is dedicated to each of the three distinct functions – specific transcription, global transcription and DNA repair – still needs to be determined. Additionally, it will be equally important to assess whether certain conditions, such as cell type or growth conditions, promote or potentiate one function of ATF2 over another.

The recently discovered role of ATF2 in the cytoplasm is likely to be only the tip of an unexplored iceberg. Although the phosphorylation of ATF2 by PKCε is a key determinant of its subcellular localization and function, the precise mechanism underlying its nuclear export and localization at the mitochondria has yet to be determined. Similarly, it will be important to determine which ATF2 domains are required for its mitochondrial function and their possible relationship to the Bcl-2 family protein signaling that is associated with mitochondrial-based death programs. Finally, given that the cytoplasmic localization of ATF2 has been reported under diverse cellular conditions, it seems unlikely that the function of ATF2 at the mitochondria is its sole activity in the cytoplasm.

ATF2 possesses several functions and properties that might serve as a paradigm for other transcription factors (Fig. 2). First, ATF2 is one of several transcription factors, including Notch, β-catenin and Myc, that have been identified to elicit either oncogenic or tumor suppressor functions (Koch and Radtke, 2007; Larsson and Henriksson, 2010). In the past, this characteristic has been primarily explained on the basis of tissue and/or cell type specificity, or genetic alterations that conferred oncogenic activities [e.g. Notch mutation (Koch and Radtke, 2007)]. However, the example of ATF2 raises the possibility that post-translational modifications and
non-transcriptional functions also have a role in tilting the balance between these opposing functions. A second important concept is the recognition that a single phosphorylation event, such as that of Thr52 in the example of ATF2, might suffice to alter the subcellular localization and primary function of a transcription factor. The control of ATF2 localization by PKCε is also likely to be relevant for other transcription factors and regulatory proteins that elicit diverse activities in distinct subcellular localizations. The third notion is that this regulation can be modified in cancer and other diseases and could thus direct or limit its specific function(s).

Understanding the regulation and function of ATF2 might also prompt the re-evaluation of many transcription factors that, until now, have been unexplored or considered uninteresting. The loss of the cytosolic function of ATF2 in melanomas that express high levels of PKCε offers just a glimpse into the rich, yet to be explored, role of ATF2 and of many transcription factors that might be subjected to similar regulatory cues.

**Acknowledgements**
We thank the members of the Ronai laboratory for advice and our collaborators, Anindita Bhoumik, Meera Shah, Harriet Kluger, Immo Scheffler and Trey Ideker.

**Funding**
The work of our laboratory is supported by the National Cancer Institute (NCI) [grant numbers CA099961, CA117927, CA051995 to Z.R.]. E.L. is supported in part by an ACS Postdoctoral Fellowship, Illinois Division [grant number 117090-PF-09-112-01-GMC]; and an NCI T32 training grant [grant number T32-CA121949]. Deposited in PMC for release after 12 months.

**References**


ATF2 nuclear and cytosolic functions


