The role of ATF2 in insulin action

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ATF2, a novel player in insulin action and insulin resistance?

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Chapter 2

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ATF2, a novel player in insulin action and insulin resistance?

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Activating transcription factor 2 (ATF2) is strongly associated with the cellular response to stress stimuli, such as viral infection, pro-inflammatory cytokines, osmotic stress and DNA damaging agents. However, ATF2 has also been identified as a component of the insulin signaling system, both *in vitro* and *in vivo*. Studies in rodents and *D. melanogaster* have implicated ATF2 in the regulation of glucose and lipid metabolism via induction of PPARγ coactivator 1α (PGC1α) and phosphoenolpyruvate-carboxykinase (PEPCK) expression, suggesting that ATF2 contributes to metabolic control. Conversely, ATF2 also regulates the expression of genes implicated in the development of insulin resistance, β-cell dysfunction and complications associated with type 2 diabetes. This suggests that ATF2 not only participates in insulin action, but that deregulation of ATF2 activity may contribute to the pathogenesis of type 2 diabetes mellitus. This review sheds light on this dual role of ATF2 in metabolic control, insulin action and insulin resistance.

The ATF/CREB family of transcription factors. Activating transcription factor 2 (ATF2; also referred to as cAMP-Responsive Element (CRE) Binding Protein 2 (CREB2) or CRE-Binding Protein-1 (CREBP-1)) is part of the mammalian ATF/CREB family of transcription factors ((1;2) and Figure 1). All members of the ATF/CREB family share the ability to bind to the ATF/CRE consensus site 5'-TG/TACNTCA-3' and contain a basic leucine zipper (bZIP) domain, which is responsible for DNA-binding and dimerization (1;2). Whereas the CREB-subfamily members ATF1, CREM and CREB respond primarily to cAMP/PKA activation, most of the other members of the ATF/CREB family are associated with cellular stress-responses. For example, ATF2-transactivation and ATF3-expression are predominantly induced by stress stimuli (3;4), whereas ATF4 and ATF6 are critical elements of the unfolded protein response induced by endoplasmic reticulum (ER) stress (5).

Structure of ATF2. The human ATF2 gene is located on chromosome 2q32 and encodes a 505 amino acid protein (6). Figure 2 shows a schematic representation of the ATF2 protein, which consists of an amino-terminal transactivation domain (TAD) and a carboxyterminal bZIP-domain, interconnected by a proline-enriched stretch and a histone acetylase (HAT) domain (7;8). ATF2 also contains two nuclear localization signals, a nuclear export signal and multiple sites that can be modified by phosphorylation, ubiquitination and glycosylation (7;8).

ATF2 is highly conserved among species. For example, the mouse ATF2 protein is highly similar to the human counterpart, in that it lacks the first 18 amino acids and differs in only 2 amino acids in the remaining part of the protein. The ATF2 homolog from *D. melanogaster*, dATF2, displays ~50% sequence similarity with mammalian ATF2 and shows conservation of the entire bZIP-domain and part of the TAD-domain (9).

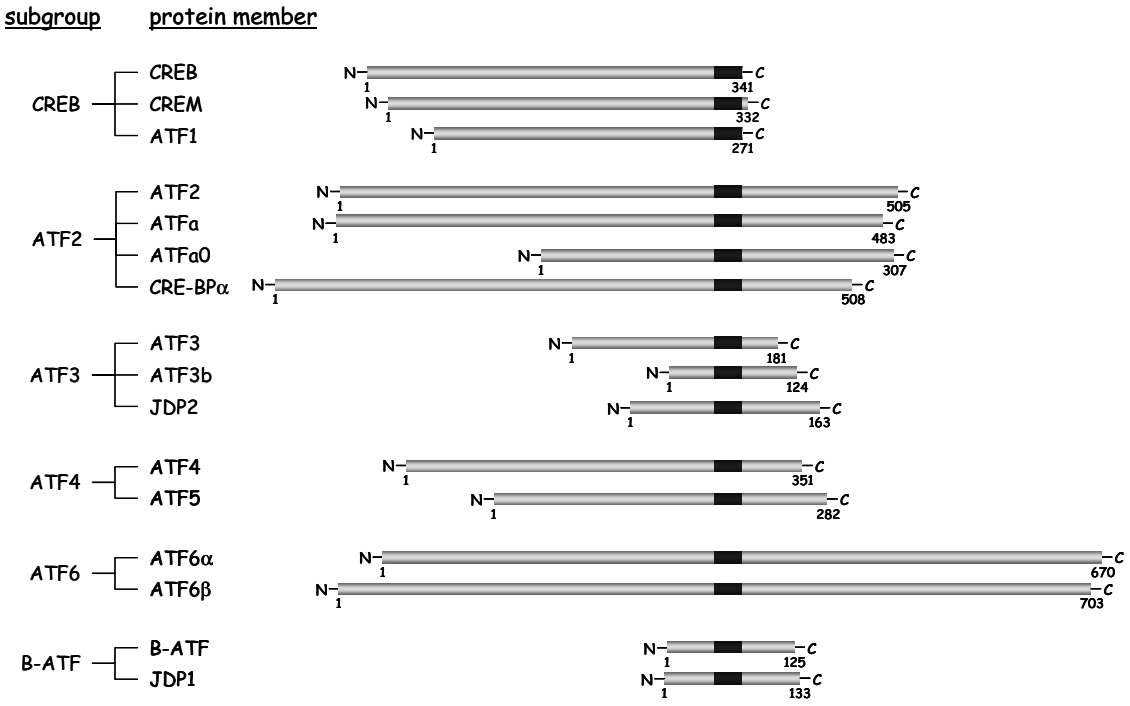


Figure 1. The ATF/CREB family of transcription factors. The protein members can be divided into six subgroups, according to their sequence similarity. The box indicates the bZIP domain. Adapted from (7).

The transactivation domain. Deletion studies using fusion proteins of the DNA-binding domain of the yeast transcription factor GAL4 and ATF2 have delineated the minimal TAD to amino acids 19-96 ((3), see Figure 2). Structural analysis of amino acids 1-105 divides the ATF2-TAD into two subdomains (10). The aminoterminal subdomain (Met19-Gly56) contains a Zn-finger motif with a structure very similar to the Zn-fingers found in the DNA-binding domains of many transcription factors. In contrast to the amino acids responsible for interaction with the phosphate backbone of DNA, the two Cys- (Cys27 and Cys32) and two His-residues (His45 and His49) that coordinate the binding of the Zn-ion, as well as the amino acids that form the hydrophobic core, are well conserved between ATF2 and Zn-finger motifs of other transcription factors. Although point-mutations of the crucial Zn-binding residues or complete deletion of the Zn-finger decreased both basal and serum-induced transcriptional activity of GAL4-ATF2 (11;12), some transcriptionally active mammalian isoforms (see Figure 3 and below) and dATF2 lack the Zn-finger motif (9;13-15). Therefore, the role of the Zn-finger domain in the regulation of ATF2 transactivation *in vivo* remains to be established.

The structure of the carboxyterminal TAD subdomain (Pro57-Lys105) is highly flexible (10). Phosphorylation of several residues within this region, most notably Thr69 and Thr71 (Figure 2), increases the transcriptional activity of ATF2 (3;16-21), suggesting that this subdomain is likely to undergo conformational changes in response to stimuli that promote ATF2 activation. Furthermore, the carboxyterminal part of the TAD, including the regulatory phosphorylation sites, is well conserved in dATF2 (9).

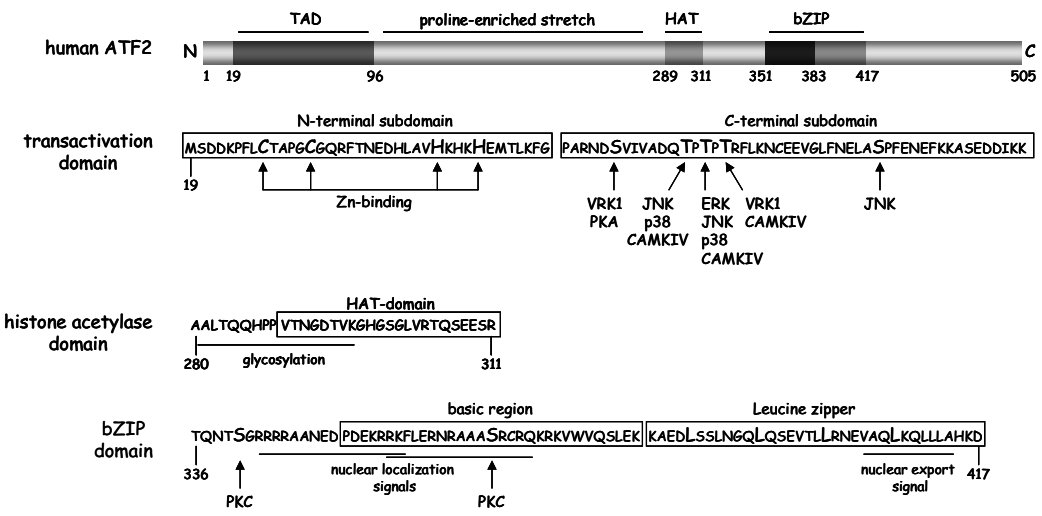


Figure 2. Structure of the human ATF2 protein and its subdomains. ATF2 consists of an amino-terminal transactivation domain (TAD) and a carboxyterminal bZIP-domain, interconnected by a proline-enriched stretch and a histone acetylase domain (HAT). ATF2 also contains two nuclear localization signals, a nuclear export signal and multiple sites that can be modified by phosphorylation and glycosylation. The arrows indicate the residues critical for zinc binding or residues that can be phosphorylated by protein kinase A (PKA), protein kinase C (PKC), extracellular signal regulated kinase (ERK), cJun N-terminal kinase (JNK), p38, or vaccinia-related kinase (VRK).

The histone acetylase domain. ATF2 has been found to acetylate histones H2B and H4 *in vitro* (22). The putative HAT domain of ATF2 is located between amino acids 289-311 (22). Ectopic expression of mutant ATF2 proteins, in which the amino acids critical for *in vitro* HAT activity were replaced by alanines, destroyed the ultraviolet (UV) irradiation-induced activation of a CRE-dependent luciferase reporter gene (22;23). A role for the HAT region in *in vivo* ATF2 function, however, has not yet been demonstrated. It should be noted in this respect that the HAT domain is not conserved in the highly-related ATFa protein (also known as ATF7), which can partially compensate for the loss of ATF2 in ATF2-/- mice (24).

The bZIP domain. The bZIP domain is located between Pro351 and Asp417 (Figure 2). The basic region (Pro351-Lys382) and leucine zipper (Lys383-Asp417) in the bZIP-domain direct DNA-binding and dimer formation, respectively (1;2). Overlapping with the bZIP domain, two nuclear localization signals (Arg342-Lys357 and Arg356-Gln371) and a leucine-rich nuclear export signal (Val405-Ala414) have been identified (25).

Based on sequence similarities, the bZIP proteins identified in the human genome have been arranged in 12 families, that form 3 subgroups on the basis of their dimerization properties (26). Within this classification, ATF2 forms a small family with ATFa and CRE-BPa. The ATF2-family belongs to the subgroup of bZIP transcription factors that can form both homo- and heterodimers (26). ATF2 itself can form heterodimers with several members of the CREB/ATF and Jun/Fos families, such as ATF3, cJun and JunD (7). Furthermore, several other types of transcription factors, including C/EBP and Smad-proteins have been found to bind to DNA in association with ATF2 (27-29).

In 'in gel-retardation' assays, the various ATF2-containing dimers all bind the ATF/CRE consensus site: 5'-TG/TACNTCA-3' (13;30;31), but cause a different degree of DNA

bending, which may contribute to regulatory specificity (32). Compared to the ATF2/ATF2 homodimer, the ATF2/cJun dimer combination is reported to be a more potent transcriptional activator on minimal promoters (13;30;31). However, the relative activities of the various dimers on more complex promoters, such as the c-jun promoter (33) or the proximal element of the interferon γ (IFNγ) promoter (34) is less clear.

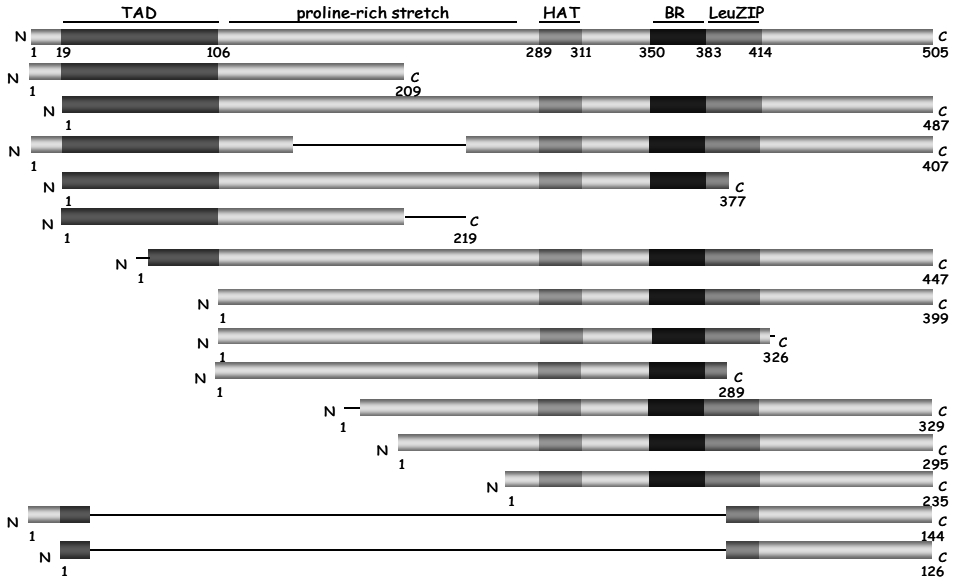
Isoforms. In all mammalian species examined, distinct ATF2 isoforms exist due to differential splicing or alternate promoter usage of the ATF2 gene (9;13-15;35;36). As shown for the human and mouse variants in Figure 3, most isoforms contain the bZIP domain, but differ in the length of the TAD, the presence of the Zn-finger and the presence of the proline-enriched stretch linking the TAD- and HAT-domain (13-15;35). Only one described variant of ATF2, termed ATF2sm, lacks most of the bZIP domain. It contains the first part of the aminoterminal TAD, including the regulatory residues Thr69 and Thr71 and the complete carboxyterminus from the last portion of the bZIP domain (36). Currently, only a few isoforms have been analyzed for biological activity (13-15;35;36). However, the presence of the bZIP domain in almost all variants suggests normal dimerization and DNA-binding properties.

Post-translational modification. ATF2 contains multiple sites that can be modified by posttranslational modification, including phosphorylation, ubiquitination and glycosylation. Table 1 lists the various phosphorylation sites in ATF2 as well the kinases responsible for inducing phosphorylation on these sites.

Table 1. Modification of ATF2 by phosphorylation

Residue	*Function*	*Kinase*	*Reference*
Ser62	transactivation	Protein kinase A VRK1	(37) (38)
Thr69	transactivation	CAMKIV JNK p38	(39) (3;16;17;19) (17;18;21)
Thr71	transactivation	CAMKIV ERK JNK p38	(39) (18;20) (3;16;17;19) (17;18;21)
Thr73	transactivation	CAMKIV VRK1	(39) (38)
Ser90	unknown	JNK	(3;19;20)
Ser121	transactivation	Protein kinase Cα	(40)
Ser340	unknown	Protein kinase C	(37)
Ser367	unknown	Protein kinase C	(2)
Ser490	DNA damage	ATM	(41)
Ser498	DNA damage	ATM	(41)

A. Human ATF2 isoforms



B. Mouse ATF2 isoforms

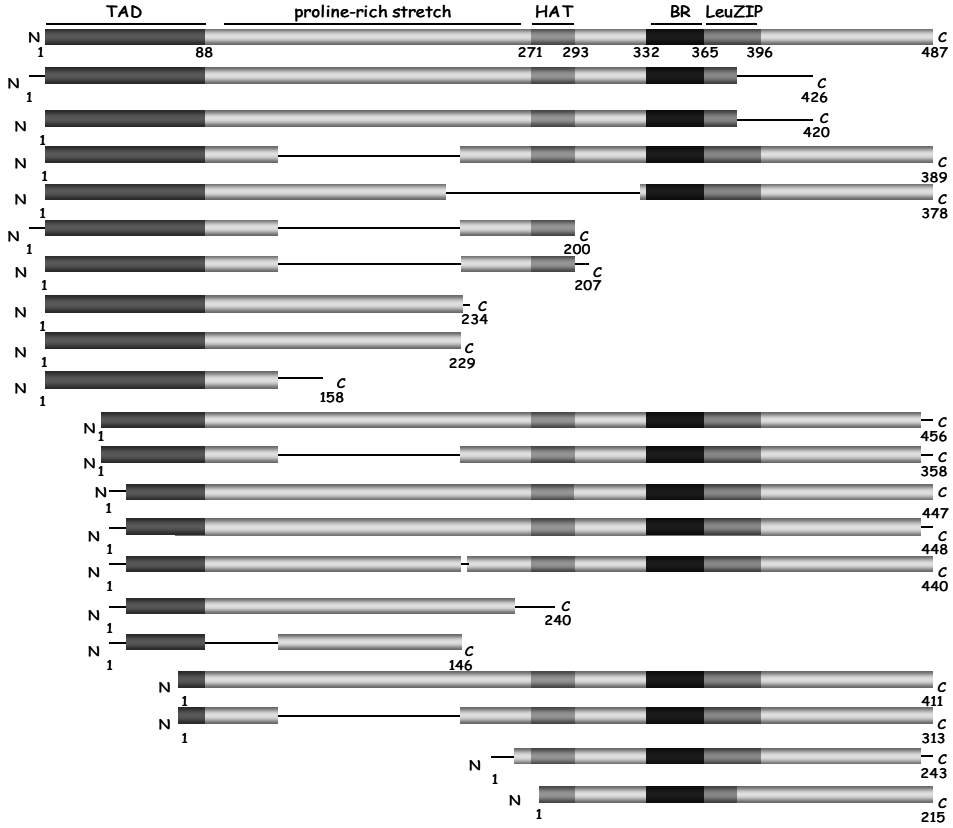


Figure 3. ATF2 isoforms. Alignment of the ATF2 variants in the human (A) and mouse (B) genome.

As will be discussed in more detail under "Regulation of ATF2 activity", phosphorylation of Thr69 and Thr71 by mitogen-activated protein kinases (MAPK) (see Figure 4 (3;16-21)) or Ca2+/calmodulin-dependent kinase IV (CaMKIV (39)) and phosphorylation of Ser62 and Thr73 by vaccinia-related kinase 1 (VRK1 (38)) enhances the transcriptional activity of ATF2. Ser490 and Ser498 are phosphorylated by ataxia telangiectasia mutated (ATM) kinase (41). However, these phosphorylations do not affect ATF2 transcriptional activity, but link ATF2 to the DNA damage response. The function of Ser90, Ser121, Ser340 and Ser367 phosphorylation is less well defined (Table 1).

In addition to phosphorylation, ATF2 might also be regulated by glycosylation. ATF2 was among the proteins identified in a high-throughput analysis of O-linked β-N-acetylglucosamine glycosylated proteins from the brain (42). The region of ATF2-glycosylation (Ala280-Lys296) overlaps in part with the HAT domain, but it remains to be determined whether glycosylation affects ATF2 function. Finally, ubiquitination of ATF2 targets the protein for degradation (43).

Regulation of ATF2 activity. The transcriptional activation capacity of ATF2 is promoted by a large number of distinct stimuli associated with cellular stress, such as viral protein products, oncogenes, pro-inflammatory cytokines, amino acid starvation, heat shock, osmotic stress and DNA damaging agents (3;44), but also mitogenic stimulation with epidermal growth factor (EGF), insulin or serum induces ATF2 transactivation (18;45).
Regulation of ATF2 activity by phosphorylation. The predominant mechanism causing ATF2 transactivation involves the phosphorylation of Thr69 and Thr71 (3;19), but phosphorylation of other residues may also result in activation of ATF2 (Table 1). The MAPK members cJun N-terminal kinase (JNK) or p38 are responsible for the cellular stress-induced phosphorylation of both Thr69 and Thr71 (3;16;17;19;20;46). In response to growth factors, ATF2-Thr69 and Thr71-phosphorylation is induced via a two-step mechanism that requires cooperation of two Ras-dependent MAPK-pathways (18). The ERK1/2-pathway induces phosphorylation of Thr71, whereas subsequent Thr69 phosphorylation is mediated by either p38 or JNK, in a cell type-dependent manner ((18;45) see Figure 4). ATF2 knock-in mice that express an ATF2 mutant in which Thr69 and Thr71 were replaced by alanines ($ATF2^{AA}$), have a phenotype strikingly similar to that of the complete ATF2 knock-out mouse (24;47). Also, in *D. melanogaster*, the p38-mediated phosphorylation of Thr59 and Thr61, the equivalents of mammalian Thr69 and Thr71, was found to be required for transcriptional activation of dATF2 (9;48). These studies highlight the importance of Thr69 and Thr71 for ATF2 function.

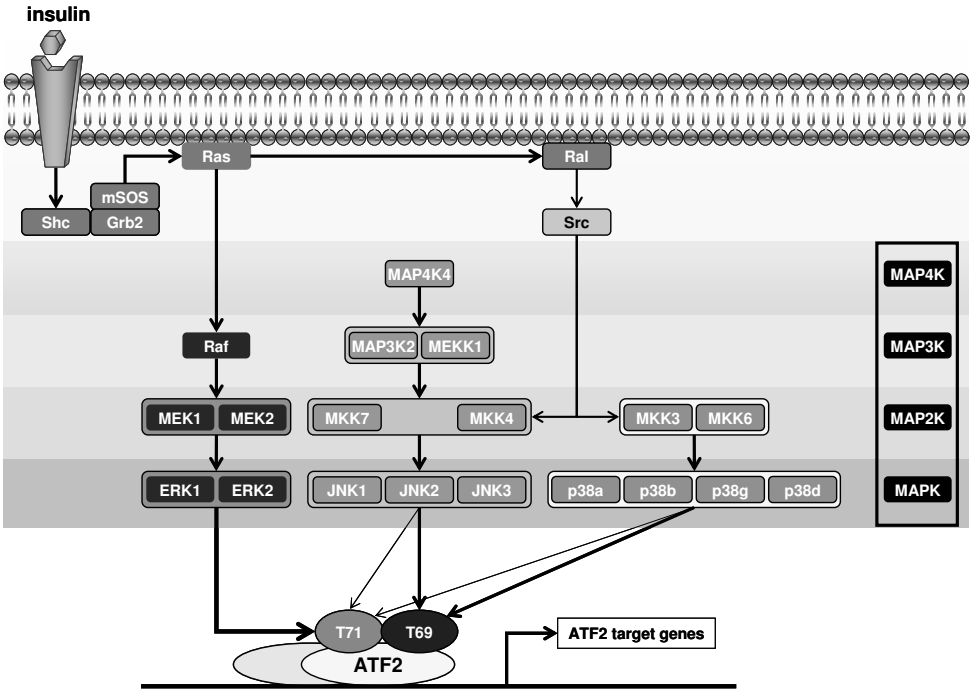


Figure 4. Regulation of ATF2 activation by insulin and MAPkinases. Activation of the Ras–Raf–MEK–ERK1/2 pathway by insulin induces phosphorylation of Thr71, whereas subsequent Thr69 phosphorylation requires the Ral–RalGDS–Src–p38 pathway (18;45).

How phosphorylation of the TAD leads to initiation of ATF2-dependent transcription is still unclear. Reporter-assays with GAL4-ATF2 fusion proteins have suggested that ATF2 is held in an inactive conformation by a direct interaction of the Zn-finger in the TAD with the bZIP domain (49;50). Consequently, transactivation of ATF2 in response to extracellular stimuli has been proposed to involve disruption of this inhibitory interaction through phosphorylation or by association with viral or cellular proteins (44;49). Also other mechanisms have been suggested via which phosphorylation of Thr69 and Thr71 leads to initiation of ATF2-dependent transcription. For example, in response to amino acid starvation ATF2-Thr69+71-phosphorylation has been found to precede *in vivo* acetylation of histone H2B and H4 (51). Histone acetylation is thought to facilitate transcription by altering the accessibility of DNA to transcriptional activators or chromatin remodelling enzymes (52). Future studies should clarify whether the acetylation of H2B and H4 can be ascribed to the putative intrinsic HAT activity of ATF2 (22) or results from ATF2-dependent recruitment of histone acetyltransferases, such as p300/CBP (40;53). Finally, phosphorylation of Thr69 and Thr71 has been implicated in ATF2-dimerization and has been reported to prevent, but also to promote ubiquitin-dependent degradation of ATF2 in different experimental systems (43;54;55).

Regulation of ATF2 activity by protein-protein interactions. Association of ATF2 with transcriptional co-activators or repressors may also affect the transcriptional activity. Proteins that activate ATF2 via protein-protein interactions include, in addition to the p300/CBP-protein mentioned previously (40;53), the adenoviral E1A protein (12;56), the hepatitis B pX protein (57), the nuclear chaperones bZIP enhancer factor and Tax (58) and

the co-activator undifferentiated embryonic cell transcription factor 1 (59). In contrast, ATF2-dependent recruitment of the repressive histone-variant macroH2A has been reported to prevent transcriptional activation of the IL8 gene in a cell-specific manner (60). In addition, ATF2-interaction with TBP-interacting protein 49b (61), Jun dimerization protein 2 (62) and interferon regulatory factor-2-binding protein-1 (63) have been found to suppress ATF2 transcriptional activity.

Subcellular localization. One report suggests that the nuclear transport signals in ATF2 direct the trafficking of the protein between the nucleus and the cytosol. In certain cell lines, heterodimerization with cJun serves to sequester ATF2 in the nucleus (25). Also, although ATF2 itself is not sumoylated, sumoylation of ATF2 dimer partners may have an impact on ATF2 subcellular localization. Sumoylated cJun and ATFa are transcriptionally inactive and at least sumoylated ATFa is primarily localized in the cytosol (64;65). Studies in other cell types, however, do not confirm a cytoplasmic localization of ATF2 (45).

ATF2-dependent gene expression. The various stimuli and protein-protein interactions regulating ATF2-activity as well as the multiple DNA sequences that can bind the different ATF2-containing dimers provide numerous levels on which ATF2-dependent target gene transcription can be regulated. Accordingly, studies aimed at characterizing ATF2-regulated genes using knock-out mouse models, gel-retardation assays, as well as 'ChIP-on-chip' analysis using (phosphospecific) ATF2 antibodies in cisplatin-treated cells, have generated an enormous list of potential target genes (7;8;24;44;66;67). It should, however, be noted that ATF2-dependence has not been validated for all of the identified genes.

Previous reports addressing the function of ATF2 in oncogenesis and DNA repair (7;8;44) have divided the (potential) ATF2 target genes into functional groups, including (i) transcription factors (ATF3, c-jun, CHOP, CREB1, Egr1, fosB, FOXO3a, HIF1α, PPARα, SREBP1c and TCF7L2), (ii) cell cycle intermediates (CDKN1B, CDKN2A cyclin A and cyclin D1), (iii) chemokines and pro-inflammatory cytokines (FasL, IFNβ, IFNγ, IGF2, IGFBP6, IL1β, IL4, IL6, IL8, MT3, TGFβ and TNFα), (iv) signaling proteins (AKT1, APS, MAP4K4, MKP1, PTEN and SHC1), (v)proteins engaged in the response to cellular stress (ATF3, CHOP and Grp78/BiP) and (vi) proteins involved in the DNA damage response (ERCC1, ERCC3, XPA, ATM, RAD23B, FOXD1 and GADD45), (vii) regulators of apoptosis (Bcl2, TBcl2-like 11 and TRAF3), (viii) adhesion (E-selectin, P-selectin, VCAM-1 and collagen), (ix) invasion (MMP2, uPA and iNOS) and (x) metabolism (apolipoproteins A1 and C3, insulin, PEPCK and PCG1α)(24;34;39;48;66-93).

As shown in Table 2, a large number of these (potential) ATF2 target genes also play regulatory roles in insulin action, β-cell (dys)function and/or glucose- and lipid metabolism. Other potential ATF2-regulated genes have been linked to the pathogenesis of type 2 diabetes (T2D) and diabetes-related complications. Intriguingly, phosphorylation of ATF2 is not only increased by insulin *in vitro and in vivo*, but also in rodent models of high-fat diet induced insulin resistance. Below, we will discuss the possible functions of ATF2 in relation to normal metabolic control and the pathogenesis of T2D.

Table 2. Functional classification of (potential) ATF2-target genes involved in insulin action, β-cell function and type 2 diabetes

Function	*Genes*	*References*
Adipocyte dysfunction	HIF1α, IGF2, IL1β, IL6, MMP2, MT3, TNFα	(66;67;83;84)
Candidate genes for T2D	CDKN2A, MAP4K4, TCF7L2	(66;88)
ER stress	ATF3, CHOP, Grp78/BiP	(68;71;78)
Glucose metabolism	PEPCK, PCG1α, SREBP1c	(48;69;70;72;73)
Inhibition of insulin signaling	IL1β, IL6, PTEN, TNFα	(67;83;84;92;93)
Insulin signaling	AKT1, APS, FOXO3a, MKP1, SHC1	(24;66)
Lipid metabolism	apolipoprotein A1, apolipoprotein C3, PPARα, SREBP1c	(66;91)
Vascular complications and fibrosis	collagen, Egr1, E-selectin, HIF1α, iNOS, P-selectin, TGFβ, VCAM-1	(66;67;77;80;81)
β-cell (dys)function	ATF3, IL1β, insulin	(39;67;68;90)

ATF2 and metabolic regulation.

Lessons from animal models. Various mouse models with deletions in the ATF2 gene have been generated. The first mouse described, ATF2m/m, expresses low levels of an ATF2-variant lacking amino acids 277-329 (47;94). These mice were born, displayed lower viability and growth in addition to bone abnormalities and reduced numbers of Purkinje cells in the brain (94). Mice completely lacking ATF2 die shortly after birth due to respiratory problems (47), while knock-out mice lacking both ATF2 and its closest homologue ATFa (also known as ATF7), or mice expressing ATF2 mutated at Thr69 and Thr71 on an ATFa-/- background, are not born due to developmental abnormalities in heart and liver, both in hepatocytes and the hematopoietic cells, already apparent at E12.5 (24). Unfortunately, experiments performed with these mouse models do not provide information on the role of ATF2 in metabolic control.

Selective ablation of dATF2 in the fat body of *D. melanogaster*, however, does identify a metabolic function for dATF2 (48). The fat body serves to sense energy and nutrient availability and coordinates the appropriate metabolic response. Knockdown of dATF2 in the fat body severely reduced phosphoenolpyruvate carboxykinase (PEPCK) expression. PEPCK is a crucial enzyme in hepatic glucose production and in lipid homeostasis in adipose tissue (95;96). In the *D. melanogaster* fat body, the ATF2/PEPCK pathway was found to regulate lipid metabolism and more specifically the synthesis of triglycerides from glycerol-3-phosphate (48).

Lessons from in vivo and in vitro studies. Studies in rat hepatoma cells have confirmed the regulation of PEPCK expression through the p38/ATF2 pathway in response to arsenite and retinoic acid (72;73). Furthermore, the ATF2 target gene and potential dimer-partner ATF3 has been linked to suppression of PEPCK expression *in vivo* (97). Insulin is known to decrease PEPCK levels in the liver (95), but an involvement of the ATF2/ATF3 pathway in this process remains to be determined. ATF2 has further been implicated in the regulation of PPARγ co-activator 1α (PGC1α). This transcriptional co-activator is activated by signals that control energy and nutrient homeostasis (98;99). In brown adipose tissue and skeletal muscle, the p38/ATF2 pathway contributes to PGC1α induction in response to a β-adrenergic stimulus and exercise, respectively (69;70;100).

A gene profiling study on chromatin immunoprecipitated from cisplatin-treated breast cancer cells with a phosphospecific ATF2 antibody identified more enzymes involved in the regulation of glucose and lipid metabolism, such as sterol regulatory element binding protein 1c (SREBP1c), peroxisome proliferator activated receptor α (PPARα) and the apolipoproteins A1 and C3, as well as components of the insulin signaling system, including Akt1, adaptor protein containing PH and SH2 domains (APS), FOXO3a, Map kinase phosphatase 1 (MKP1) and Src homologous collagen-1 (SHC1) (66). Except for apolipoprotein C3 and MKP1, a role for ATF2 in the regulation of these genes has not been validated yet. Apolipoprotein C3 is a very low density lipoprotein (VLDL) protein and inhibits lipoprotein lipase and hepatic lipase, thereby delaying the breakdown of triglyceride-rich particles. DNAseI footprinting analysis and gel retardation studies have demonstrated ATF2 binding to the apolipoprotein C3 promoter (91). Also, ATF2 stimulates apolipoprotein C3 promoter activity in reporter assays in HepG2 cells (91).

In cultured cell lines, the expression of MKP1 is induced by insulin and blunted by shRNA-mediated silencing of ATF2 ((101); BB and DMO, unpublished results: see *Chapter 5*). Also studies in embryonic livers from mice lacking both ATF2 and ATFa (also known as ATF7) confirm MKP1 as an ATF2-regulated gene (24). MKP1 is a negative regulator of the upstream activators of ATF2, i.e. the MAP kinases p38, JNK and ERK1/2 (24;102). In adipocytes, MKP1 has an inhibitory effect on the production of monocyte chemoattractant protein 1 (MCP1), a chemokine highly associated with the development of adipocyte dysfunction in insulin resistance (103). Conversely, mice lacking the MKP1 gene are resistant for diet-induced obesity (104). Therefore, further studies are clearly required to clarify the role of MKP1 in *in vivo* insulin action.

ATF2 has also been implicated in the regulation of β-cell function and proliferation. In isolated rat islets, Ca^{2+}-induced activation of CAMKIV leads to phosphorylation of ATF2 and induction of ATF2-dependent insulin expression (39). Transfection studies in rat insulinomas confirm ATF2 as crucial regulator of glucose-induced insulin transcription by demonstrating ATF2 binding to a CRE-binding element in the human insulin promoter (90). ATF2 further regulates the induction of the cyclin D1 gene in response to regenerating gene product stimulation of β-cells (105). Finally, gene variants in two other cell cycle regulators, i.e. cyclin-dependent kinase inhibitor 2A (CDKN2A) and transcription factor 7 like 2 (TCF7L2, also known as TCF4), that associate with type 2 diabetes and reduced insulin secretion (106;107), were also identified as potential ATF2-regulated genes in a ChIP-on-chip analysis using (phosphospecific) ATF2 antibodies in cisplatin-treated cells (66).

ATF2 and insulin resistance. Insulin resistance in combination with insufficient compensatory insulin secretion due to β-cell dysfunction characterizes type 2 diabetes (T2D) (108). One of the features of insulin resistance is elevated activity of the stress kinase JNK in several insulin target tissues including liver, fat and adipose tissue ((109;110) reviewed in (111)). High fat diet (HFD)-induced insulin resistance in rodent models is mitigated by pharmacological or shRNA-mediated inhibition of JNK activity (112-114). Furthermore, it was found that JNK1-, but not JNK2-knockout mice are resistant to the development of insulin resistance induced by HFD (109;115). JNK activation has been described to inhibit insulin signaling directly via serine phosphorylation of IRS proteins, thereby interfering with tyrosine phosphorylation of the proteins and subsequent activation of downstream signaling pathways (116). Alternatively, JNK may affect insulin

sensitivity indirectly via expression of pro-inflammatory cytokines (116;117). In the insulin resistant state, macrophages infiltrate major insulin target tissues, such as liver and adipose tissue, and various cytokines, including IL6 and TNFα, are released by both macrophages and adipose tissue (118). In hepatocytes, IL6 has been linked to the induction of suppressor of cytokine signaling 3 (SOCS3) (119), a protein that binds to and inhibits the insulin receptor, but also targets the IRS proteins for proteasomal degradation (120;121). In 3T3L1 adipocytes, TNFα increases the expression of MAP4K4 (88). MAP4K4 is an upstream activator of JNK (Figure 4), has been identified as T2D candidate gene (122) and is reported to inhibit adipocyte differentiation and glucose uptake in adipocytes (123). In primary muscle cells isolated from human subjects with T2D, silencing of MAP4K4 restored insulin sensitivity and prevented TNFα-induced insulin resistance (124).

ATF2 has been implicated in induction of IL6, TNFα and MAPK4K4. Silencing ATF2 in 3T3L1 adipocytes abrogates the induction of MAP4K4 (88) and expression of wildtype ATF2 is required for maximal induction of IL6 and TNFα in mouse models (67). Also, studies in *D. melanogaster* support the role of ATF2 in the immune response (9). Interestingly, ATF2-phosphorylation is elevated in both the liver and white adipose tissue from HFD-fed mice (125), suggesting that ATF2 may participate in the pathogenesis of insulin resistance, in addition to and possibly as a downstream effector of JNK.

Furthermore, increased JNK activity was also found in the hypothalamus of HFD-induced insulin resistant mice (126). As ATF2 is highly expressed in the brain (2), amongst others in the arcuate nucleus (127), a region of the hypothalamus that is associated with the control of food intake (128;129), this finding potentially links neuronal ATF2 with the metabolic disturbances found in the insulin resistant state.

An important question is whether activation of the JNK/ATF2-axis within a specific organ might be responsible for the development of insulin resistance. In contrast to ATF2, mice with tissue-specific alterations in JNK1 activity have been generated and analyzed for alterations in insulin sensitivity following high-fat feeding. Whereas overexpression or ablation of JNK in skeletal muscle does not affect glycogen metabolism, or protein levels of key molecules regulating glucose metabolism (130;131), liver-specific knockdown of JNK1 led to a significant reduction in plasma glucose and insulin levels and enhanced insulin-mediated phosphorylation of PKB/Akt (132). In line with these data, mice with antisense oligonucleotide-mediated JNK1-silencing in liver failed to develop insulin resistance and steatohepatitis in response to high-fat feeding (114). Mice with specific ablation of JNK1 in adipose tissue show a greatly reduced susceptibility to insulin resistance induced by high-fat feeding (119). In addition, the HFD-induced expression and secretion of IL6 in adipose tissue was abrogated in adipose-specific JNK1 knock-out mice (119). As described above, IL6-induced expression of hepatic SOCS3 may contribute to insulin resistance. In support of this model, mice with a fat-specific deletion of JNK1 are protected against HFD-induced increase in SOCS3 expression and abrogation of insulin-mediated PKB/Akt-phosphorylation in the liver (119). As the accumulation of macrophages in adipose tissue may also trigger the inflammatory changes seen in insulin resistance and T2D, various groups have generated mice with JNK1 deficiency in myeloid and hematopoietic cells. One report shows that ablation of JNK1 in myeloid cells ameliorates obesity-induced insulin resistance, but subsequent studies did not confirm this finding (119;133-135). Interestingly, all reports found a reduction in the expression of the ATF2 target genes TNFα and IL6 in adipose and hepatic tissue of macrophage-specific JNK-deficient mice.

Despite these suggestive data, the involvement of ATF2 in JNK-dependent processes associated with insulin resistance has not been thoroughly examined. Future studies on mice with a tissue-specific ablation of ATF2 are required to detail the contribution of ATF2 to these JNK-dependent processes.

ATF2 and β-cell dysfunction. Islet cells from patients with T2D show increased inflammation characterized by the presence of immune cell infiltration, amyloid deposits, cytokines and apoptotic cells (136). Various studies implicate ATF2 and ATF2-regulated genes in β-cell dysfunction and apoptosis. Exposure of β-cells to amylin induces a p38-dependent increase in ATF2-phosphorylation (137). Furthermore, amylin-induced apoptosis is prevented by silencing of ATF2 (137). The increase in ATF2 phosphorylation induced by IL1β is mediated by JNK and inhibition of JNK prevents IL1β-induced apoptosis (138;139). Future studies should detail whether the induction of ATF2-regulated genes, IL1β, ATF3, CHOP and BiP/Grp78 (66;68;71) also contributes to β-cell dysfunction and apoptosis. Increased expression of ATF3 in β-cells has been found to lower the levels of IRS2, a key regulator of β-cell survival (140). The induction of IL1β by ATF2 may cause a vicious cycle resulting in progressive β-cell loss. Furthermore, IL1β exposure of β-cells results in endoplasmic reticulum stress, amongst others via the induction of ATF3, CHOP and BiP/Grp78 (141).

ATF2 and T2D-associated complications. Endothelial dysfunction is a co-morbidity in T2D and insulin resistance. Insulin regulates vasodilation by increasing endothelial nitric-oxide synthase (eNOS) activity through the PI3K-PKB/Akt-pathway (142). In insulin resistance, defects in insulin-induced endothelium-dependent vasodilation have been reported (143). ATF2 has been found in complex with the promoter of phosphatase and tensin homolog (PTEN). Furthermore, two potential inducers of endothelial dysfunction in T2D, resistin and free fatty acids, have been found to increase PTEN levels in endothelial cells via activation of the p38/ATF2-pathway (92;93). PTEN acts as a phosphatase to dephosphorylate the product of PI3K, phosphatidylinositol (3,4,5)-triphosphate (PIP3). Thus, activation of ATF2 can contribute to endothelial dysfunction by inhibiting the PI3K-PKB/Akt- dependent phosphorylation of eNOS (142).

The characterization of ATF2-target genes in cisplatin-treated breast cancer cells also identified several genes (66) including collagen, early growth response 1 (Egr1), E-selectin, IGF2, inducible nitric oxide synthase (iNOS), P-selectin, transforming growth factor β (TGFβ) and vascular cell adhesion molecule 1 (VCAM-1), that are associated with endothelial dysfunction as well as other T2D-associated complications, like tissue fibrosis and diabetic nephropathy (144-147). However, further clarification of the involvement of ATF2 in these processes is still needed.

Conclusions and perspectives. ATF2 has been identified as component of the insulin signaling system *in vitro* and *in vivo* (18;45;125). The nature of the ATF2-regulated genes suggests a function for ATF2 in the regulation of glucose and lipid metabolism, as well as β-cell function. Genetic evidence from *D. melanogaster* supports a function for ATF2 in lipid metabolism. Intriguingly, in obesity and insulin resistance, a sustained increase in the activity of the p38/ATF2- and JNK/ATF2-signalling pathways is observed, which may contribute to the development of T2D and associated complications. Future studies should elucidate whether insulin or conditions associated with insulin resistance and obesity induce distinct sets of ATF2-target genes.

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