

## Gene Section

### Review

# ATF4 (activating transcription factor 4 (tax-responsive enhancer element B67))

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## Identity

**Other names:** CREB-2; CREB2; TAXREB67; TXREB

**HGNC (Hugo):** ATF4

**Location:** 22q13.1

**Note:** ATF4 has a genomic size of 2122 bp.

The mouse ATF4 has been classified as a member of the ATF4 subgroup due to 55% identity to mATF4.

## DNA/RNA

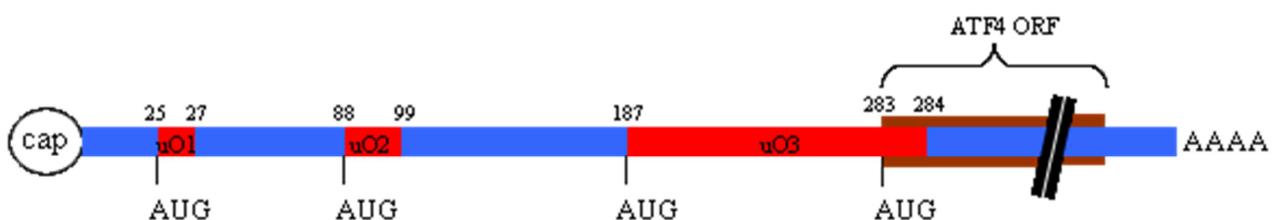
### Note

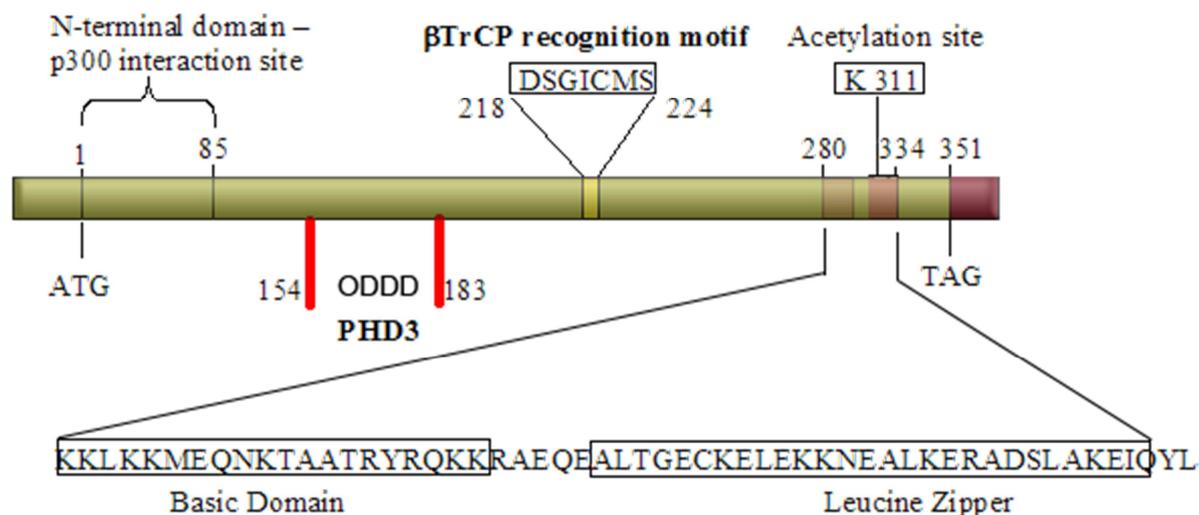
ATF4 gene is transcribed at very high levels (according to ACEview). Several stress conditions such as hypoxia, anoxia, and glucose deprivation result in endoplasmic reticulum stress (ER stress), initiating the unfolded protein response pathway (UPR pathway) that increases the synthesis (increased mRNA translation) of ATF4.

### Description

The mouse ATF4 mRNA contains two upstream

open reading frames, uORF1 and uORF2, and the human ATF4 contains three open reading frames, uORF1 (uO1), uORF2 (uO2), and uORF3 (uO3) that are located 5' to the ATF4 coding sequence. These uORFs are translated in non-stressed conditions, which result in exclusion of ATF4 translation. In mouse, uORF2, or in humans, uORF3 overlap ATF4 ORF in an out of frame manner. After translation of uORF1, sufficient eIF2-GTP makes it possible to reinitiate translation from the uORF2 in mouse, and uORF3 in human, and therefore ATF4 synthesis is minimized. During ER stress, PERK phosphorylates eIF2 $\alpha$  resulting in a decrease of functional eIF2 complex. Stress-induced p-eIF2 $\alpha$  leads to limited eIF2-GTP and prolongs the duration for the scanning ribosome to reinitiate following uORF1, 2, and 3. Consequently ribosome scanning bypasses the mouse uORF2 or human uORF3, and translation re-initiation occurs at the ATF4 ORF (initiation at the ATF4 coding region is increased). Therefore, translation of ATF4 is increased in response to stress including hypoxia, anoxia, nutrition deprivation, including amino acid limitation and glucose deprivation.





## Protein

### Note

ATF4 protein consists of 351 amino acids and is 38,590 Da. The protein is structured into several domains/motifs.

### Description

ATF4 protein consists of 351 amino acids. The protein is unstable and structured into several domains/motifs that are essential for ATF4 homo/heterodimerization, DNA binding, and the regulation of ATF4 at the protein stability level. The organization of the motifs modulating ATF4 protein stability is potentially essential for the regulation of ATF4 stability in response to stress, including hypoxic and anoxic insult. ATF4 has an oxygen dependent degradation domain (ODDD) motif which is recognized by the orthologs of *C. elegans* Egl-9, designated as PH (prolyl hydroxylase) domain containing enzymes (PHD) [also called HIF Prolyl Hydroxylase, HPH], specifically PHD3. The betaTrCP recognition motif is another degradation motif, which when phosphorylated, is recognized by betaTrCP and targeted for proteasomal degradation.

### Expression

ATF4 mRNA is transcribed ubiquitously, but protein expression and level is increased in cells that are exposed to various stress factors such as hypoxia, anoxia, lack of nutrition, as well as during development.

### Localisation

ATF4 protein is targeted to the nucleus. Single point mutations of basic amino acids within the basic region of ATF4 identified the sequence KKLKK (amino acids 280 to 284) as important for nuclear targeting.

### Function

ATF4 protein can function as a transcriptional activator, as well as a repressor. It is also a protective

gene regulating the adaptation of cells to stress factors such as anoxic insult, endoplasmic

reticulum stress and oxidative stress. ATF4 plays an essential role in development, and is particularly required for proper skeletal and eye development as well as haematopoiesis. ATF4 is also involved in proper function of memory. Furthermore, ATF4 is also a major factor in nutrition sensing, and has also been recently implicated in extreme hypoxia/anoxia mediated metastasis.

**Metabolism:** ATF4 is a conserved regulator of metabolism and carbohydrate homeostasis, and provides a mechanistic link between nutrients, insulin resistance, and diabetes, and has been described as a major mediator of nutrition-sensing response pathway, regulating the expression of asparagine synthetase (ASNS). In addition to regulating the expression of ASNS during lack of nutrition, ATF4 also regulates several aspects of mammalian metabolism, such as fat storage, energy expenditure, and glycemic control. The TOR pathway regulates invertebrate and vertebrate metabolism, and ATF4 mutant mice have reduced TOR signaling, and consequently reduced expression of genes important in the intracellular concentration of amino acids. Therefore, lack of ATF4 results in reduced concentration of amino acids, attributed to reduced TOR input. Thus, there is a close relationship between ATF4 function, the TOR pathway, and metabolism. This function of ATF4 also explains why type I collagen synthesis is specifically reduced in primary osteoblast cultures lacking ATF4, which can be rescued by adding nonessential amino acids to the culture. Thus, ATF4 is required for efficient amino acid import into osteoblasts.

**Bone metabolism:** ATF4 is being considered as a global regulator of osteoblast biology and bone metabolism and formation. ATF4 supports bone formation through two mechanisms, which depend on its phosphorylation by RSK2. ATF4 regulates osteoblast-specific gene transcription and the synthesis of type I collagen, the main component of the bone

extracellular matrix (ECM). ATF4 does this by favoring amino acid import, and therefore is a critical determinant of the synthesis of proteins in osteoblasts. Type I collagen is the most abundant protein of the bone ECM, and therefore, ATF4 is a major regulator of bone formation and of bone ECM mineralization.

Consequently, ATF4-deficient mice are runted and harbor low bone mass, reduced osteoblast activity, decreased type I collagen synthesis, and inhibited osteocalcin and bone sialoprotein gene transcription.

**Skeletogenesis:** ATF4 plays an important role in assuring that osteoblasts fulfill their function. Rsk2-deficient mice display decreased bone mass due to impaired bone formation. ATF4 is more strongly phosphorylated by Rsk2 than any other proposed substrate. ATF4-deficient mice have revealed that this transcription factor plays several crucial roles in osteoblast differentiation and function. ATF4-deficient mice display a delayed skeletal development and result in a severe low-bone-mass phenotype caused by decreased bone formation.

**Osteoclast differentiation:** ATF4 regulates osteoclast differentiation and ultimately bone resorption through its expression in osteoblasts. ATF4 binds to the promoter of the receptor activator of nuclear factor- $\kappa$ B ligand (RankL) gene, which encodes a factor secreted by osteoblasts that promotes osteoclast differentiation. Accordingly, ATF4-deficient mice have decreased osteoclast numbers owing to reduced RankL expression.

**Fetal liver hematopoiesis:** A knockout mutation of ATF4 has demonstrated severe fetal anemia in mice. ATF4<sup>-/-</sup> Fetal livers are pale and hypoplastic, and the number of hematopoietic progenitors of multiple lineages is decreased more than 2 fold. Therefore, ATF4 is essential for the normal, high-level proliferation required for fetal-liver hematopoiesis.

**Memory:** ATF4 is a memory repressor that blocks the new expression of genes needed for memories, which appears to be a conserved mechanism. Decreasing the activity of ATF4 in mice or ApCREB2 (the ortholog of ATF4) in the sea slug *Aplysia* lowers the threshold for long-lasting changes and memory.

### Homology

*Drosophila*: Cryptocephal (CRC) gene.

*C. elegans*: According to WormBase, the *C. elegans* homologue of the human ATF4 gene is *atf-5* (T04C10.4). The binding site of *C. elegans* ATF-5 is uncharacterized.

## Mutations

### Note

A frameshift mutation is present in one allele of the ATF4 gene in F9 embryonal carcinoma stem cells. The mutation gives rise to the fusion of a short 5' open

reading frame to the coding sequence of ATF4. Overexpression of mutant ATF4 suppresses ras-induced transformation.

## Implicated in

### Note

Implication of ATF4 in disease comes mainly from transgenic and in vitro studies. Studies in transgenic animals have indicated that ATF4 is required for skeletal and eye development, cellular proliferation, hematopoiesis, and neurological disorders, including memory. ATF4 has also been observed in greater levels in tumors than in normal tissue, suggesting that ATF4 signaling in hypoxic and anoxic areas of tumors might regulate processes relevant to cancer progression.

### Various cancers

### Note

ATF4 is a major factor induced by tumor hypoxia and anoxia, as well as lack of nutrition including low glucose levels. The expression of ATF4 has been noted to be greater in patient cancer compared to paired normal tissue. ATF4 is important for cellular survival under conditions of extreme hypoxia, including anoxia. Recently it has been shown that antiangiogenic treatment with avastin results in induction of ATF4 in vivo.

ATF4 renders cells resistant to multiple anti-cancer drugs and it has been implicated to be a multidrug resistant gene in cancer, and is involved in metastasis, by regulating the expression of the metastasis associated gene LAMP3.

### Oncogenesis

ATF4 is a major factor in regulating the expression of asparagine synthetase (ASNS) during hypoxia and nutritional deprivation (lack of amino acids and glucose). ASNS is associated with drug resistance in leukemia and oncogenesis triggered by mutated p53.

### Skeletal abnormalities of neurofibromatosis

### Note

There has been a link between increased Rsk2-dependent phosphorylation of ATF4 and the development of the skeletal abnormalities in human patients suffering from neurofibromatosis. This disease of tumor development in the nervous system, is caused by inactivating mutations of the neurofibromatosis 1 (NF 1), which plays a major physiological role in bone remodeling. The Nf1ob<sup>-/-</sup> (NF knockout specifically in osteoblasts) mice display a high bone mass phenotype. NF1 induces an increased production of type I collagen, attributed to Rsk2-dependent activation of ATF4. Thus, transgenic mice overexpressing ATF4 in osteoblasts display a phenotype similar to the Nf1ob<sup>-/-</sup> mice.

## Alzheimer's

### Note

In human brains, ATF4 and phospho-eIF2 $\alpha$  levels are tightly correlated and up-regulated in Alzheimer disease, most probably representing an adaptive response against disease-related cellular stress rather than a correlate of neurodegeneration.

## Coffin-lowry syndrome

### Note

Coffin-Lowry Syndrome (CLS) is an X-linked mental retardation condition associated with skeletal abnormalities. ATF4 has been identified as a critical regulator of osteoblast differentiation and function, and lack of ATF4 phosphorylation by RSK2 may contribute to the skeletal phenotype of CLS.

## Vascular disease

### Note

ATF4 can be induced by both vascular injury and fibroblast growth factor-2 (FGF-2) and can serve as a conduit for the inducible expression of one growth factor by another during the process of intimal thickening.

## Joubert syndrome

### Note

The centrosomal protein, nephrocystin-6 (NPHP6), is disrupted in Joubert syndrome. NPHP6 interacts physically with and activates ATF4 as a signaling component on the level of transcriptional regulation in this disease group.

## Microphthalmia

### Note

Lack of ATF4 results in severe microphthalmia due to complete aphakia (absence of the eye lens). The effects of lack of ATF4 is attributed to p53 mediated apoptosis of anterior lens epithelial cells.

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