

Physiological Relevance of Antioxid/Redox Genes; Learning from Genetically Modified Animals

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Thioredoxin binding protein (TBP)-2/Txnip and α -arrestin proteins in cancer and diabetes mellitus

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Thioredoxin binding protein –2/ thioredoxin interacting protein is an α -arrestin protein that has attracted much attention as a multifunctional regulator. Thioredoxin binding protein –2 expression is downregulated in tumor cells and the level of thioredoxin binding protein is correlated with clinical stage of cancer. Mice with mutations or knockout of the thioredoxin binding protein –2 gene are much more susceptible to carcinogenesis than wild-type mice, indicating a role for thioredoxin binding protein –2 in cancer suppression. Studies have also revealed roles for thioredoxin binding protein –2 in metabolic control. Enhancement of thioredoxin binding protein –2 expression causes impairment of insulin sensitivity and glucose-induced insulin secretion, and β -cell apoptosis. These changes are important characteristics of type 2 diabetes mellitus. Thioredoxin binding protein –2 regulates transcription of metabolic regulating genes. Thioredoxin binding protein –2-like inducible membrane protein/ arrestin domain containing 3 regulates endocytosis of receptors such as the β_2 -adrenergic receptor. The α -arrestin family possesses PPXY motifs and may function as an adaptor/scaffold for NEDD family ubiquitin ligases. Elucidation of the molecular mechanisms of α -arrestin proteins would provide a new pharmacological basis for developing approaches against cancer and type 2 diabetes mellitus.

Key Words: thioredoxin binding protein –2/ thioredoxin interacting protein, thioredoxin binding protein –2-like inducible membrane protein/ arrestin domain containing 3, α -arrestin, cancer, diabetes mellitus

We identified thioredoxin binding protein (TBP)-2 as a protein that interacts with thioredoxin in the yeast-two hybrid assay.⁽¹⁾ TBP-2 is identical to vitamin D₃ upregulated protein (VDUP)-1⁽²⁾ and also is referred to as thioredoxin interacting protein (Txnip).⁽³⁾ Several groups reported interaction between thioredoxin and this protein.^(4,5) We and others showed that TBP-2 acts as a negative regulator of thioredoxin to inhibit its protein-reducing activity.⁽¹⁾ Reports also show a reciprocal repression pattern between TBP-2 and thioredoxin.^(1,6,7) Although redox-regulating activity of TBP-2 is one aspect of its function, TBP-2 seems to exert functions redox-independently. TBP-2 belongs to the α -arrestin family and has arrestin domains with similarity to those of β -arrestins.⁽⁸⁾ Since β -arrestins are known to serve as adaptor and scaffold proteins, TBP-2 may have similar functions.

TBP-2 is mainly located in the nucleus⁽⁹⁾ and its expression is induced by various stimuli. TBP-2 has a wide variety of biological functions including regulation of cell death, growth, and differentiation.

Although TBP-2 is reported to interact with JAB1,⁽¹⁰⁾ histone deacetylase (HDAC) 1, Fanconi anemia zinc-finger (FAZF), promyelocytic leukemia zinc-finger (PLZF),⁽¹¹⁾ pVHL,⁽¹²⁾ and Dnajb5,⁽¹³⁾ the physiological significance of each interaction has not been fully clarified. In addition, the molecular and biochemical mechanisms responsible for the pleiotropic functions of TBP-2 have not been elucidated. Here, we review the mechanisms of action and roles of TBP-2 in cancer suppression, immune and inflammatory responses, and metabolism. In this review, animal models in which the TBP-2/Txnip/VDUP1 gene is targeted are termed as TBP-2^{-/-} mice, Txnip knockout mice, Txnip-null mice, and VDUP1^{-/-} mice; the variations of each phenotype are displayed in Table 1.

TBP-2 and Thioredoxin

Several reports show that TBP-2 interacts with thioredoxin *in vitro* and *in vivo*.^(1,4,5) The finding that TBP-2 bound to wild-type thioredoxin but not to a mutant with altered active site cysteines suggests that TBP-2 attaches close to the active site of thioredoxin, and that this interaction inhibits thioredoxin's protein-reducing activity. Indeed, overexpression of TBP-2 inhibited the reducing activity of thioredoxin.^(1,7,9) The C247 residue of TBP-2 is necessary for the interaction between TBP-2 and thioredoxin, since TBP-2 C247S did not interact with thioredoxin *in vitro*.^(14,15)

Interestingly, TBP-2 and thioredoxin sometimes display a reciprocal expression pattern at the RNA level. In interleukin (IL)-2-dependent CTLL-2 cells, deprivation of IL-2 caused upregulation of TBP-2 expression, while thioredoxin expression was downregulated (Ahsan *et al.*, unpublished observation). TBP-2 expression was augmented and thioredoxin expression downregulated in HL60 cells treated with vitamin D₃⁽¹⁾ or peroxisome proliferator-activated receptor (PPAR) ligands⁽⁷⁾ and in MCF-7 breast cancer cells treated with the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA).^(6,16) The molecular mechanism under-

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lying this reciprocal gene expression pattern is currently unknown. It may be that TBP-2 regulates transcription of the thioredoxin gene. There are reports that upregulated TBP-2 expression causes suppression of thioredoxin's reducing activity resulting in oxidative stress, which may lead to apoptosis. Based on the finding that thioredoxin interacts with and regulates apoptosis signaling kinase 1 (ASK1),^(17,18) it has been argued that TBP-2 binds to and oxidizes thioredoxin or thioredoxin2, allowing for ASK1's activation.⁽¹⁹⁻²³⁾ The physiological significance of these findings should be verified further.

The function of TBP-2, especially its metabolic regulating function, however, is not explained by mere inhibition of thioredoxin, as discussed below. It should be noted that TBP-2-like inducible membrane protein (TLIMP)/arrestin domain containing 3 (ARRDC3), another α -arrestin, does not bind to thioredoxin and suppress its reducing activity. Among the TBP-2 family, only TBP-2 interacts with thioredoxin,⁽⁷⁾ a result confirmed by another group.⁽¹⁴⁾ These results suggest that α -arrestin proteins have distinct roles other than suppression of thioredoxin.

TBP-2 Gene Expression

Expression level of TBP-2 is influenced by a variety of conditions, suggesting roles in several biological processes. Currently known stimuli and pathways are summarized in Fig. 1. Expression of TBP-2 was upregulated in HL-60 cells stimulated with $1\alpha,25$ -dihydroxyvitamin D₃,^(1,2) and was augmented by glucose, adenosine-containing molecules,⁽²⁴⁾ the HDAC inhibitor SAHA,⁽⁶⁾ 5-fluorouracil,⁽²⁵⁾ deprivation of serum or IL-2 in cell culture,^(26,27) carcinogens,⁽²⁸⁾ ceramide, etoposide,^(20,29) hydrogen peroxide (H₂O₂), heat shock,^(5,29) transforming growth factor (TGF)- β ,⁽¹¹⁾ ultraviolet light,⁽³⁰⁾ glucocorticoid (GC),^(31,32) allse treatment⁽³³⁾ and lipopolysaccharide (LPS).⁽³⁴⁾ An element containing two CCAAT motifs in the gene regulatory region is required for a response to vitamin D₃⁽³⁵⁾ and NF-YA plays a role in vitamin D₃-induced activation of the TBP-2 gene (Masutani *et al.*, unpublished observation). Upregulation of TBP-2 gene expression by SAHA is also reported mediated by the CCAAT element and NF-YA.⁽⁶⁾

TBP-2 expression induced by glucose is mediated by carbo-

hydrate-response elements (ChoREs)^(36,37) and the associated transcription factors Max-like protein X (MLX) and Mondo (MondoA or ChoRE-binding protein [ChREBP; also known as MondoB]).⁽³⁸⁻⁴⁰⁾ Glutamine inhibits transcriptional activation of TBP-2 by triggering recruitment of an HDAC-dependent corepressor to the amino terminus of MondoA.⁽³⁹⁾ TBP-2 and ARRDC4 expression is upregulated under lactic acidosis.⁽⁴¹⁾ Glucose-stimulated TBP-2 expression is regulated by forkhead box O1 transcription factor (FOXO1) and p38 mitogen-activated protein kinase (MAPK).⁽⁴²⁾ Overexpression of Krüppel-like factor 6 (KLF6) increased expression and promoter activity of TBP-2, which was inhibited by concurrent exposure to a PPAR- γ agonist.⁽⁴³⁾ In contrast, insulin is a potent repressor of TBP-2 expression.⁽⁴⁴⁾ Inhibitors of mitochondrial oxidative phosphorylation downregulated TBP-2 expression at the transcriptional level.⁽⁴⁵⁾ Tandem CCAAT boxes and ChoRE motifs and associated NF-Y and Mondo/MLX transcription factors are required for enhancing TBP-2 promoter activity.⁽⁴⁶⁾ Glucose-induced upregulation of TBP-2 expression was suppressed by an inhibitor of p38 MAPK.^(47,48) One interesting report indicates the involvement of microRNA in transcriptional regulation of TBP-2.⁽⁴⁹⁾ TBP-2 gene expression was enhanced by a PPAR- α ligand and PPAR- γ agonists but not by a PPAR- β/δ ligand.⁽⁷⁾ An analysis of TBP-2 showed the presence of a functional PPAR- γ response element in the promoter.⁽⁵⁰⁾ TBP-2 levels were elevated in skeletal muscle of patients with impaired glucose tolerance (IGT) or type 2 diabetes mellitus (T2DM).⁽⁵¹⁾ A thorough understanding of the mechanism regulating expression of TBP-2 in glucose metabolism seems very important for developing approaches against T2DM (discussed below). A bilberry anthocyanin-rich extract altered TBP-2 expression in the aorta of apo E-deficient mice.⁽⁵²⁾ *Trans*-resveratrol suppressed upregulation of TBP-2 expression occurring during liver ischemia-reperfusion.⁽⁵³⁾ These natural products may be beneficial for controlling levels of TBP-2.

Expression of TBP-2 has been analyzed in various stages of the cellular differentiation process. TBP-2 expression was upregulated in mesenchymal stem cells by glucose.⁽⁵⁴⁾ Blockade of the *N*-methyl-D-aspartate (NMDA) receptor increased TBP-2 levels *in vivo* and *in vitro*.⁽⁵⁵⁾ TBP-2 mRNA expression was augmented

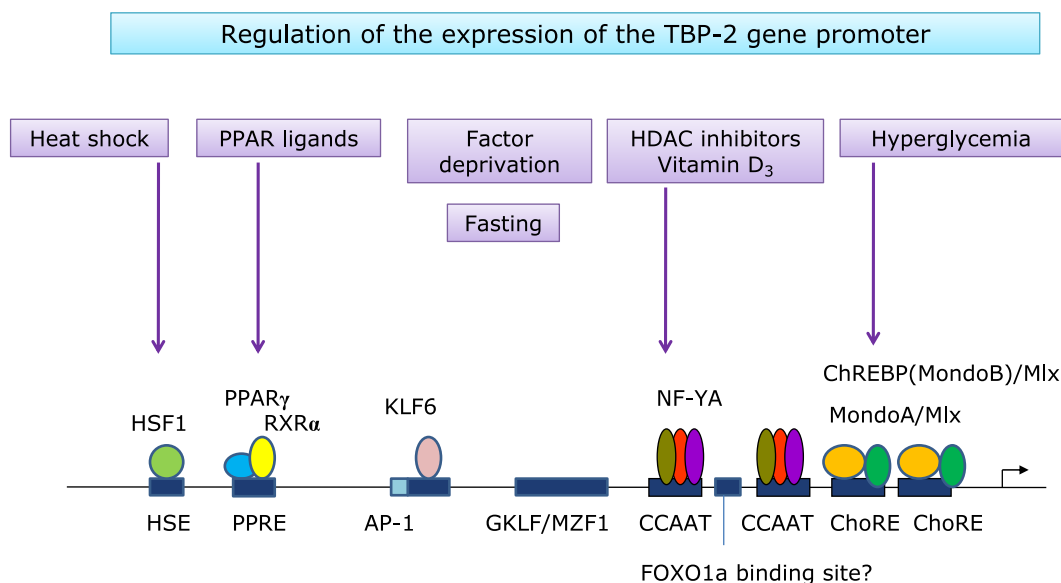


Fig. 1. Transcriptional regulation of TBP-2. Gene expression of TBP-2 is enhanced by a wide variety of stimuli. For each stimulus, the specific binding sequence and transcription factor are indicated. HSE, heat shock element;⁽²⁹⁾ PPRE, peroxisome proliferator-activated receptor (PPAR) response element;⁽⁵⁰⁾ AP-1, activator protein-1; GKLF, gut-enriched Krüppel-like factor; MZF1, myeloid zinc finger 1, putative FOXO1a-binding site;⁽⁶⁶⁾ and ChoRE: carbohydrate response element.^(36,38) HSF1: heat shock factor 1; RXR α , retinoid X receptor α ; KLF6, Krüppel-like factor 6;⁽⁴³⁾ NF-YA, nuclear transcription factor Y subunit α ; ChREBP, carbohydrate response element-binding protein; Mlx, Max-like protein X.

in cerebellar granule neurons following their withdrawal from a high-potassium medium.⁽⁵⁶⁾ Expression of TBP-2 decreased during hematopoietic stem cell activation. A study on VDUP1^{-/-} mice suggests that TBP-2 may be essential for hematopoietic stem cell quiescence.⁽⁵⁷⁾ TBP-2 expression is significantly induced in fetal lung, suggesting that TBP-2 play some role in proliferation and differentiation during pulmonary development.⁽⁵⁸⁾ The *cis*-regulatory element of *dvdup1*, the *drosophila* TBP-2 homologue gene, contains consensus binding sites for the glial fate gene reversed polarity (*repo*)⁽⁵⁹⁾ and hedgehog-dependent transcription factor,⁽⁶⁰⁾ suggesting that TBP-2 may be linked to neuronal growth and development.

Several stimuli and conditions can cause suppression of TBP-2 expression. Expression of TBP-2 decreased in the presence of soluble receptor activator of NF- κ B ligand (sRANKL) and overexpression of TBP-2 inhibited osteoclastogenesis.⁽⁶¹⁾ Platelet-derived growth factor (PDGF) and H₂O₂ treatment suppressed TBP-2 expression in human aortic smooth muscle cells.⁽⁶²⁾ Calcium channel blockers suppressed cardiac expression of TBP-2, which may enhance cardiomyocyte survival.⁽⁶³⁾ Exenatide reduced expression of TBP-2 and protected INS-1 β -cells against apoptosis.⁽⁶⁴⁾ Expression of TBP-2 in rat cardiomyocytes was rapidly suppressed by biomechanical strain or H₂O₂.⁽⁶⁵⁾ One interesting study found that TBP-2 expression was downregulated in human compared with chimpanzees and TBP-2 may be a direct target of FOXO1a.⁽⁶⁶⁾ TBP-2 expression was markedly suppressed by LPS and interferon (IFN)- γ in macrophages, an effect that was partially inhibited by an inducible nitric oxide synthase (iNOS) inhibitor.⁽⁶⁷⁾ Trans-synaptic activation of synaptic NMDA receptors (NMDARs) suppressed TBP-2 transcription by causing dissociation of FOXO from the TBP-2 promoter via a phosphoinositide 3-kinase (PI3K)-Akt pathway.⁽⁶⁵⁾ Knockdown of HDAC10 significantly increased TBP-2 mRNA expression.⁽⁶⁸⁾ These results collectively imply roles of TBP-2 for various cellular processes and disease conditions (also discussed below). Studies are required to analyze the mechanism behind suppression of TBP-2 gene expression (also discussed below).

TBP-2 in Cancer Suppression

TBP-2 has growth-suppressive activity^(1,9,11,26,69) and its augmented expression is associated with apoptosis.^(6,23,36,64,70-73) Fibroblasts from VDUP1^{-/-} mice proliferated more rapidly than wild-type cells with reduced expression of p27 (kip1).⁽¹⁰⁾ TBP-2 is also a target for mediating GC-induced apoptosis.^(31,32)

Many reports have shown downregulation of TBP-2 expression in tumor cells and correlation of TBP-2 levels with clinical stages of cancer. For instance, TBP-2 mRNA expression is downregulated in colorectal cancer.⁽⁷⁴⁾ TBP-2 expression was decreased in colorectal and gastric cancer, and significantly lower in stage III and IV tumors than stage II tumors.⁽⁷⁵⁾ The expression was also decreased in primary breast and colon tumors,^(6,75) human breast, lung, and stomach cancers,⁽¹¹⁾ and 7,12-dimethylbenz(α)anthracene (DBMA)-induced rat mammary adenocarcinomas.⁽⁷⁶⁾ Patients with diffuse large B cell lymphomas who had the worst prognosis had decreased levels of TBP-2.⁽⁷⁷⁾

TBP-2 expression was abrogated in human T cell leukemia virus type 1 (HTLV-I)-infected IL-2-independent T cells but maintained in HTLV-I-infected IL-2-dependent T cells as well as in HTLV-I-negative T cells.⁽²⁶⁾ Epigenetic silencing of the TBP-2 gene results in loss of responsiveness to IL-2.⁽²⁷⁾ Knockdown of TBP-2 at an IL-2-dependent stage attenuated GC-induced cell death, suggesting that TBP-2 mediates GC-induced cell death.⁽³²⁾ In ferric nitrilotriacetate-induced carcinogenesis in rat kidney, TBP-2 is silenced by epigenetic mechanisms during tumorigenesis.⁽²⁸⁾ A recent report found that recruitment of HDAC1 to the TBP-2 promoter was mediated by a complex consisting of the RET finger protein and NF-Y. A high level of RET finger protein

was correlated with downregulation of TBP-2 expression in human colon cancers and associated with poor clinical outcome.⁽⁷⁸⁾

TBP-2 was expressed more markedly in nonmetastatic melanomas than in parental metastatic cells.⁽⁷⁹⁾ Metastasis-free intervals in 788 cases of breast cancer revealed that TBP-2 expression was associated with a better prognosis.⁽⁸⁰⁾ TBP-2 expression was downregulated significantly in malignant vs benign pheochromocytoma.⁽⁸¹⁾ These results suggest that TBP-2 is associated with protection against metastasis.

The HcB-19 mouse strain, which has a spontaneous mutation in the TBP-2 gene, was found associated with increased incidence of hepatocellular carcinoma.⁽⁸²⁾ VDUP1^{-/-} mice showed impaired NK cell development and reduced tumor rejection.⁽⁸³⁾ TBP-2 expression is suppressed during human hepatic carcinogenesis, and mice lacking TBP-2 (VDUP1^{-/-}) were much more susceptible to diethylnitrosamine-induced hepatocarcinogenesis than wild-type mice.⁽⁸⁴⁾ These results suggest that a decrease of TBP-2 is associated with tumorigenesis. Considering the retarded onset of cancer in mice with disrupted expression of the TBP-2 gene, the cancer-suppressive effect of TBP-2 seems attributable to the progression phase of cancer development but not the initiation phase. Although a regulatory role for TBP-2 in the tumor necrosis factor (TNF)- α -NF- κ B pathway is suggested during tumorigenesis,⁽⁸⁴⁾ the underlying molecular mechanism of cancer suppression by TBP-2 remains unclear.

TBP-2 in the Fasting Response

The clinical relevance of TBP-2 was first indicated by a surprising report in 2002, demonstrating that TBP-2 is responsible for the familial combined hyperlipidemia (FCHL) in HcB-19 mice, which have a nonsense mutation in the TBP-2 gene and lack TBP-2 expression.⁽³⁾ Although mutation of TBP-2 was not detected in human families with hyperlipidemia,⁽⁸⁵⁻⁸⁷⁾ the finding was an important indication that TBP-2 plays a role in metabolic control.

TBP-2^{-/-} mice prepared by Oka *et al.*⁽⁸⁸⁾ are fertile and of normal appearance. On fasting, however, they are predisposed to death from hyperlipidemia, hypoglycemia, a bleeding tendency, and hepato-renal insufficiency.⁽⁸⁸⁾ In mutant HcB-19 mice, plasma free fatty acids levels are increased.^(3,89) Fasted HcB-19 mice are also hypoglycemic and hypertriacylglyceridemic and have higher levels of ketone bodies.^(90,91) These results show that HcB-19 mice and TBP-2^{-/-} mice exhibit very similar phenotypes and that TBP-2 is a critical regulator of fasting responses.

Ablation of pancreatic β -cells with streptozotocin (STZ) completely blocked the fasting-induced hypoglycemia and hypertriacylglyceridemia, suggesting that these abnormalities are due to excess insulin secretion. Expression of insulin-inducible sterol-responsive element-binding protein (SREBP)-1c and its target genes was elevated.⁽⁹⁰⁾ Similar results were obtained in TBP-2^{-/-} mice. Gene chip analyses of the liver of TBP-2^{-/-} mice showed that considerable numbers of TBP-2-regulated genes are also fasting-response genes. TBP-2 itself is one of the genes most upregulated on fasting. A feature of TBP-2^{-/-} mice is a change in gene expression of insulin and targets of SREBP such as fatty acid synthetase and lipoprotein lipase in the fasted state. Intraperitoneal glucose tolerance tests (IP-GTT) revealed increased insulin secretion in TBP-2^{-/-} mice. In intraperitoneal insulin tolerance tests (IP-ITT), TBP-2^{-/-} mice were demonstrated exposed to prolonged insulin effects. These results suggest a role for TBP-2 in regulation of insulin secretion and sensitivity, providing a possible link to T2DM.⁽⁹²⁾

Another feature of TBP-2^{-/-} mice was upregulation of PPAR target genes in the fed state.⁽⁹²⁾ Sheth *et al.*⁽⁹¹⁾ also reported that expression of PGC-1 α was augmented in the liver of HcB-19 mice.⁽⁹¹⁾ These results suggest that TBP-2 plays a role in PPAR- α expression and signaling.

Txnip-null mice did not show hyperlipidemia,⁽⁹³⁾ unlike HcB-19

Table 1. Phenotypes of mice with mutations or knockout of the TBP-2/Txnip/VDUP1 gene

1) HcB-19 ^(3,82,90,91)	Fasting-induced hypoglycemia, hypertriacylglyceridemia, and hyperinsulinemia
2) TBP-2 ^{-/-} ^(88,92)	Fasting-induced hypoglycemia, hypertriacylglyceridemia, and hyperinsulinemia
3) Txnip knockout mice (Txnip-TKO) ⁽⁹⁴⁾	Fasting-induced hypoglycemia and hypertriacylglyceridemia; normal insulin levels
Liver-specific Txnip knockout (Txnip-LKO)	No fasting-induced changes
Muscle-specific Txnip knockout (Txnip-MKO)	Fasting-induced hypoglycemia and hypertriacylglyceridemia; normal insulin levels
4) Txnip-null mice ^(100,118,130)	Hypoglycemic and hypoinsulinemic; normal lipid levels
Liver-specific Txnip-null mice	No change of glucose clearance
5) VDUP ^{-/-} mice ^(83,112)	Improved glucose and insulin tolerance
6) β -Cell-specific Txnip KO (Txnip-bTKO) ⁽⁷¹⁾	Normal serum levels of triacylglycerides and ketones; reduced fasting blood glucose levels
7) BTBRlep ^{ob/ob} txnip ^{hcb/hcb} ⁽⁷¹⁾	Obese, protected against diabetes
8) C57BL6 lep ^{ob/ob} TBP-2 ^{-/-} ⁽⁹⁹⁾	Obese, protected against diabetes

mutant mice,^(3,89,90,91) TBP-2^{-/-} mice⁽⁸⁸⁾ and total Txnip knockout mice (Txnip-TKO).⁽⁹⁴⁾ The reason for the varied insulin levels during fasting among the different mutants and constructs with a disrupted TBP-2/Txnip/VDUP1 gene is unknown. It could be caused by differences in experimental conditions for fasting and susceptibility. Modifier gene effects resulting from strain differences among these models or contributions specific to the different TBP-2 gene deletions may also play a role in the differences in β -cell function.⁽⁹³⁾ The differences in metabolism among several lines of mutant and knockout mice are summarized in Table 1. Meanwhile, overexpression of TBP-2 in mice caused changes of lipid metabolism, mirroring those in TBP-2^{-/-} mice,⁽⁹⁵⁾ underlining the significance of TBP-2 in metabolic control.

TBP-2 in T2DM

TBP-2 expression is elevated in skeletal muscle of patients with impaired glucose tolerance or T2DM.⁽⁵¹⁾ The frequency of a 3'UTR single nucleotide polymorphism in TBP-2 was investigated in subjects with normal glucose tolerance, impaired glucose tolerance, and T2DM. The frequency of the variation did not differ among groups, but within the diabetic subjects, carriers of the T variant had 1.6-fold higher triacylglyceride concentrations and a higher diastolic blood pressure than homozygous carriers of the common C-allele, whereas in nondiabetic subjects fasting glucose was lower in carriers of the T-allele. These results imply the involvement of TBP-2 in T2DM.⁽⁹⁶⁾

Hyperglycemia and activation of receptor for advanced glycation end products induced expression of TBP-2 in cultured rat retinal endothelial cells. TBP-2 overexpression in rat retinal endothelial cells abolished H2K9 tri-methylation and increased H3K9 acetylation, implying a role for TBP-2 in ocular inflammation and endothelial dysfunction in patients with diabetic retinopathy.⁽⁹⁷⁾ TBP-2 expression was rapidly induced in mesangial cells in the kidney. Overexpression of TBP-2 resulted in induction of type IV collagen alpha1 chain (COL4A1) mRNA expression and accumulation of type IV collagen, indicating TBP-2 to be a molecular mediator/marker for fibrosis in diabetic cases of nephropathy.⁽⁹⁸⁾ As discussed above, studies have demonstrated that TBP-2 expression is induced by glucose⁽³⁶⁻⁴⁰⁾ and PPAR ligands.^(7,50) Results obtained with HcB-19 mutant mice⁽⁹⁰⁾ and TBP-2^{-/-} mice⁽⁹²⁾ indicate a regulatory role for TBP-2 in insulin secretion and sensitivity. Crossing ob/ob mice with HcB-19 mice provided protection against diabetes,⁽⁷¹⁾ whereas disruption of TBP-2 in ob/ob mice completely ameliorated hyperglycemia.⁽⁹⁹⁾ These reports collectively demonstrate involvement of TBP-2 in the pathophysiology of T2DM.

Regulation of Insulin Sensitivity by TBP-2

Knockdown of TBP-2 expression enhanced glucose uptake in cultured adipocytes and primary human skeletal muscle myocytes, whereas TBP-2 overexpression inhibited glucose uptake.⁽⁵¹⁾

Disruption of TBP-2 improves glucose tolerance and insulin sensitivity.⁽⁹²⁾ HcB-19 mice crossed with ob/ob mice⁽⁷¹⁾ and TBP-2^{-/-} mice crossed with ob/ob mice showed improved glucose tolerance.⁽⁹⁹⁾ Augmented glucose transport was identified in adipose tissue and skeletal muscle of Txnip-null mice.⁽¹⁰⁰⁾ Txnip-TKO mice produced by Hui *et al.*⁽⁹⁴⁾ exhibited increased Akt signaling and insulin sensitivity in skeletal muscle and hearts but not liver and adipose tissue. Skeletal muscle glucose uptake was increased in the Txnip-TKO mice. Muscle and heart-specific Txnip knockout mice (Txnip-MKO) showed a similar metabolic phenotype to the total knockout mice, but liver-specific Txnip knock mice (Txnip-LKO) did not show metabolic changes.⁽⁹⁴⁾ These results collectively demonstrate that TBP-2 deficiency improves insulin sensitivity mainly in muscle.

Although altered production of adipocytokines accompanied with obesity is implicated in insulin resistance and glucose intolerance, TBP-2 deficiency improved insulin sensitivity without any amelioration of obesity and adipocytokine levels.⁽⁹⁹⁾ TBP-2 deficiency in ob/ob mice restored Akt phosphorylation in the skeletal muscle and heart but not liver. Several insulin signaling-related genes such as Irs-1 (insulin receptor substrate-1; IRS-1) were upregulated by the deficiency. IRS-1 protein levels were downregulated in ob/ob mice, whereas IRS-1 levels and the phosphorylation of Akt in response to insulin were restored by TBP-2 deficiency in skeletal muscle. Thus TBP-2 regulates expression of molecules involved in insulin signaling, which may enhance insulin sensitivity in skeletal muscle.⁽⁹⁹⁾ The molecular mechanism by which TBP-2 regulates expression of insulin signaling-related genes remains to be elucidated.

Regulation of Insulin Secretion by TBP-2

A determinant of declining glucose tolerance is a progressive decrease in glucose-stimulated insulin secretion (GSIS).⁽¹⁰¹⁾ HcB-19 and TBP-2^{-/-} mice showed enhancement of insulin secretion *in vivo*.^(90,92) Decrease in β -cell mass after treatment with STZ was greater in HcB-19 mice than control mice, suggesting that control of β -cell mass may be one function of TBP-2.⁽¹⁰²⁾ However, no significant change in islet mass between ob/ob·TBP-2^{-/-} mice and ob/ob mice or between TBP-2^{-/-} and wild-type mice was observed. Serum insulin levels after glucose loading *in vivo* were enhanced in ob/ob·TBP-2^{-/-} mice compared with ob/ob mice. In isolated pancreatic islets, TBP-2 deficiency enhanced GSIS in wild-type and ob/ob mice.⁽⁹⁹⁾ In addition, silencing of TBP-2 enhanced GSIS in INS-1 cells, whereas TBP-2 overexpression suppressed GSIS.⁽⁹⁹⁾ These results clearly revealed the regulatory role of TBP-2 in GSIS.

Deficiency of TBP-2 enhanced mitochondrial ATP production and GSIS in pancreatic β -cells.⁽⁹⁹⁾ Mitochondrial uncoupling protein (UCP)-2 is a key regulator of ATP production and insulin secretion in pancreatic β -cells and UCP-2 deficiency has been shown to improve GSIS and glucose-induced ATP production in ob/ob mice.⁽¹⁰³⁾ UCP-2 expression is upregulated with increases

in the activity of PGC-1 α .⁽¹⁰⁴⁾ TBP-2 enhanced expression and transcriptional activity of UCP-2 by recruiting PGC-1 α to the UCP-2 promoter.⁽⁹⁹⁾

While TBP-2 was reported to interact with various proteins,^(1,4,5,10,11) Mybbp1a was identified as a novel candidate binding protein of TBP-2.⁽⁹⁹⁾ Mybbp1a is reported to inhibit PGC-1 α function and transcription of PGC-1 α target genes through direct protein-protein interaction.⁽¹⁰⁵⁾ As discussed below, TBP-2 may interact with the WW domain of HECT domain-containing ubiquitin ligases through its PPXY motifs. TBP-2 may negatively regulate substrates such as Mybbp1a by protein degradation through E3 ubiquitin ligases. The molecular mechanism by which TBP-2 regulates metabolism should be investigated further.

Regulation of β -Cell Apoptosis by TBP-2

TBP-2 is an important regulator of β -cell apoptosis.⁽¹⁰⁶⁾ TBP-2 is overexpressed in T2DM and induced by glucose to induce β -cell apoptosis.^(36,107) HcB-19 mouse islets were protected against glucose-induced apoptosis.⁽¹⁰⁸⁾ Lack of TBP-2 inhibits the mitochondrial death pathway underlying β -cell glucotoxicity, but has very few protective effects against endoplasmic reticulum (ER) stress-mediated apoptosis.⁽⁷²⁾ HcB-19 mice crossed with ob/ob mice against the BTBR background were protected from diabetes and β -cell apoptosis at age 9 months, resulting in a 3-fold increase in β -cell mass. β -Cell-specific Txnip knockout mice (Txnip-bKO) also showed enhanced β -cell mass and revealed an approximately 50-fold reduction of β -cell apoptosis on STZ treatment.⁽⁷¹⁾ In C57BL/6J mice, TBP-2 deficiency also suppressed β -cell apoptosis at age 36 weeks, although β -cell apoptosis did not occur significantly in ob/ob and ob/ob-TBP-2^{-/-} mice at age 10 weeks of age.⁽⁹⁹⁾ These results clearly demonstrate that TBP-2 deficiency protects against β -cell apoptosis in aged mice.

Collectively, TBP-2 deficiency ameliorates insulin sensitivity in skeletal muscle and insulin secretion from pancreatic β -cells and protects against β -cell apoptosis. Conversely, in obesity or under conditions with augmented levels of free fatty acids and hyper-

glycemia the expression of TBP-2 is augmented, resulting in impairments of insulin sensitivity and insulin secretion and enhanced β -cell apoptosis. Augmented expression of TBP-2 might also result in suppression of thioredoxin, which plays a protective role against oxidative stress in β -cells^(109,110) (Fig. 2). Thus TBP-2 seems an attractive target of drug development against T2DM.

Regulation of Immunity and Inflammation by TBP-2

TBP-2 is also involved in regulation of immunity and inflammation. VDUP1^{-/-} mice showed minimal changes in the development of T and B cells but exhibited marked reduction in natural killer (NK) cells as well as decreased NK activity. Expression of CD122 was reduced, suggesting that TBP-2 is critical for development and function of NK cells *in vivo*.⁽⁸³⁾ Dendritic cells (DCs) derived from TBP-2^{-/-} mice are defective in T cell activation. In a mixed leukocyte reaction, and with LPS stimulation, IL-12 production from Txnip^{-/-} DCs was significantly lower than that from wild-type DCs. The proliferation of T cells cultured with TBP-2^{-/-} DC was poorer than that with wild-type DCs.⁽¹¹¹⁾ The percentage of hepatic NK T (NKT) cells decreased in TBP-2^{-/-} mice but increased in TBP-2 transgenic mice. TBP-2^{-/-} mice were resistant to concanavalin A-induced hepatitis.⁽⁹⁵⁾ TBP-2^{-/-} mice injected with LPS did not show higher serum levels of TNF α , IL-6, IL-10, IFN- β , IFN- γ , monocyte chemoattractant protein (MCP) 1, and macrophage inflammatory protein (MIP) 2 compared with wild-type mice but exhibited fat accumulation in liver as well as hypoglycemia and hyperinsulinemia similar to the phenotypes of the fasted mice. Thus although TBP-2 may not directly regulate LPS-induced inflammatory signaling, it influenced signaling involved in metabolic control during LPS-induced endotoxemia.⁽³⁴⁾

Very recently, there was a report that TBP-2 plays an important role in inflammasome activation. The inflammasome is an emerging concept of a mechanism of host defense. Macrophages have a recognition system against pathogenic microbes and cellular stress to activate caspase 1, leading to IL-1 secretion. TBP-2 interacts with a nod-like receptor protein (NLRP) 3 inflammasome

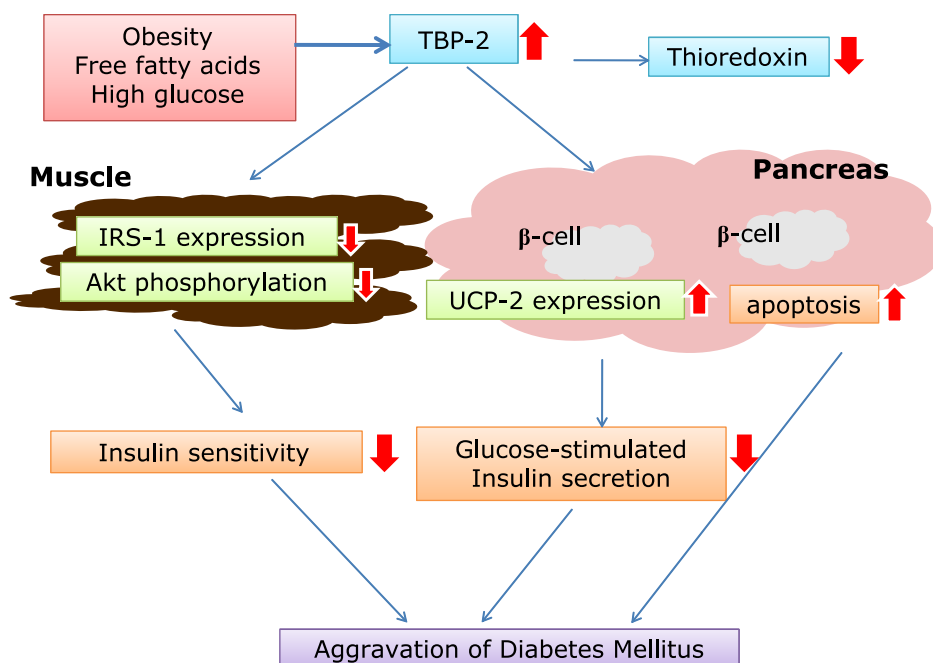


Fig. 2. Aggravation of T2DM by TBP-2. In obesity, free fatty acids and hyperglycemia may augment expression of TBP-2. TBP-2 suppresses 1) insulin sensitivity by decreasing Akt phosphorylation and expression of insulin signal-regulating genes such as insulin receptor substrate-1 (IRS-1) gene in muscle; and 2) glucose-stimulated insulin secretion from pancreatic β -cells by enhancing expression of uncoupling protein (UCP)-2; augments 3) pancreatic β -cell apoptosis; and also causes 4) suppression of thioredoxin. These changes may together lead to aggravation of diabetic phenotypes.

complex in macrophages leading to the NLRP3 inflammasome's activation in a reactive oxygen species (ROS)-sensitive manner.⁽¹¹²⁾ Subsequent study showed that TBP-2 is not involved in islet amyloid polypeptide-induced activation of the NLRP3 inflammasome, analyzed in macrophage.⁽¹¹³⁾ The significance of the role of TBP-2 in regulation of inflammasome should be further verified.

Regulation of Vascular and Cardiac Function by TBP-2

The role of TBP-2 in vascular and cardiac function has been investigated. Incubation of rat pulmonary artery smooth muscle cells with the NO donor S-nitroso-glutathione suppressed TBP-2 expression.⁽¹¹⁴⁾ TBP-2 has marked antiproliferative effects in vascular smooth muscle cells.⁽⁶²⁾ TBP-2 protein expression is upregulated in the lungs of 1-day-old newborn mice compared with E19 embryos, showing an inverse correlation with expression of vascular endothelial growth factor (VEGF). Overexpression of TBP-2 inhibited hypoxia-inducible factor (HIF)-mediated reporter activity, suggesting that TBP-2 is a potential regulator of HIF-mediated gene transcription in the murine lung.⁽¹¹⁵⁾ Exposure of rabbit aortae or cultured endothelial cells to normal flow was associated with decreased TBP-2 expression compared with exposure to low flow. In aortae from HcB-19 mice, TNF-induced vascular cell adhesion molecule (VCAM) 1 expression was inhibited, indicating a role for TBP-2 in TNF signaling and inflammation in endothelial cells in fluid shear stress.⁽¹¹⁹⁾ A role for TBP-2 in angiogenesis was also suggested.⁽¹¹⁶⁾ The role and the mechanism of involvement of TBP-2 in vasculature physiology need further investigation.

Aortic constriction reduced TBP-2 expression. Cells over-expressing TBP-2 develop less hypertrophy after aortic constriction than control cells, suggesting that TBP-2 is a critical regulator of biomechanical signaling.⁽¹¹⁷⁾ Txnip-null mouse hearts had attenuated cardiac hypertrophy and preserved left ventricular (LV) contractile reserve through 4 weeks of pressure overload, whereas TBP-2 deletion ultimately led to maladaptive LV remodeling at 8 weeks of pressure overload.⁽¹¹⁸⁾ Therefore, the role of TBP-2 in cardiac hypertrophy seems complex and requires further investigation. Importantly, TBP-2 expression was significantly increased in rat hearts following acute myocardial ischemia. Direct intracardiac injection of sequence-specific DNA enzyme to down-regulate TBP-2 mRNA at the time of acute cardiac infarction reduced myocardial TBP-2 mRNA expression and resulted in a prolonged reduction in cardiomyocyte apoptosis, accompanied by a significant reduction in cardiac expression of pro-collagen type I alpha2 mRNA as well as marked reduction of myocardial scar formation. These results suggest a role for TBP-2 in the adverse effects of ischemia.⁽²²⁾

Involvement of TBP-2 in Other Diseases

TBP-2 deficiency enhances methionine–choline-deficient diet-induced hepatic steatosis and inhibits nonalcoholic steatohepatitis (NASH), suggesting that TBP-2 is involved in the pathogenesis of NASH.⁽¹¹⁹⁾ Recently, it was shown that in VDUP1^{-/-} mice, liver regeneration is accelerated after partial hepatectomy.⁽¹²⁰⁾ Another report showed that TBP-2 expression was significantly lower in ulcerative colitis colonic mucosa specimens than in normal tissue.⁽⁷⁴⁾ TBP-2 protein level was increased in retinal ganglion cells at 2 and 5 weeks after experimental glaucoma induction.⁽¹²¹⁾ Therefore, TBP-2 may be involved in the pathophysiology of these diseases.

TBP-2 and α -Arrestin Proteins and their Cellular Distribution

Phylogenetic analysis revealed a large number of unrecognized arrestins in eukaryotes termed α and β class arrestins.⁽⁸⁾ β -Arrestin

Table 2. α -Arrestin family proteins

	Other name	Localization
TBP-2	Txnip, VDUP1	Nucleus
ARRDC1		Membrane
ARRDC2		Nucleus
ARRDC3	TLIMP	Inner membrane, endosome, lysosome
ARRDC4	DRH1	Cytosol
ARRDC5		

is a scaffold/adaptor protein that interacts with various signaling regulators such as MAPK and ASK1 and has a wide variety of functions including the regulation of endocytosis and degradation of surface receptors such as G coupled-protein receptors (GPCR).

The α -arrestin family has arrestin domains and conserved PPXY motifs and is found in all multicellular organisms except plants. Since a subfamily (TBP-2, ARRDC2, ARRDC3, and ARRDC4) has highly homologous protein sequence signature, it seems reasonable to consider that TBP-2 belongs to a subfamily of the α -arrestin family (Table 2). TBP-2 family members share 80% amino acid similarity. ARRDC3 was originally reported as TLIMP. We showed that TLIMP/ARRDC3 is a novel vitamin D₃- or PPAR- γ ligand-inducible membrane-associated protein and plays a regulatory role in cell proliferation and PPAR- γ activation.⁽⁷⁾ Another homolog of TBP-2, DRH1, is identical to ARRDC4 and reported downregulated in hepatocellular carcinoma cells.⁽¹²²⁾

A clue to the functions of individual α -arrestin proteins is provided by their cellular distribution. Although some reports suggest that TBP-2 is expressed in mitochondria,^(21,28,123) many others indicate that it is expressed and functions primarily in the nucleus.^(9,12,21,92,99) Without stimulation in MCF-7 cells, TBP-2 was hardly detectable in immunohistochemical analysis. On treatment with the HDAC inhibitor SAHA, TBP-2 was detected mainly in the nucleus.⁽⁹⁾ TBP-2-EGFP displays scattered expression in about 30% of cells, indicating that TBP-2 functions mainly in the nucleus. TBP-2 interacts with importin α , an important shuttle between the cytosol and nucleus, *in vitro* and *in vivo*.

In contrast to the nuclear expression of TBP-2, expression of TLIMP/ARRDC3 is on the inner side of the plasma membrane, lysosome and endosome. These observations suggest that TLIMP/ARRDC3 plays a role in the endocytotic process.⁽⁷⁾ The membrane-associated and endosomal expression was also confirmed by other studies.^(124,125) ARRDC4 occurs in the cytosol, whereas ARRDC2 is expressed in the nucleus (our unpublished observation). ARRDC1 is located in the plasma membrane.⁽¹²⁵⁾

TBP-2 family members have several similar characteristics. All have growth suppressive activity. TBP-2 and TLIMP/ARRDC3 expression is induced by vitamin D₃ and PPAR ligands.⁽⁷⁾ On the other hand, there may be differences among the TBP-2 family members, including binding of thioredoxin as discussed above.^(7,14) Thus members of the TBP-2 family occur in each cellular compartment and may have both common regulatory functions and distinct functions. Further characterization of these proteins is needed.

TBP-2 and NEDD Family Proteins

TBP-2 and other α -arrestin proteins have conserved PPXY sequences that are known binding motifs for the WW domain^(7,8,14,126) (Fig. 3). Arrestin-related trafficking adaptors (ARTs) contain PPXY motifs that interact with Rsp5/Nedd4-like ubiquitin ligase, regulating the internalization of plasma membrane proteins (cargos) and degradation in the lysosome in the yeast system.⁽¹²⁷⁾ Art1 has considerable homology around the PPXY motif to mammalian ARRDC proteins. It is worth noting that TLIMP/ARRDC3 is also associated with the inner cellular membrane and endosome/lysosome,⁽⁷⁾ supporting the notion that ARRDC

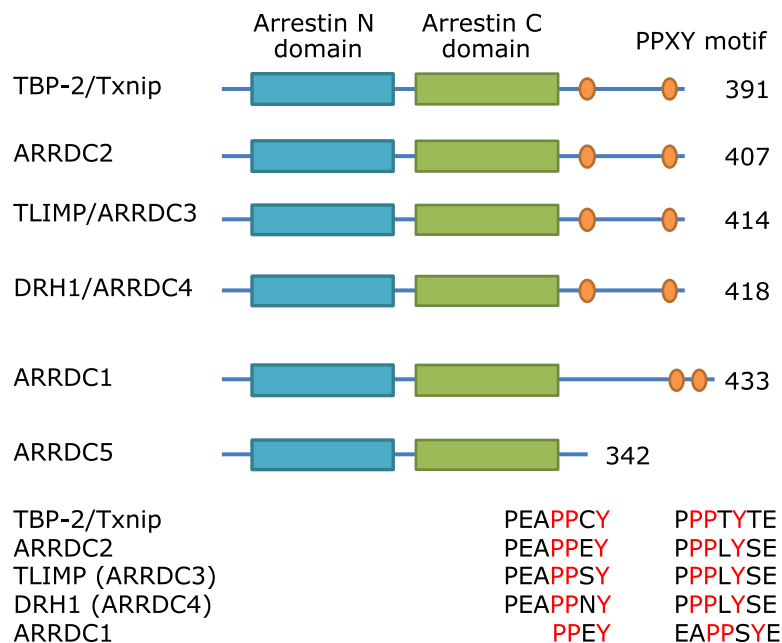


Fig. 3. α -Arrestin family proteins. TBP-2/Txnip, ARRDC2, TLIMP/ARRDC3, and DRH1/ARRDC4 share high sequence homology. TBP-2 and ARRDC1, 2, 3, 4, and 5 all contain arrestin N and C domains. All but ARRDC5 possess conserved PPXY motifs.

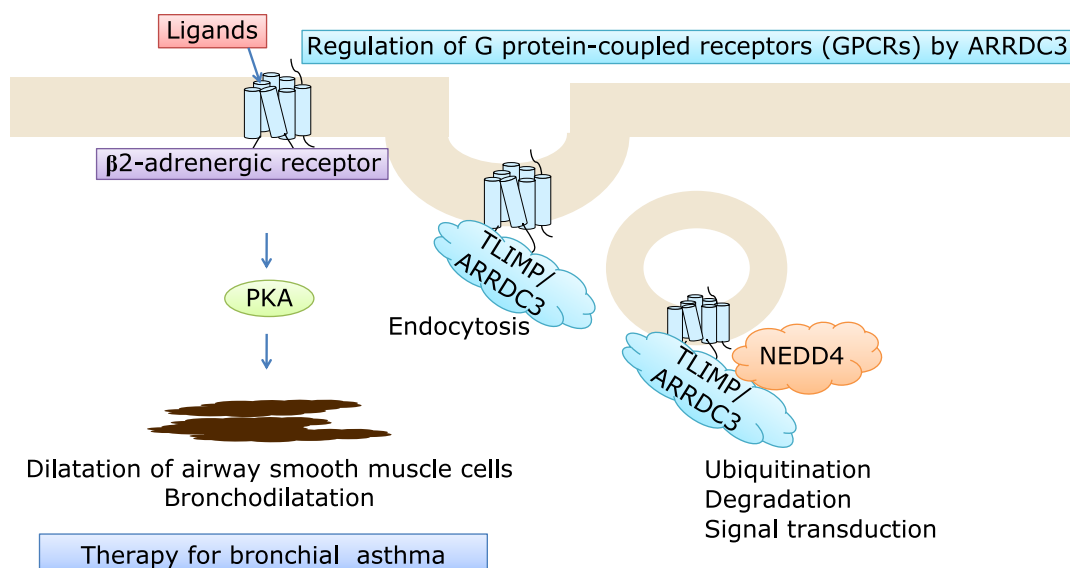


Fig. 4. Regulation of G protein-coupled receptors by TLIMP/ARRDC3. TLIMP/ARRDC3 is reported to interact and co-localize with the β 2-adrenergic receptor.⁽¹²⁵⁾ The β 2-adrenergic receptor is a pharmacologically important G protein-coupled receptor (GPCR) for regulation of dilation of bronchial smooth muscle cells and therapy for bronchial asthma. TLIMP/ARRDC3 may regulate endocytosis, signaling, ubiquitination, and degradation of the β 2-adrenergic receptor.

proteins interact with NEDD family ubiquitin ligases to play a regulatory role in endocytosis. Indeed, TLIMP/ARRDC3 directly binds to a phosphorylated form of β 4 integrin leading to its internalization, ubiquitination, and ultimate degradation.⁽¹²⁴⁾ Furthermore, TLIMP/ARRDC3 recruits NEDD4 to mediate ubiquitination of the β 2-adrenergic receptor⁽¹²⁵⁾ (Fig. 4). In human, the NEDD family has nine members such as NEDD4, NEDD4L, WWP1, WWP2, ITCH, SMURF1, SMURF2, HECW1, and HECW2 (Table 3). One possibility is that as in the yeast system, human α -arrestin proteins act as adaptors for ubiquitin ligases (Fig. 5). On the other hand, human α -arrestin may be regulated through degradation by the NEDD family. Various reports clearly

Table 3. NEDD family proteins

	Other name
NEDD4	NEDD4-1
NEDD4L	NEDD4-2
WWP1	AIP5
WWP2	AIP2
ITCH	AIP4
SMURF1	
SMURF2	
HECW1	NEDL1
HECW2	NEDL2

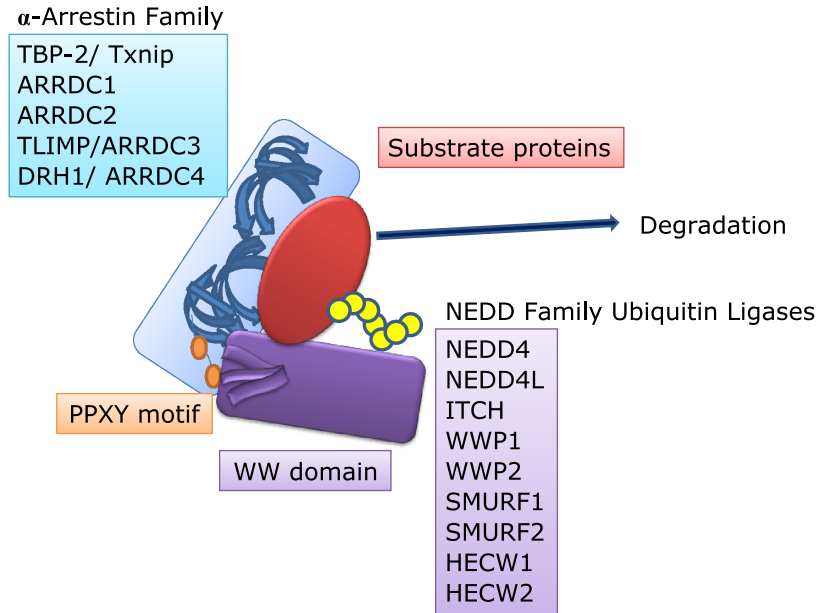


Fig. 5. Hypothetical model of the interaction between α -arrestin family proteins and NEDD family ubiquitin ligases. α -Arrestin proteins containing conserved PPXY motifs may interact with the WW domain of NEDD family ubiquitin ligases to regulate ubiquitination and degradation of substrate proteins.

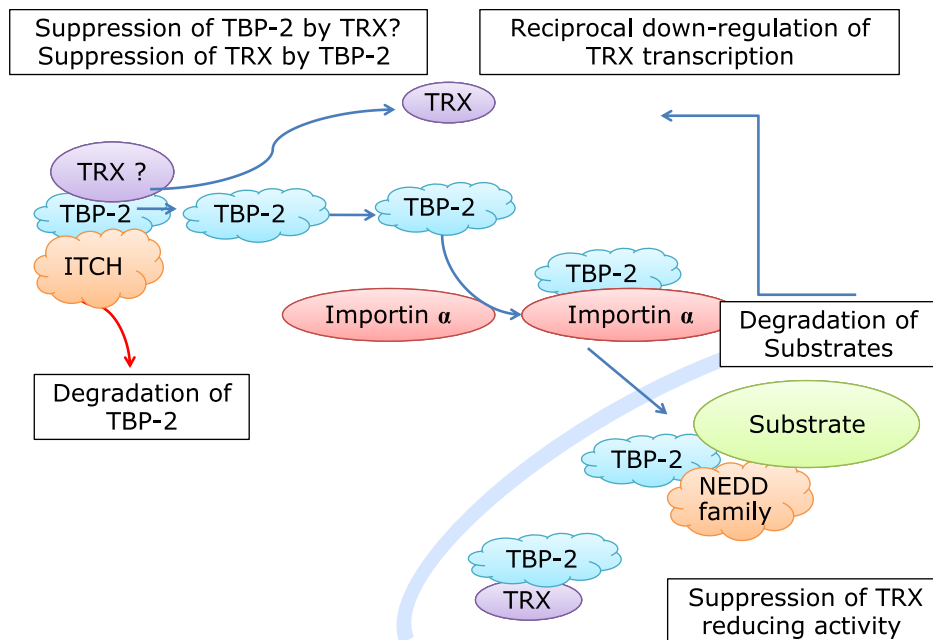


Fig. 6. Hypothetical model of reciprocal regulation between thioredoxin and TBP-2. TBP-2 is maintained at low levels and may be degraded by NEDD family ubiquitin ligases such as ITCH. TBP-2 binds to importin α , moves to the nucleus, interacts with substrates, and may induce their degradation by the NEDD family. TBP-2 also acts as a negative regulator of thioredoxin (TRX). Interaction between thioredoxin and TBP-2 might regulate each other's stability. TBP-2 might also transcriptionally regulate expression of thioredoxin.

show that TBP-2 protein expression is rapidly turned over. It was recently found that ITCH targets TBP-2 for ubiquitin-dependent degradation.⁽⁷³⁾ Thus it is tempting to speculate that TBP-2 itself is rapidly degraded by ubiquitin ligases and maintained at low levels, and TBP-2 is stabilized by appropriate signal recognition and

translocated into the nucleus to interact with substrates for facilitating subsequent ubiquitination of the proteins. The interaction between α -arrestin proteins and NEDD family ubiquitin ligases in each cellular compartment should be investigated further.

Regulation of Cellular Signals by Reciprocal Regulation between Thioredoxin and TBP-2

Although TBP-2 seems to regulate metabolism mainly redox-independently, TBP-2 could contribute to modulation of redox status. In VDUP1^{-/-} mice, the hepatomito-gen-induced response was associated with a considerable increase in the release of TNF- α and subsequent enhancement of NF- κ B activation on TBP-2 deficiency.⁽⁸⁴⁾ Primary hepatocytes isolated from VDUP1^{-/-} mice displayed increased activation of ERK1/2 and Akt in response to hepatocyte growth factor (HGF) and TGF- α .⁽¹²⁰⁾ These results suggest that TBP-2 has a suppressive effect on growth factor-induced signaling. Thioredoxin knockdown suppressed epidermal growth factor (EGF)-induced ERK1/2 activation, suggesting that thioredoxin plays a positive regulatory role in ERK1/2 signaling.⁽¹²⁸⁾ It is tempting to speculate that TBP-2 deficiency results in augmentation of thioredoxin function, leading to facilitation of growth factor signaling. It remains to be elucidated how the stability of TBP-2 is regulated under various forms of stress, during which the level of TBP-2 protein is increased. Once stabilized, TBP-2 binds to importin α to transfer to the nucleus and may interact with substrates, inducing them into subsequent degradation by NEDD family protein ligases. A schematic model of how TBP-2 regulates protein stability and signals is displayed in Fig. 6. The possible mutual regulation between TBP-2 and thioredoxin should be investigated further.

Perspectives

Accumulating evidence suggests that TBP-2 is a multifunctional protein associated with various disease conditions such as cancer, hyperlipidemia, T2DM, inflammatory diseases, cardiac and vascular diseases, and NASH. The NEDD family is involved in a variety of processes including transcription, receptor endocytosis, membrane budding, and budding of viruses.⁽¹²⁹⁾ Further elucidation of the molecular function of not only TBP-2 and TLIMP/ARRDC3 but also other arrestin proteins will provide new insights into clinical biochemistry and diseases such as cancer and T2DM.

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Abbreviations

ARRDC3	arrestin domain containing 3
ASK1	apoptosis signaling kinase 1
ChoRE	carbohydrate response element
DC	dendritic cell
FAZF	Fanconi anemia zinc-finger
FOXO1	forkhead box O1 transcription factor
GSIS	glucose-stimulated insulin secretion
HDAC	histone deacetylase
HIF	hypoxia-inducible factor
HTLV-I	human T cell leukemia virus type 1
IFN	interferon
IGT	impaired glucose tolerance
IL-2	interleukin-2
IRS-1	insulin receptor substrate-1
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MLX	Max-like protein X
NASH	nonalcoholic steatohepatitis
NEDD	neuronal precursor cell-expressed developmentally downregulated
NF-Y	nuclear transcription factor Y
NK	natural killer
NLRP3	nod-like receptor protein 3
NMDA	N-methyl-D-aspartate
NO	nitric oxide
PGC-1 α	PPAR γ co-activator-1 α
PLZF	promyelocytic leukemia zinc-finger
PPAR	peroxisome proliferator-activated receptor
SAHA	suberoylanilide hydroxamic acid
SREBP	sterol-responsive element-binding protein
TBP-2	thioredoxin binding protein-2
T2DM	type 2 diabetes mellitus
TGF	transforming growth factor
TLIMP	TBP-2 like inducible membrane protein
TNF	tumor necrosis factor
Txnip	thioredoxin interacting protein
UCP-2	uncoupling protein-2
VDUP1	vitamin D ₃ upregulated protein-1
WT	wild-type

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