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The signalling from endoplasmic reticulum-resident bZIP transcription factors involved in diverse cellular physiology

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Eukaryotic cells can adapt to endoplasmic reticulum (ER) dysfunction by producing diverse signals from the ER to the cytosol or nucleus. These signalling pathways are collectively known as the unfolded protein response (UPR). The canonical branches of the UPR are mediated by three ER membrane-bound proteins: PERK, IRE1 and ATF6. These ER stress transducers basically play important roles in cell survival after ER stress. Recently, novel types of ER stress transducers that share a region of high sequence similarity with ATF6 have been identified. They have a transmembrane domain, which allows them to associate with the ER, and possess a transcription–activation domain and a bZIP domain. These membrane-bound bZIP transcription factors include Luman, OASIS, BBF2H7, CREBH and CREB4. Despite their structural similarities with ATF6, differences in activating stimuli, tissue distribution and response element binding indicate specialized functions of each member on regulating the UPR in specific organs and tissues. Here, we summarize our current understanding of the biochemical characteristics and physiological functions of the ER-resident bZIP transcription factors.

Keywords: bZIP transcription factor/endoplasmic reticulum stress/OASIS family/regulated intramembrane proteolysis/unfolded protein response.

Abbreviations: AIbZIP, androgen-induced bZIP; APC, antigen-presenting cell; APR, acute phase response; ATF6, activating transcription factor 6; BBF-2, box B-binding factor-2; BBF2H7, BBF2 human homolog on chromosome 7; BFA, brefeldin A; BiP, immunoglobulin heavy chain-binding protein; bZIP, basic leucine zipper; CLSD, cranio-lenticulo-sutural dysplasia; CNS, central nervous system; Col1, type I collagen; CRE, cyclic AMP-responsive element; CREB, cyclic AMP-response element-binding protein; CREB4, cyclic AMP-response element-binding protein 4; CREBH, cyclic AMP-response element-binding protein H; CRP, C-reactive protein; DC, dendritic cell; DC-STAMP, dendritic cell specific TrAnsMembrane protein; ECM, extracellular matrix; EDEM, endoplasmic reticulum degradation-enhancing mannosidase-like protein; eIF2, eukaryotic initiation factor-2; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERSE, endoplasmic reticulum stress response element; HCF, host cell factor; Herp, homocysteine-induced ER protein; IRE1, inositol requiring 1; KA, kainic acid; LPS, lipopolysaccharide; LRF, Luman recruitment factor; OASIS, old astrocyte specifically induced substance; PEPCK, phosphoenolpyruvate carboxykinase; PERK, PKR-like endoplasmic reticulum kinase; PKR, double-stranded RNA-dependent protein kinase; PSA, prostate-specific antigen; RAMP4, ribosome associated membrane protein; RIP, regulated intramembrane proteolysis; S1P, Site-1 protease; S2P, Site-2 protease; SAP, serum amyloid P-component; UPR, unfolded protein response; UPRE, unfolded protein response element.

The endoplasmic reticulum (ER) is a central cellular organelle responsible for the synthesis, folding and post-translational modifications of proteins destined for the secretory pathway. The ER is also a critical organelle for calcium homeostasis. Various pathophysiological conditions, such as ER-calcium depletion, oxidative stress, hypoglycaemia, expression of mutated proteins and hypoxia, interfere with the correct folding of proteins and these misfolded or unfolded proteins accumulate in the ER lumen. These conditions, which are collectively termed ER stress, have the potential to induce cellular damage. The ER responds to these perturbations by activating an integrated signal transduction pathway, called the unfolded protein response (UPR) (*1*–*3*). Activation of the UPR leads to a transient translational attenuation to decrease the demands made on the organelle, transcriptional induction of genes encoding ER-resident chaperones to facilitate protein folding, and ER-associated degradation (ERAD) to degrade the unfolded proteins that have accumulated in the ER. In mammalian cells, ER stress sensing and signalling involves three well-established and ubiquitous ER stress transducers: double-stranded RNA-dependent protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) (*4*), inositol-requiring enzyme-1 (IRE1) (*5*, *6*) and activating transcription factor 6 (ATF6) (*7*, *8*). PERK directly phosphorylates the α−subunit of eukaryotic initiation factor (eIF2α) and this event

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leads to a dramatic reduction in cellular protein synthesis (*9*). IRE1, ATF6 and ATF4, which is downstream of the PERK-eIF2α pathway, are involved in transcriptional regulation for ER molecular chaperones, ERAD-related genes, amino acid transport and metabolism proteins. If these effects are not sufficient to relieve the protein folding demands, and unfolded/misfolded proteins continue to accumulate, the cell activates apoptotic signalling events (*2*, *3*).

Recently, novel types of ER stress transducers, ER-resident transcription factors that share a region of high sequence similarity with ATF6, have been identified. They have a transmembrane domain, which allows them to associate with the ER, and possess both a transcription-activation domain and a basic leucine zipper (bZIP) domain. The new types of ER stress transducers include Luman/LZIP/CREB3 (*10*, *11*), OASIS/CREB3L1 (*12*), BBF2H7/CREB3L2 (*13*), CREBH/CREB3L3 (*14*) and CREB4/AIbZIP/Tisp40/CREB3L4 (*15*, *16*). In this review, these bZIP transmembrane transcription factors are referred to as the OASIS (old astrocyte specifically induced substance) family members. Despite structural similarities among these proteins and ATF6, differences in activating stimuli, tissue distribution and response element binding indicate that these proteins play specific functions in regulating the UPR in specific organs and tissues. Here, we summarize our current understanding of the biochemical characteristics and physiological functions of the ER-resident bZIP transcription factors. A growing body of new work indicates that the UPR branches regulated by OASIS family members play essential roles in cell differentiation and maturation or maintenance of basal cellular homeostasis in mammals.

Structures and distribution of ER-resident transmembrane bZIP transcription factors

In humans, there are more than 55 known bZIP transcription factors (*17*). By sequence similarity in the coiled-coil region, these transcription factors can be divided into 16 different families. Luman/LZIP/CREB3, OASIS/CREB3L1, BBF2H7/CREB3L2, CREBH/CREB3L3 and CREB4/AIbZIP/Tisp40/CREB3L4 are identified as members of the OASIS family. Members of the OASIS family are distinguishable from the ATF6 α/β family group; however, their domain structures are considerably similar within each group (Fig. 1). They have a transmembrane domain, which allows them to associate with the ER, and possess both a transcription–activation domain and a bZIP domain. bZIP domains are extremely well conserved within these members. An additional region of ~30 residues is located adjacent to the N-terminal end of the bZIP region, and is conserved in the OASIS family members, but missing in ATF6. As this region is not a conserved site of the typical consensus bZIP DNA binding domain, distinct and particular roles are provided in this unique sequence. Further, each of them commonly contains the consensus sequence for cleavage by site-1 protease (S1P) in its lumenal segment, indicating that they are processed within the

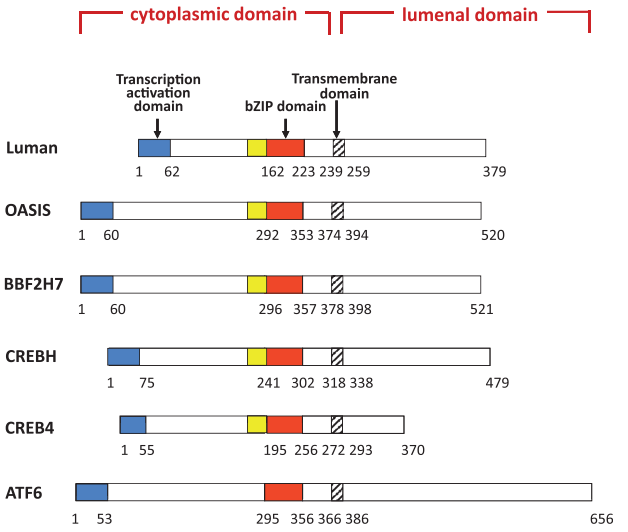

Fig. 1 Predicted peptide features of mouse bZIP transmembrane transcription factors. OASIS family members including Luman, OASIS, BBF2H7, CREBH and CREB4 share regions of high sequence similarity with ATF6. They have a transmembrane domain, a bZIP domain, and a transcription activation domain. About 30 amino acids adjacent to the N-terminal end of the bZIP region are conserved in the Luman, OASIS, BBF2H7, CREBH and CREB4 proteins (yellow box), but are absent in ATF6.

transmembrane regions by regulated intramembrane proteolysis (RIP) (*18*), as observed for ATF6.

Comparison of the sequences between OASIS family members and ATF6 show that the lumenal domains of each OASIS family member have little homology with that of ATF6. The lumenal domain in ATF6 has the significant sites for immunoglobulin heavy chain-binding protein (BiP) binding and Golgi localization signalling that are important for sensing the unfolded proteins and translocalization from the ER to the Golgi, respectively (*19*). Therefore, the sensing of unfolded proteins and translocation system from the ER to the Golgi are quite different between OASIS family members and ATF6.

OASIS family proteins (except Luman) reveal unique cell or tissue-specific expression patterns (Table I). OASIS, BBF2H7 (BBF2 human homolog on chromosome 7), CREBH (cyclic AMP-response element-binding protein H) and CREB4 (cyclic AMP-response element-binding protein 4) are preferentially expressed in osteoblasts and astrocytes (*12*, *20*), chondrocytes (*21*), liver cells (*14*, *22*), and testis and prostate (*15*, *23*, *24*), respectively, indicating that these transcription factors may be associated with a distinct physiological response that is dependent on the cell or tissue.

Evolutionally, orthologues of ATF6 and OASIS family proteins exist in organisms higher than *Caenorhabditis elegans*. *Caenorhabditis elegans* contains three bZIP transcription factors, and although the degree of homology is less than that within the mammalian family, these proteins are most closely related to ATF6, OASIS and Luman (*25*, *26*). In Drosophila, two bZIP transcription factors are closely related to ATF6 and OASIS (*25*). In vertebrates, cells

Table I. Biological characteristics of OASIS family members.

	Expressing cells/tissues	Target elements	Target genes	ER stress-dependent RIP	Biological functions	Phenotypes of KO mice
Luman	Various tissues (mRNA), TGN[a], monocyte, dendritic cells (protein)	ERSE-II, UPRE-like	Herp, EDEM	–	Anti-apoptotic, dendritic cell maturation	Not reported
OASIS	Osteoblast, astrocyte, intestine, salivary glands, etc.	CRE-like	Col1a1	+	Osteoblast differentiation, bone formation	Osteopenia, decreased bone density, spontaneous fracture
BBF2H7	Chondrocyte, lung, spleen, gonad, neuron	CRE-like	Sec23a	+	Chondrogenesis, formation of COPII vesicle	Chondrodysplasia, abnormal expansion of rough ER
CREBH	Liver, intestine, stomach	CRE, box-B, ATF6-site, ERSE-I,II	CRP, SAP, hepcidin	+	Induction of APR pathway, iron homeostasis	No morphological/ developmental defects, failure of APR and iron homeostasis
CREB4	Prostate, pancreas, brain (human), testis, intestine (mouse)	UPRE	RAMP4, EDEM?	–	Spermatogenesis	Apoptosis of spermatozoa

[a]Trigeminal ganglional neuron.

differentiate to play roles for various biological phenomena, and also adapt to environmental parameters. Vertebrate cells adjust the functionality and capacity of their ER. This adjustment is dependent on the diversity of the cell types. Thus, the signalling of the vertebrate UPR, which is regulated by canonical ER stress transducers (PERK, IRE1 and ATF6) and OASIS family members, has considerable complexity and is highly developed in cell-type specific patterns.

Luman/CREB3

Luman/CREB3 (also known as LZIP) was first identified through its association with herpes simplex virus-related host cell factor (HCF) (*27–29*). The Luman transcript is present in a wide range of adult and fetal tissues (*29*), but its translated product has only been found in trigeminal ganglional neurons and monocytes, and dendritic cells (DCs) (*30–32*), which are the professional antigen-presenting cells (APCs). Processing of Luman at the transmembrane domain is highly stimulated by brefeldin A (BFA), a compound that causes the reflux of the Golgi apparatus enzymes to the ER. In addition, co-expression of Luman with S1P containing a KDEL ER retrieval signal results in virtually quantitative cleavage of Luman in the absence of any treatment, providing evidence that Luman undergoes proteolytic processing as a RIP step (*33*). However, the RIP of Luman is not induced by ER stressors including tunicamycin, an inhibitor of N-linked glycosylation, and thapsigargin, a drug that blocks the ER calcium-ATPase pump (*33*, *34*). Only the report by Liang *et al.* (*35*) suggested that the activation of Luman may be induced by thapsigargin. Thus, it is unclear what types of stimuli are physiologically needed for the RIP of Luman.

ERAD-related gene Herp (homocysteine-induced ER protein) was identified as one of the direct targets of Luman. The transcription by Luman is activated by acting on the ER stress response element II (ERSE-II; ATTGG-N-CCACG) in the promoter region of Herp via physical association with the second half-site (CCACG) of ERSE-II (*35*). Luman can also induce another ERAD gene, endoplasmic reticulum degradation-enhancing mannosidase-like protein (EDEM) (*36*), likely through an unfolded protein response element (UPRE)-like element found in its promoter (*34*). Expression of Luman enhances cellular tolerance of ER stress and protection of cells from ER stress-induced cell death (*35*). Herp is known to associate with the components of the ERAD pathway (*37*). Further, Herp also prevents ER stress-induced apoptotic cell death in a similar fashion as Luman (*38*, *39*). Taken together, Luman may have an adaptive response to perturbations in the ER through the activation of the ERAD pathway.

A novel Luman recruitment factor (LRF), which is a UPR-responsive basic-region leucine zipper protein that is prone to proteasomal degradation, was identified as an interacting partner of Luman (*40*). LRF directly binds to proteolytically processed Luman through the leucine zipper region. LRF recruits the nuclear form of Luman to discrete nuclear foci and promotes Luman degradation followed by repression of its transactivation activity. It is therefore proposed that LRF is a negative regulator in the mammalian UPR by repressing the transactivation activity of Luman, especially in the EDEM- and Herp-related ERAD pathways. The Dendritic Cell-Specific TrAnsMembrane protein (DC-STAMP) was also identified as a physically interacting partner of Luman (Fig. 2) (*32*). DC-STAMP is a dendritic cell-specific multi-membrane spanning protein composed of 470 amino acids that is localized to the ER (*41–43*). Mapping of the interaction domains of DC-STAMP and Luman by a yeast two-hybrid system suggests that the amino acid sequence of Luman required for binding to DC-STAMP involves the central part of the

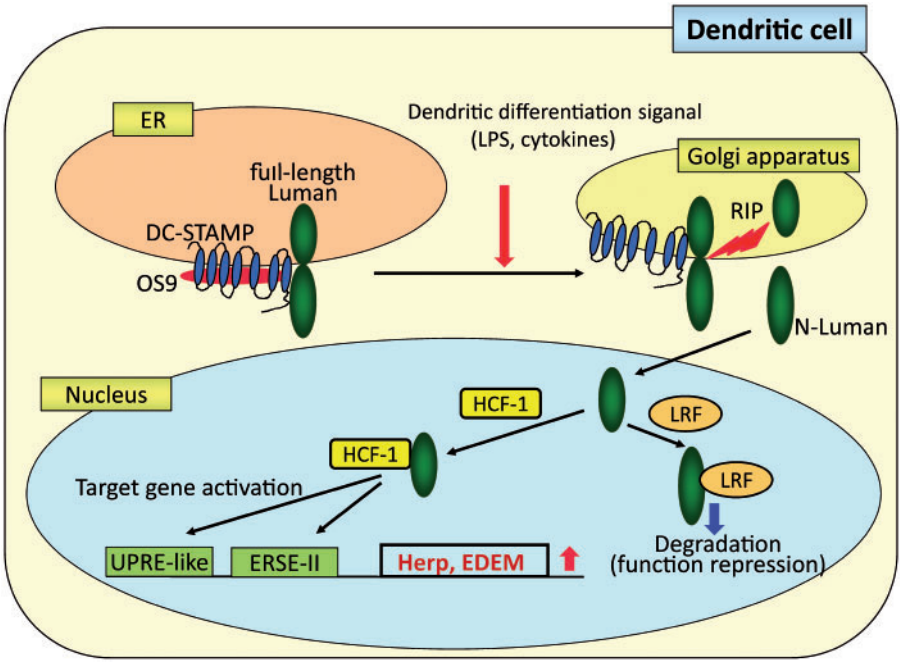

Fig. 2 The putative signalling pathways and cellular functions regulated by Luman. Luman, DC-STAMP and OS9 interact with each other at the cytosolic site of the ER. Upon dendritic maturation, the Luman/DC-STAMP complex translocates to the Golgi apparatus and Luman is subjected to RIP. The Cleaved N-terminal fragment of Luman is moved into the nucleus. In the absence of the interaction with LRF, Luman forms a complex with the co-factor HCF-1, and binds to UPRE-like or ERSE-II sequences to promote transcription of Herp and EDEM. When Luman interacts with LRF in the nucleus, Luman is rapidly degraded by the proteasome and transcription of the target genes is suppressed.

sequence, including its transmembrane region. DC-STAMP not only co-immunoprecipitates with Luman, but also with the previously identified DC-STAMP-interacting protein OS9 (*44*), indicating that Luman, DC-STAMP and OS9 are part of the same complex. Luman is expressed in the ER of immature DCs. Interestingly, upon DC maturation, Luman is proteolytically activated and its cleaved fragments translocate to the nucleus. DC-STAMP is also localized to the ER in immature DC, and upon DC maturation, it translocates towards the Golgi compartment rather than the nucleus. Taken together, the following model is proposed. In immature DC, Luman interacts with DC-STAMP and OS9 in the ER. Upon DC maturation, the Luman/DC-STAMP complex is released from the ER and the complex translocates to the Golgi. Luman is then subjected to the RIP process and its N-terminal portion relocates to the nucleus (*32*). This model implies that DC-STAMP fulfils a role as a transport protein, much akin to the role of SCAP in SREBP activation (*45*). Although this model is attractive, the mechanisms responsible for sensing and triggering translocation of the complex from the ER to the Golgi apparatus remain unclear. Furthermore, it is not known how the pathway regulated by Luman and its interacting partner proteins are implicated in the DC differentiation and maturation process.

OASIS/CREB3L1

The *OASIS* gene was identified originally as a gene specifically induced in long-term cultured astrocytes (*46*). The OASIS protein is a bZIP transcription factor of the cyclic AMP-responsive element (CRE)-binding protein (CREB)/ATF family, with a transmembrane domain that allows it to associate with the ER as well as Luman. The N-terminus of the OASIS protein that contains the transmembrane domain is 31% identical to ATF6, but its C-terminus, the portion that is within the ER lumen, does not show homology to ATF6. In the lumenal segment, OASIS contains the sequence RSLL (beginning at residue 423), which fits the R (x) x L consensus for S1P whose active site faces the Golgi lumen. Indeed, under ER stress conditions, OASIS is cleaved at the site by S1P and subsequently at the transmembrane domain by site-2 protease (S2P) (*12*, *47*). ATF6 contains a stretch of amino acids in its lumenal domain that is essential for translocation from the ER to the Golgi (*48*). All deletion mutants for the lumenal domain of OASIS, however, reveal that proteolytic processing and translocation to the Golgi remain intact, indicating that OASIS does not have significant sequences for Golgi localization in its luminal domain (*47*). Therefore, the translocation system from the ER to the Golgi is quite different between OASIS and ATF6 under ER stress conditions. In a well-functioning and stress-free ER, ATF6 is constitutively bound by an ER resident chaperone, BiP, in its lumenal domain and rendered inactive (*19*, *48*). Accumulation of improperly folded proteins in the ER results in the dissociation of BiP from the lumenal domain of ATF6 and ATF6 is subsequently translocated to the Golgi apparatus. Interaction of the lumenal domain of OASIS with BiP has not been detected (Kondo *et al.*, unpublished data). Consequently, the

mechanisms responsible for sensing ER stress by OASIS remains unresolved.

OASIS is highly expressed in osteoblasts of bone tissues (*20*) and astrocytes in the central nervous system (CNS) (*12*, *49*), and considerably expressed in the intestine, salivary glands and prostate (*46*, *50*). In an injured brain induced by kainic acid (KA), OASIS is up-regulated in astrocytes of the hippocampus (*49*). In C6 glioma cell lines or primary cultured osteoblasts, OASIS is induced at the transcriptional level following treatment with ER stressors such as tunicamycin or thapsigargin (*12*).

OASIS deficient mice are born at the expected Mendelian ratios, but exhibit severe osteopenia involving a decrease in bone density at all skeletal sites, and often spontaneous fractures as well (*20*). Type I collagen, a major component of osseous tissues, was identified as one of the targets for OASIS. OASIS activates the transcription of Col1a1 (type I collagen a1 gene) through direct binding to a CRE-like sequence that exists in the Col1a1 promoter regions. OASIS deficient osseous tissues have shown that type I collagen levels are markedly reduced. The defect of bone formation involving decreased amounts of type I collagen is completely rescued by osteoblast-specific overexpression of OASIS, indicating that osteopenia in OASIS deficient mice is primarily caused by deletion of the OASIS gene in osteoblasts (*51*).

ER stress is necessary for the activation of OASIS. When and in what manner does ER stress occur in osteoblasts? In a culture model system, ER stress actually occurs during differentiation from mesenchymal stem cells to mature osteoblasts (*20*). However, the expression levels of ER stress markers, including BiP, CHOP and EDEM, in differentiating osteoblasts are comparably lower than those in cells treated with ER stressors. Thus, ER stress that occurs in immature osteoblasts is very mild and at levels that do not cause cell death. Although the detailed mechanisms of why ER stress occurs during osteoblast differentiation processes remains unclear, it is possible that when a bone matrix production switch is pressed at the stage of differentiation, nascent protein is delivered in amounts that exceed the capacity of the ER. Such an event may serve as a trigger for mild ER stress conditions (physiological ER stress, Fig. 3).

As mentioned above, OASIS is also highly expressed in astrocytes of the CNS. In hippocampal astrocytes injured by intraperitoneal injection of kainic acid, ER stress is induced and OASIS mRNA is strongly up-regulated (*49*). Primary cultured OASIS deficient astrocytes reveal reduced vulnerability to ER stress. Pyramidal neurons in the hippocampi of OASIS deficient mice are more susceptible to the toxicity induced by KA than those of wild-type mice, suggesting that OASIS plays important roles in protection against the neuronal damage induced by KA (*49*). Astrocytes are well known to produce and secrete various neurotrophic factors to protect the surrounding neurons under neurodegenerative conditions (*52*). To provide sufficient amounts of proteins, such as neurotrophic factors for the survival of neurons, it is quite possible that the ER is mildly burdened and this mild ER stress triggers the activation of OASIS.

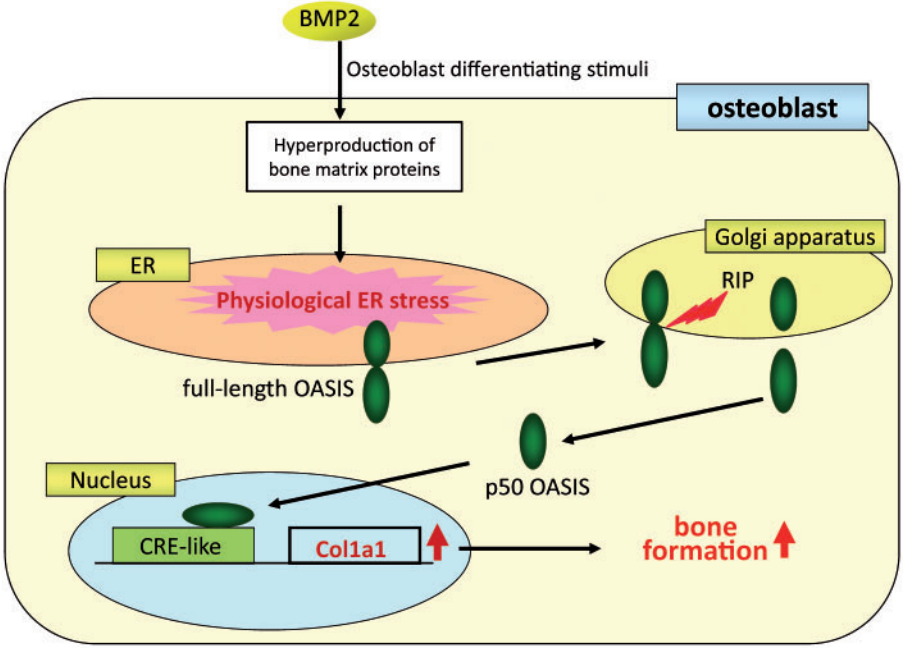

Fig. 3 Schematic representation of the putative mechanisms responsible for bone formation by OASIS. BMP2, which is required for bone formation, induces mild ER stress (physiological ER stress) in developing osteoblasts, and results in the translocation of OASIS from the ER to the Golgi apparatus and cleavage at the transmembrane region of OASIS. Physiological ER stress in developing osteoblasts induced by the BMP2 signalling pathway is presumed to be associated with a high demand for synthesis and secretion of bone matrix proteins. P50 OASIS directly binds to the CRE-like sequence in the *Col1a1* promoter region, and induces its transcription for bone formation.

BBF2H7/CREB3L2

BBF2H7 was identified by virtue of its involvement in a gene translocation in low-grade fibromyxoid sarcoma, resulting in a fusion event between the FUS gene on chromosome 16 and the BBF2H7 gene on chromosome 7 (*53*). BBF2H7 is a transmembrane transcription factor that is closely related to the structure to OASIS (Fig. 1), and is also cleaved at the membrane region by RIP in response to ER stress (*13*). Where it differs from OASIS is in its expression patterns. It is most strongly expressed in the proliferating zone of cartilage during the development of long bones (*21*). BBF2H7 is also expressed in the lungs, spleen, gonad and nervous system (*13*) although it is not expressed at all in osseous tissues (*21*).

BBF2H7-deficient mice reveal severe chondrodysplasia and die by suffocation shortly after birth, because of an immature chest cavity (*21*). In BBF2H7-deficient cartilage, the typical columnar structure is lack in the proliferating zone and the size of the hypertrophic zone is reduced because of a significant reduction in the amount of extracellular matrix (ECM) proteins. Surprisingly, proliferating chondrocytes show abnormally expanded rough ER containing aggregated type II collagen and cartilage oligomeric matrix protein (COMP), indicating that secretion of ECM proteins from chondrocytes in the proliferating zone is prevented in BBF2H7-deficient mice, and this defect in protein secretion leads to the disruption of the ECM network in cartilage, and of cartilage zone formation.

Chondrocytes secrete cartilage ECM proteins during differentiation, and corresponding with the secretion, ER stress markers BiP, PDI, GRP94 and EDEM are slightly up-regulated, indicating that mild ER stress is induced in chondrocytes during their normal differentiation (*21*). Induction of BBF2H7 mRNA and RIP of BBF2H7 are initiated at this stage in the differentiation of chondrocytes. Thus, BBF2H7 activation depends on mild ER stress caused by the augmented production of cartilage matrix proteins. In BBF2H7-deficient chondrocytes, the expression of mature differentiation markers delays its peak. Further, the expression of ER stress-related genes increases and induction is prolonged. Taken together, deletion of the *BBF2H7* gene disturbs chondrocyte differentiation and causes severe ER stress with the accumulation of ECM proteins in the ER lumen.

Sec23a was identified as a target for BBF2H7 (*21*). Active forms of BBF2H7 (N-terminal domain cleaved at the transmembrane domain) directly bind to the CRE-like sequence in the promoter region of Sec23a (Fig. 4). Sec23a recruits other components of the COPII vesicle including Sec13/31, and completes the complex before transporting secretory proteins from the ER to the Golgi (*54*, *55*). Recently, it has been reported that Sec23a is a genetic cause of craniolenticulo-sutural dysplasia (CLSD), which is characterized by a malformed craniofacial structure involving

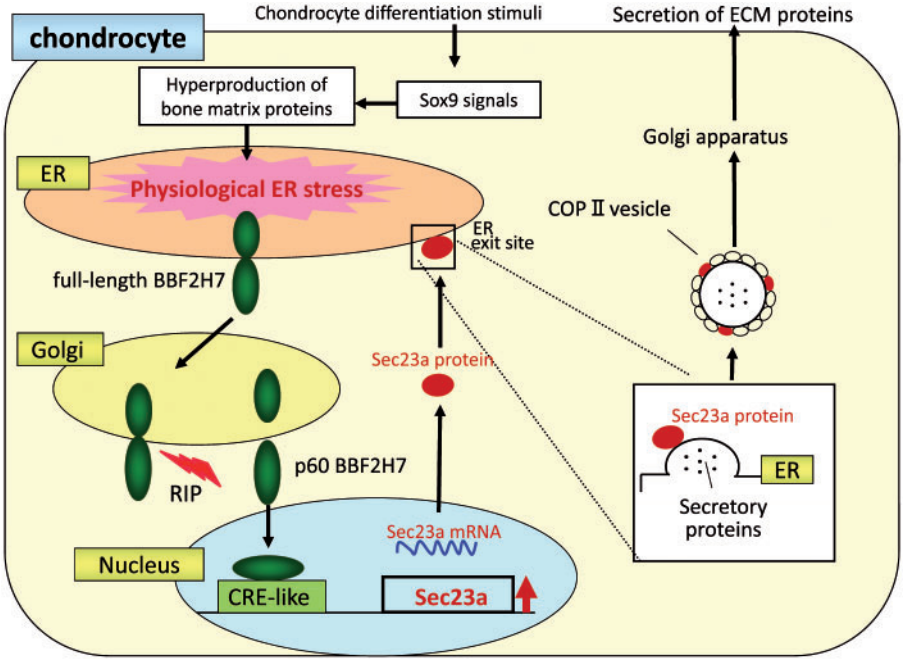

Fig. 4 Schematic representation of the putative signalling pathway and function of BBF2H7. Transcription factor Sox9 is essential for chondrocyte differentiation. Sox9 facilitates the synthesis of various proteins for chondrocyte differentiation and induces physiological ER stress in chondrocytes. BBF2H7 is cleaved at the transmembrane region in response to physiological ER stress. Activated N-terminal BBF2H7, p60 BBF2H7, directly binds to the CRE-like sequence within the Sec23a promoter region and facilitates its transcription. Sec23a has crucial roles in COPII vesicle formation and anterograde transport of cargo proteins from the ER to the Golgi. Sec23a recruits other components of the COPII vesicle, including Sec13 and Sec31, and completes the complex before transporting secretory proteins from the ER to the Golgi. The axis of the BBF2H7-Sec23a pathway is essential for the ER stress-coupled protein transport system from the ER to the Golgi in chondrocytes for the abundant secretion of ECM proteins.

chondrodysplasia and cataracts (*56*). The fibroblasts of CLSD patients have abnormally expanded rough ER, and secretory proteins are retained inside. These morphological changes are very similar to those observed with BBF2H7 deficient chondrocytes (*21*). Thus, Sec23a has crucial roles in COPII vesicle formation and anterograde transport of cargo proteins from the ER to the Golgi. For chondrocytes to secrete large amounts of cartilage ECM proteins, it is necessary to smoothly transport secretory materials from the ER to either the Golgi or cell membranes. It is inevitable that such functions will be acquired during the process of growing from an undifferentiated state into mature chondrocytes, and activation of the BBF2H7-Sec23a pathway is essential for developing a series of secretory machinery.

CREBH/CREB3L3

CREBH was identified as a hepatocyte-specific bZIP transcription factor belonging to the CREB/ATF family (*57*). CREBH was also known as a gene whose expression is dependent on hepatocyte nuclear factor 4α (*58*), which is a nuclear hormone receptor that is essential for initiating and maintaining hepatocyte differentiation and liver function (*59–61*). Although CREBH is robustly expressed in the liver, it is also expressed in the small intestine and stomach at lower levels (*57*, *58*). The R (x) x L sequence, which is hallmark for S1P recognition, is located in the lumenal domain of CREBH at sites 18 residues distal from the transmembrane domain. Furthermore, CREBH has a proline in its hydrophobic transmembrane domain at a location similar to the conserved proline for S2P cleavage identified in SREBP and ATF6. Indeed, in response to ER stress, CREBH is cleaved by S1P and S2P to liberate an amino-terminal fragment (*14*). Mutant CREBH abrogated N-linked glycosylation sites are unstable, unfolded or aggregated (*62*). Unglycosylated CREBH is largely uncleaved even if treated with brefeldin A, suggesting that N-linked glycosylation is essential for the activation of CREBH by intramembrane proteolysis.

The fragment of CREBH binds to the CRE, box B and ATF6-binding element. CREBH also binds less potently to the CRE located in the rat hepatic gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) gene, ERSE-I and ERSE-II (*63*), and activates the promoter of PEPCK. The PEPCK promoter activities by CREBH are further stimulated by cyclic AMP and protein kinase A, indicating PEPCK represents a physiological target by CREBH in the liver.

To explore the physiological functions of CREBH, CREBH-knockdown mice were generated by injection of CREBH-specific RNAi lentivirus into single-cell mouse embryos (*14*). Although histopathological analysis of the knockdown embryos at E14.5 did not reveal any morphological or developmental defects, expression of genes involved in the acute phase response (APR) including C-reactive protein (CRP) and serum amyloid P-component (SAP) was significantly reduced. Both CRP and SAP are inducible by stimulation with proinflammatory cytokines or bacterial lipopolysaccharide (LPS). However, the induction of mRNAs and serum CRP and SAP after injection of IL6 plus IL1 or LPS were significantly inhibited in the CREBH knockdown mice, indicating that CREBH is required to activate transcription of CRP and SAP genes in response to proinflammatory cytokines and LPS. ER stress increased expression of the CRP and SAP mRNA. Further, the stimulation of proinflammatory cytokines or LPS promoted the cleavage of CREBH in the liver of wild-type mice; the cleavage of which showed very similar patterns to those of upregulation of UPR-related genes such as BiP and spliced XBP1 mRNA. In contrast, full-length and cleaved CREBH were not detected in the livers of CREBH knockdown mice under the same conditions (*14*). These results provide evidence that proinflammatory cytokines and LPS induce ER stress and cause cleavage of CREBH followed by the activation of the APR pathway in the liver.

The N-terminal portion of CREBH has the potential to interact with the cleaved fragment of ATF6, and synergistically activate transcription of CRP and SAP (*14*). The results provide new insight into ATF6 functions that serve as a potent enhancer to augment CREBH-mediated acute inflammatory responses (Fig. 5). The functions of the other transmembrane bZIP transcription factors, such as Luman, OASIS, BBF2H7 and CREB4, may also be modulated by forming a heterodimer with ATF6.

Both LPS and IL6 are also potent inducers of hepcidin (*64*, *65*), which is a peptide hormone that is secreted by the liver and controls body iron homeostasis (*66*). ER stress is also demonstrated to induce hepcidin, and the induction is defective in CREBH knockout mice (*67*). Further, CREBH binds to and transactivates the hepcidin promoter, indicating that CREBH plays roles in ER stress-regulated hepcidin expression. The regulation of hepcidin expression by CREBH may link iron metabolism to inflammatory responses due to ER stress (Fig. 5).

CREB4/AIbZIP/Tisp40/CREB3L4

CREB4 was originally cloned during large-scale sequencing analysis of human cDNA libraries (*68*), with transcripts highly detected in human prostate, brain and pancreas tissues. A second group identified a transcription factor that is highly expressed in human prostate and prostate tumours, and is induced by treatment with androgen. The gene was named androgen-induced bZIP (AIbZIP) (*69*). At almost the same time, the mouse homologue of CREB4, also referred to as Tisp40 (*70*) or ATCE1 (*16*), was identified as a factor involved in spermatogenesis, and is specifically expressed in mouse testis. Consequently, existing reports on the expression of CREB4 in murine and human tissues are somewhat controversial.

The mouse CREB4 gene generates two types of proteins, CREB4α and CREB4β, differing by an N-terminal extension of 55 amino acids (*15*). Both isoforms contain a transmembrane domain and localize to the ER. In the lumenal domain, CREB4 contains

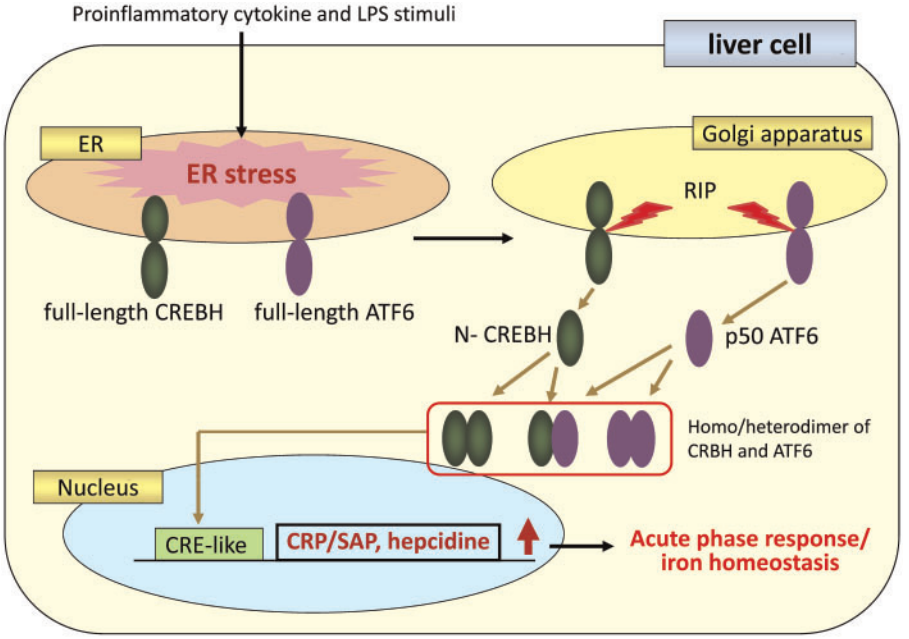

Fig. 5 Putative roles of CREBH in the APR and iron homeostasis. Proinflammatory cytokines and LPS induce ER stress followed by cleavage of CREBH and ATF6. These activated fragments of CREBH and ATF6 form homodimers/heterodimers and promote transcription of APR genes such as CRP and SAP. Hepcidine, which is a peptide hormone that is secreted by the liver, and controls body iron homeostasis, is also induced by CREBH after proinflammatory stimuli or ER stress.

the cleavage site for S1P adjacent to the transmembrane domain, indicating that CREB4 may also be cleaved by RIP, as is the case for other transmembrane bZIP transcription factors. However, CREB4 is resistant to brefeldin A-induced cleavage, and is not cleaved by co-expression with S1P-KDEL. The cleavage is detected only after deletion or substitution of its C-terminal lumenal domain (*16*). Deletion of the CREB4 transmembrane domain results in nuclear accumulation and a truncated N-terminal form of CREB4 potently activates expression of target genes. These results indicate that the CREB4 C-terminal lumenal domain acts as a negative regulator of S1P-mediated cleavage, and cleavage of CREB4 within the transmembrane region is regulated by mechanisms that are different from those found in other bZIP transcription factors.

The N-terminals of both CREB4α and β have the potential to bind to a UPRE sequence (TGACGTGG), but not to closely related CRE sequences (TGACGTCA) (*15*). CREB4β, but not CREB4α, activates transcription of a reporter gene carrying 5×UPRE. Overexpression of N-terminal CREB4β strongly induces the expression of EDEM, which is presumably mediated by a putative UPRE. In contrast, CREB4α appears to weakly induce expression of EDEM, suggesting that the CREB4 gene generates not only an inactive transcription factor, CREB4α, but also an active transcription factor, CREB4β. CREB4 knockout mice reveal that the mRNA levels of EDEM are unchanged (*71*), indicating that the endogenous targets of CREB4 are another set of genes different from EDEM. In the testis of CREB4 knockout mice, RAMP4 (ribosome associated membrane protein 4), which acts to stabilize transmembrane proteins (*72*), is up-regulated. The mechanisms responsible for the up-regulation of RAMP4 in the testis of CREB4 knockout mice are presumed to involve CREB4α suppressing the transcription of the RAMP4 gene by covering the UPRE in its enhancer region. As such, CREB4α can no longer suppress the region in CREB4 knockout testis, and this leads to the up-regulation of the RAMP4 gene (Fig. 6).

CREB4 knockout mice are born at the expected ratios, are healthy and display normal survival rates (*72*, *73*). The breeding of knockout males with wild females is productive and the average litter size is not altered although the number of spermatozoa in the epididymis of CREB4 knockout mice is significantly reduced. The seminiferous tubules of CREB4 knockout mice contain all of the developmental stages, but apoptosis of meiotic/post-meiotic germ cells is increased (*72*, *73*). The apoptosis is presumed to be due to excess ER stress and resultant activation of caspase 12 (*72*), suggesting that CREB4 is involved in the ER stress response process during spermiogenesis and plays a role in male germ cell development.

CREB4 is also expressed in Paneth and goblet cells in the intestines (*74*). The ETS-domain transcription factor SPDEF (SAM-pointed domain-containing ETS-like factor) acts downstream of Math1 (*75*, *76*) and promotes terminal differentiation of a secretory progenitor pool into Paneth and goblet cells (*74*, *77*). Maturation of Paneth and goblet cells is impaired in SPDEF deficient mice, whereas immature secretory progenitors are accumulated in the intestine (*74*). At the same time, the expression of CREB4 is abolished in SPDEF knockout Paneth and goblet cells. SPDEF has been known to bind to a GGAT core

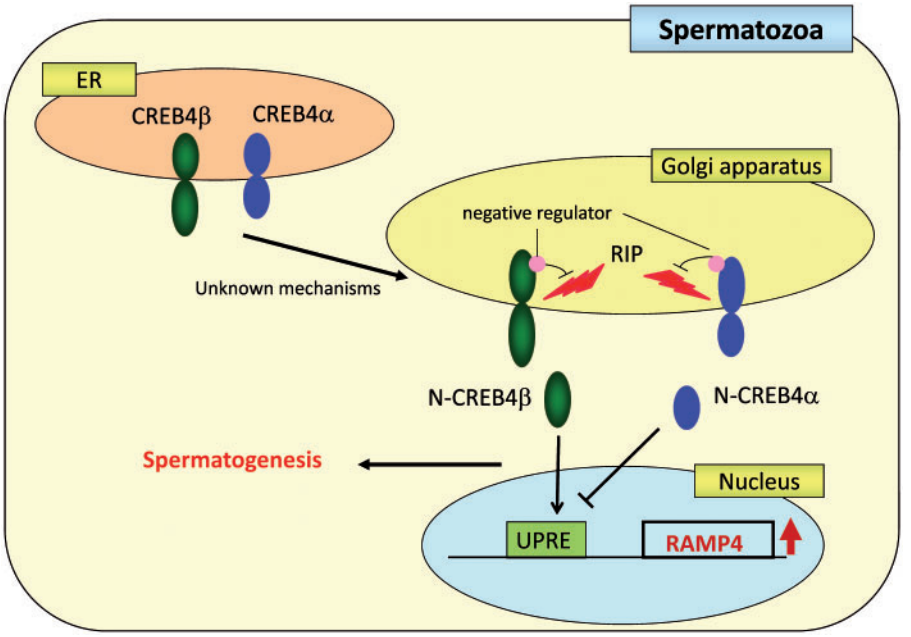

Fig. 6 Putative signalling mediated by CREB4 in spermatozoa. The murine CREB4 gene is preferentially expressed in testis and generates two types of proteins, CREB4α and CREB4β, differing by an N-terminal extension of 55 amino acids. C-terminal lumenal domains of both isoforms act as negative regulators of S1P-mediated cleavage. CREB4β has the potential to bind to the UPRE sequence (TGACGTGG) and promote transcription of RAMP4 (ribosome associated membrane protein 4), which acts to stabilize transmembrane proteins. In contrast, CREB4α suppresses the transcription of RAMP4 by masking the UPRE in RAMP4's enhancer region. The number of spermatozoa in the epididymis of CREB4 knockout mice is significantly reduced, suggesting that CREB4 plays a role in male germ cell development.

sequence in the prostate-specific antigen (PSA) promoter (*78*). Interestingly, this core sequence is scattered in the 5′-upstream of both human and mouse CREB4 genes (Saito *et al*., unpublished data). Taken together, it is possible that the expression of CREB4 is regulated by SPDEF, and CREB4 plays roles in terminal differentiation of Paneth and goblet cells from secretory progenitors downstream of SPDEF.

Conclusion

UPR signalling was originally found as an evading system from cellular damage in acute ER perturbation. However, recent advances have revealed that UPR signalling provides important signals for regulating cellular physiology. Not only canonical ER stress transducers, but also OASIS family members, act as signalling centres for numerous networks originating from the ER. In particular, OASIS family members are specialized in biological regulation including cell differentiation, maturation and maintenance of basal cellular homeostasis: Luman in dendritic cell differentiation (*32*); OASIS in osteogenesis and osteoblast maturation (*20*); BBF2H7 in secretion of matrix proteins and chondrocyte differentiation (*21*); CREBH in the acute phase response in the liver (*14*); and CREB4 in spermatogenesis (*72*, *73*) and goblet and Paneth cell differentiation in the intestine (*74*). The signalling from the ER mediated by OASIS family members is presumed to be triggered by ER perturbation. However, is the ER stress actually only the stimulation for activating OASIS family members that are required for initiating physiologically important signalling events? OASIS family members may possibly be activated by other mechanisms different from ER stress. To elucidate the physiological functions of these bZIP transmembrane transcription factors, the mechanisms responsible for their activation in specific tissues and cells requires further research effort.

It is widely known that bZIP type transcription factors can form homo- and/or heterodimers, and this dimerization often affects their binding ability. Indeed, ATF6 can bind to active forms of CREBH and serve as a potent enhancer to augment CREBH-mediated APR gene transcription for the acute inflammatory response (*14*). It is possible that changing the binding partners for each transcription factor may switch the target genes promoting the expression, and carry out fine tuning of UPR signalling for regulating physiological phenomena. OASIS family proteins except Luman reveal unique cell or tissue-specific expression patterns, but overlapping expression is also observed in each protein in the same cells or tissues. Thus, it is necessary to analyse the spatiotemporal hetero/homo dimerization of each protein *in vivo* to better understand the diversity of the UPR system in mammals.

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Conflict of interest
None declared.

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