EXPRESSIN AND REGULATED NUCLEAR TRANSPORT OF TRANSDUCERS OF REGULATED CREB 1 IN RETINAL GANGLION CELLS


Departments of Ophthalmology, Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510630, China
State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou 510060, China
Laboratory of Cell Signal and Metabolism, National Institute of Biomedical Innovation, 7-6-8, Asagi, Saito, Ibaraki, Osaka 567-0085, Japan
Institute of Neuroscience and State Key Laboratory of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China

Abstract—Calcium- and cAMP-dependent activation of CREB and transcription of a CAMP-responsive element (CRE)–target genes play critical roles in various physiological and pathological conditions. TORCs (transducers of regulated CREB) represent a new family of conserved CREB coactivators that function as intracellular calcium- and CAMP-sensitive coincidence detectors, controlling the kinetics of CRE-mediated responses and long-term potentiation of synaptic transmission. Here we examined the expression and activity-dependent translocation of TORCs in adult retinal ganglion cells (RGCs), the primary target of acute retinal ischemic injury as well as chronic retinal degenerative diseases. TORC1 is enriched in RGCs and its subcellular location could be regulated by various stimuli, including NMDA or increase of cAMP signaling by administration of forskolin. Enriched expression of TORC1 in brain tissue has been reported consistently (Zhou et al., 2006; Altarejos et al., 2007; Kitagawa et al., 2007; Hietakangas et al., 2008). It is also reported that TORC1 is required for fertility through induction of CRE-target gene transcription in responding to increased intracellular Ca2+ and/or cAMP (Conkright et al., 2003; Bittinger et al., 2004; Screaton et al., 2004). Physiologically, activation of TORCs activity is critical for the homeostasis of glucose (Koo et al., 2005), the biogenesis of mitochondrial in muscle cells (Wu et al., 2006) as well as the activation of HTLV-1 (human T-cell leukemia virus type 1) transcription by Tax (Siu et al., 2006).

Recent evidence shows that starvation triggers TORC activation in Drosophila, where it maintains energy balance through induction of CREB target genes (Wang et al., 2008). It is also reported that TORC1 is required for fertility via regulation of leptin signaling (Altarejos et al., 2008). In the CNS, TORC1 was reported as an essential regulator for the activity-dependent transcription of BDNF and the maintenance of late-phase long-term potentiation in hippocampus (Zhou et al., 2006; Kovac et al., 2007). TORCs have also been implicated in many pathological processes of the CNS, like drug addiction and ischemic injury (Wu et al., 2007; Kitagawa et al., 2007; Hietakangas et al., 2008). Enriched expression of TORC1 in brain tissue has been reported consistently (Zhou et al., 2006; Altarejos et al., 2008), however, the expression of TORC1 and its functional implication in retina have not been studied.

Fleeting episodes of neuronal activity results in long-lasting changes of structure and function of neurons, circuitry through induction of new gene expression (Badia et al., 1991). Many extracellular stimuli activate neurons via increasing intracellular Ca2+ and cAMP levels and consequently upregulate the transcription of genes containing the CAMP-responsive element (CRE) in their promoter regions (Dash et al., 1991; Bading et al., 1993; Shaywitz et al., 1999). Among the CRE-target genes, if not all, in neurons are critical for physiological processes such as neuronal survival, development, plasticity, learning and memory as well as pathological conditions such as neuroprotection and regeneration of damaged axons (Carlezon et al., 1998; Potter, 2001; Bleckmann et al., 2002; Lonze et al., 2002). Calcium- and cAMP-dependent activation of CRE-target gene transcription requires the phosphorylation of transcription factor CREB and the recruitment of its coactivators CBP and transducers of regulated CREB (TORCs) (Cardinaux et al., 2000; Mayr et al., 2001; Lonze et al., 2002; Lee et al., 2005). TORCs are recently identified as a new family of coactivators for CREB (lourenko et al., 2003). TORCs are sequestered in the cytoplasm in resting condition and translocated into nucleus to potentiate CRE-target gene transcription in responding to increased intracellular Ca2+ and/or cAMP (Conkright et al., 2003; Bittinger et al., 2004; Screaton et al., 2004). Physiologically, activation of TORCs activity is critical for the homeostasis of glucose (Koo et al., 2005), the biogenesis of mitochondrial in muscle cells (Wu et al., 2006) as well as the activation of HTLV-1 (human T-cell leukemia virus type 1) transcription by Tax (Siu et al., 2006).

Key words: TORC1, retinal ganglion cells, Ca2+, retinal ischemic injury.

*Corresponding author. Tel: +86-21-54921720; fax: +86-21-54921735 (Y. Zhou).
E-mail addresses: zhangxl2@mail.sysu.edu.cn (X.-L. Zhang), yzhou@ion.ac.cn (Y. Zhou).

Abbreviations: CRE, CAMP-responsive element; DEPC-PBS, 4% paraformaldehyde in 0.1 M phosphate buffer saline; RGC, retinal ganglion cell; RT-PCR, reverse transcription polymerase chain reaction; SC, superior colliculus; SD, Sprague-Dawley; SIK1, salt-inducible kinase 1; TORC, transducers of regulated CREB.
axons and survival of retinal ganglion cells (RGCs) (Yoshida et al., 1995; Stellwagen et al., 1999; Pham et al., 2001; Harvey et al., 2006). Under pathological states, irreversible degeneration and loss of RGCs are prominent feature of many retinal diseases (So et al., 1998; Kuehn et al., 2005). It is reported that BDNF, a well-studied CREB target gene, confers antiapoptotic effect on RGCs under acute retina ischemic injury or retinal nerve injury conditions (Unoki et al., 1994; Polo et al., 1998; Mo et al., 2002; Kuehn et al., 2005). Furthermore, expression of the CRE target gene Bcl-2 has been shown to promote regeneration of injured axon of RGCs (Jiao et al., 2005). These findings indicate that activation of CRE target genes may confer neuroprotective effects to RGCs during pathological conditions. In this study, we examined the expression TORC1 in adult RGCs as well as its regulated translocation after retinal ischemic injury.

**EXPERIMENTAL PROCEDURES**

**Animals and fluorogold labeling of RGCs**

All experiments were performed on adult male Sprague–Dawley (SD) rats (weight 200–220 g). The use and care of animals in this study are approved by the Institutional Animal and Use Committee of Shanghai Institutes for Biological Sciences. All experiments conformed to named international guidelines on the ethical use of animals. Animals were fed and maintained in cages in temperature-controlled rooms with a 12-h light/dark cycle with free access to food and water. Efforts were made to minimize the number of animals used and their suffering. Retrograde labeling of RGCs by application of fluorogold was performed as previously described (Deng et al., 1996; Li et al., 2006). Rats were anesthetized by a single intraperitoneal injection of chloral hydrate; eye lubricant ointment was applied to prevent drying of the corneas during surgery. Rats were then fixed on the head stage by a head clamp and set in the incisor bar. Supraorbital incision was positioned and skull was drilled stereotaxically, a layer of gelatin sponge pre-soaked with 6% fluorogold (Biotum, Haywood, CA, USA) was placed on the surface of SC. The incision was closed with suture, and animals were allowed to recover under incandescent lights, and experiments were performed 1 week later.

**Retina ischemic surgery and drug administration**

Rats were anesthetized with 10% chloral hydrate for stereotaxic surgery. Retinal ischemia-reperfusion injury was performed as previously described (Yokoyama et al., 2001; Nonaka et al., 2000). Briefly, the optical nerve of each eye was dissociated by making an incision in the upper conjunctival fornix and detaching the superior rectus muscle. The central retinal artery with the surrounding retinal nerve fiber was ligated for 60 min with microclips 2 mm posterior to the eyeballs. Lack of blood circulation during the ischemic period and the reperfusion of retinal vessel were confirmed by indirect ophthalmoscopy. During the recovery phase from anesthesia, animals were placed in their cages, and steroid antibiotic ointment containing neomycin and dexamethasone was applied to prevent corneal descission. Intravitreous injection experiments were performed as previously described (Yokoyama et al., 2001; Mo et al., 2002). Vehicle and drugs were administered into the left and right eyeballs of rats respectively. Ocular fluid was first released to lower intraocular pressure by paracentesis. Indicated drugs or vehicle 2 μ1 were injected into vitreous via fine glass electrodes. After the indicated time after surgery, rats under deep anesthesia were perfused with cold saline followed by 4% paraformaldehyde. Eyeballs were extracted for morphological experiments.

**Reverse transcriptional polymerase chain reaction (RT-PCR) analysis**

Total RNA of retina was extracted from adult male SD rats with TRIzol reagent (Invitrogen). Total RNA (1.5 μg) was reverse transcribed, and 1/20 of the RT products was used for PCR amplification. For the detection of specific genes, primers were used as following: TORC1 (forward primer: 5’-GGACACACGAGGAGCAGG-3’), reverse primer: 5’-CAGGACGTGGGGCCCTTGGAAC-3’; TORC2 (forward primer 5’-AGGTGATGACTGACGCTC-3’; reverse primer: 5’-TTGGTGTCTTGGCGGCCTCTT-3’); TORC3 (forward primer 5’-AGTTCCATGGAAGATCGCC-3’; reverse primer: 5’-GGGACGTTAAGAGATCGCC-3’); BDNF (forward primer: 5’-GACGCCGGAGGAGGACCC-3’; reverse primer: 5’-GAACGCACGCTATTTTCGAG-3’).

**Western blotting assay**

Retinal tissue was homogenized in RIPA solution and protein concentrations were determined by Lowry method. Approximately 10–20 μg of each sample were separated by SDS-PAGE in a 9% polyacrylamide gel and electroblotted to methanol-immersed polyvinylidene difluoride membrane. Membranes were blocked with non-fat dried milk in 0.5% Tween for 1 h. The blots were probed with antibodies diluted as follows: rabbit anti-TORC1/3 (1:3000), rabbit anti-TORC2 (1:2000), rabbit anti-CREB (1:1000; Cell Signaling Technology). Immunoblotting was performed following the standard protocol; membranes were incubated for 1 h. Immunoreacted protein bands were visualized by ECL plus Western blotting detection system (Amersham), Prestained proteins (10–250 kDa; Bio-Rad) were used as markers.

**In situ hybridization**

In situ hybridization was performed as previously described (Zhou et al., 2006). Briefly, SD rats were deeply anesthetized and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer saline (DEPC-PBS; pH 7.4). The eyeballs were removed and placed into DEPC-PBS (pH 7.4) for 4 h. Eyeballs were dehydrated in gradient of sucrose (15%, 30%, dissolved with DEPC-PBS). Eyeballs were cut into 20 μm thin slices under −20 °C with Cryostat CM1900 (Leica, Germany) and mounted onto non-Nase silane-coated slides (Fisher Scientific, USA), stored at −20 °C. The PCR products of rat TORC1 (forward primer: 5’-GCCAACCAGGAGCAGG-3’, reverse primer: 5’-CAGGACGTGGGGCCCTTGGAAC-3’); rat BDNF (forward primer: 5’-AGCTTGCCGGACCATGG3’- reverse primer: 5’-GAACGCACGCTATTTTCGAG-3’); GAD (forward primer: 5’-GGGACGTTAAGAGATCGCC-3’; reverse primer: 5’-TTGGTGTCTTGGCGGCCTCTT-3’).

**Immunohistostaining**

Frozen retina slices with 20 μm thickness were processed for immunohistochemical assays. Slices were fixed in ice-cold 4% paraformaldehyde for 30 min, permeabilized with 0.1% Triton X-100 in PBS for 30 min, blocked with 10% goat serum for 60 min. Slices were then incubated with rabbit anti-TORC1/3 IgG (1:1000), rabbit anti-phospho-CREB (1:750, Cell Signaling Technology, USA) in 10% normal goat serum in PBS at 4 °C for 36 h. After a thorough wash with PBS, slices were probed with Alexa 488–conjugated goat antirabbit secondary antibody at 4 °C for 12 h (1:2000, Molecular Probes, USA). Retinal slices were mounted with
Hoechst 33342 for nuclear staining and viewed using a Zeiss 2 photon laser confocal-scanning system (Zeiss LSM 510, Jena, Germany). Images were taken using a 40× oil immersion objective with a numerical aperture of 0.8.

Statistical analysis
Statistical data are presented as mean ± SEM. The significance of differences was determined using Student’s t-test as compared to sham group using Prism 4.0 software.

RESULTS
Expression of TORC1 in rat retina
Expression of TORCs mRNA was examined by RT-PCR using cDNA prepared from adult rat retina. TORC1 and TORC2 mRNAs were readily detected from three independent batches of cDNA preparations, while TORC3 mRNA is undetectable under the same PCR cycles (Fig. 1A). To examine the expression of TORC1 and TORC2 at protein level, Western blotting was performed using antibodies specific for TORC1 and TORC2, respectively. Specificity of TORC1 antibody had been proven in a previous study (Zhou et al., 2006). In homogenate of rat retina tissue, TORC1 antibody recognized only a single band of about 75 kD, which is consistent with the molecular weight of TORC1 protein. This protein band was further confirmed with flag-tagged TORC1 overexpressed in BHK cell as a positive control (Fig. 1B). Since mRNA of TORC2 was also detected in retinal cDNA preparation, we next examined the expression of TORC2 protein in rat retina homogenate. We found that TORC2 protein is detectable but relatively lower than that of TORC1 in rat retina (Fig. 1B).

Cellular distribution of TORC1 in rat retina
The rat retina tissue contains a mixture of pigment epithelium, photosensors, RGCs, bipolar, horizontal and amacrine cells in the inner nuclear layer as well as ganglion cells (Kolb, 1991). To examine the spatial distribution of TORC1 in rat retinal circuitry, we performed in situ hybridization experiments using specific oligonucleotide probes of rat TORC1. Strong reactivity of digoxigenin labeled anti-sense probe was detected in the RGCs layer (Fig. 2A). No obvious signal was observed using the negative control sense oligonucleotide (Fig. 2A). RGCs were characterized by its localization, large size of cell body and irregular arrangement. These results indicate that TORC1 mRNA is enriched in the RGCs layer of adult rat retina. Immunohistochemical staining further confirmed that TORC1 protein was mainly distributed in the RGCs layer of retina (Fig. 2B). The cytoarchitecture of these cell layers in the same retinal section was validated by co-staining of TORC1 with the nuclear marker Hoechst 33324 (Fig. 2B). Nominal amplified imaging of RGCs cells revealed that TORC1 was mainly located in the cytoplasm of RGCs (Fig. 2C). To further validate the distribution of TORC1 in retina, we performed retrograde labeling of RGCs using fluorogold as previously described (Dong et al., 1996; Li et al., 2006). This technology has been well demonstrated in tracing RGCs. Retinas were fixed for immunostaining 1 week later, selective labeling of RGCs was revealed by co-labeling with fluorogold (Fig. 3A, B). The subcellular localization of TORC1 in cytoplasm of RGCs is similar to our previous finding in pyramidal neurons of hippocampus (Zhou et al., 2006).

Activity-dependent nuclear translocation of TORC1 in RGCs
TORC1 activity is tightly controlled by diverse signaling pathways (Zhou et al., 2006; Altarejos et al., 2008; Siu et al., 2008). In cultured hippocampal neuron, TORC1 was mainly distributed in the cytoplasm but not nucleus in resting neurons, increasing intracellular Ca^{2+} or cAMP leads to nuclear accumulation of TORC1 (Zhou et al., 2006). To examine whether the localization of TORC1 in

---

Fig. 1. Expression of TORCs mRNA and protein in rat retina. (A) Representative RT-PCR result of TORC family genes in rat retina, GAPDH gene was used as RT-PCR control. (B) Representative Western blots of TORC1 and TORC2 protein expression in rat retina. Flag-tagged TORC1 and flag-tagged TORC2 overexpressed in HEK293 cells were used as positive controls. CREB protein was used as an internal control to validate equivalent protein loading.
RGCs could be regulated, immunohistochemistry assays were performed after intravitreous administration of drugs into the eyeballs. Ten minutes after injection of agonist of ionotrophic glutamate receptor NMDA at a concentration of 50 mM resulted in TORC1 translocation into the nucleus of RGCs as compared with vehicle administration (Fig. 4A, C). Robust nuclear accumulation of TORC1 was also observed 15 min after injection of forskolin, an activator of...
intracellular cAMP activity (Fig. 4A, B). These data demonstrated that TORC1 translocated from cytoplasm to nucleus in response to increased intracellular Ca\(^{2+}\) and/or cAMP levels in RGCs.

**Nuclear and peri-nuclear accumulation of TORC1 in RGCs after ischemic injury**

Many pathological changes of retina like retina ischemia are accompanied with abnormal Ca\(^{2+}\) and cAMP signaling. To examine whether pathological stimuli induce TORC1 nuclear accumulation, transient ischemic reperfusion injury of retina was performed in adult rats. Lack of blood circulation during the ischemic period and reperfusion of retinal vessel within 5 min after the initiation of reperfusion were confirmed by indirect ophthalmoscopy. Retinas were fixed after 15 min or 6 h of reperfusion, respectively. Six retinas from three rats were examined in each group. In sham retina that underwent surgery without ligation of optic nerve, TORC1 was located in the cytoplasm (Fig. 5A). Nuclear and peri-nuclear staining of TORC1 was observed in retinal slices prepared from rats after 15 min reperfusion injury as revealed by co-staining with nuclear marker Hoechst 33324 (Fig. 5B). Significant nuclear accumulation of TORC1 could also be observed 1 h after reperfusion injury (Fig. 5C). It was interesting that, 6 h after reperfusion injury, TORC1 was shuttled to the cytoplasm of RGCs and no obvious nuclear staining of TORC1 was observed (Fig. 5D). These results indicated that acute ischemic injury leads to transient nuclear and peri-nuclear accumulation of TORC1.

We further examined the change of phosphorylation of CREB at Ser 133 after reperfusion injury with the same time course, as CREB phosphorylation at Ser 133 is well-known as the marker of CRE-target gene transcription (Mayr et al., 2001; Lonze et al., 2002). In sham retinas that underwent surgery without ligation of the optic nerve, weak phospho-CREB signal was detectable in RGCs (Fig. 6A). Reperfusion of retina for 15 min led to a robust increase of phospho-CREB in RGCs (Fig. 6B). Increased phospho-CREB in RGCs was observed 6 h after reperfusion injury (Fig. 6C, D). These results indicated that acute ischemic injury leads to persistent increase of CREB phosphorylation in RGCs.
Increased BDNF gene expression in RGCs after ischemic injury

Previous studies indicated that activity-induced nuclear translocation of TORC1 potentiates BDNF transcription (Zhou et al., 2006; Kovacs et al., 2007). It is also reported that increasing BDNF level in retina tissue confers neuroprotective effects on RGCs under injury states (Unoki et al., 1994; Polo et al., 1998). As nuclear accumulation of TORC1 was observed 15 min and 1 h after ischemic reperfusion injury, we performed RT-PCR experiments to study whether ischemic injury induced increased transcription of the BDNF gene. As compared to the sham group, a significant increase of BDNF mRNA was observed at 1 and 6 h after reperfusion (Fig. 7A, B). In situ hybridization results further revealed that RGCs are responsible for increased BDNF gene transcription in ischemic injury (Fig. 7C, D). These results suggested that nuclear accumulation of TORC1 correlates with increased transcription of BDNF genes in RGCs under ischemic injury.

**DISCUSSION**

In this study, we have examined the expression and regulation of TORC1 in rat retina. We found that TORC1 is enriched in retina as compared with other isoforms of the TORCs family. In the resting condition, TORC1 was mainly distributed in the cytoplasm as well as processes near the soma of RGCs. This special subcellular location of TORC1 in RGCs may provide a structural basis for TORC1 in sensing synaptic signals originating from the interneurons in the inner nuclear layer, and transmit these synaptic...
signals to the nucleus of RGCs to initiate CRE-target gene transcription. Nuclear accumulation of TORC1 was observed after increased activity of RGCs by injection of NMDA and forskolin as well as after transient ischemic reperfusion injury of the retina, suggesting that TORC1 is a Ca\(^{2+}\) and cAMP sensitive detector in RGCs. The effect of NMDA on TORC1 nuclear translocation in RGCs is consistent with our previous study (Zhou et al., 2006). However, NMDA treatment is often regarded as toxic but not protective for many types of neuron, it was also reported that calcium influx via a synaptic and/or extra-synaptic NMDA receptor confers opposite effects on neuronal survival (Hardingham et al., 2002). Thus, it is possible that the location of the NMDA receptor or the intensity of the NMDA

Fig. 6. Representative pictures of phosphorylation of CREB at Ser 133 after retinal ischemic reperfusion injury. (A) Phospho-CREB in RGCs from sham group. (B) Phospho-CREB in RGCs 15 min after ischemic reperfusion injury. (C) Phospho-CREB in RGCs 1 h after ischemic reperfusion injury. (D) Phospho-CREB in RGCs 6 h after ischemic reperfusion injury. Propidium iodide staining (red signal) was used as a marker of the cellular nucleus. Scale bar=20 μm. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Fig. 7. Expression of BDNF mRNA after retinal ischemic reperfusion injury. (A) Representative RT-PCR picture of BDNF mRNA from sham, I/R 1 h and I/R 6 h groups, GAPDH mRNA was used as RT-PCR control. (B) Statistical results of BDNF mRNA in sham, I/R 1 h and I/R 6 h groups, data were collected from three independent retinas and normalized to GAPDH. ** P<0.01 as compared to sham group using Student’s t-test. (C, D) Representative in situ hybridization result of BDNF mRNA in RGCs from sham and I/R 1 h groups.
receptor activation has differential effects on RGC cell survival under injury conditions.

Our results indicated that ischemia induced a transient nuclear accumulation of TORC1 but persistent activation of phospho-CREB in RGCs. The failure of TORC1 nuclear accumulation 6 h after reperfusion is probably due to the prolonged activation of intracellular signal pathways that exporting TORC1 into cytoplasm. In cultured hippocampal neurons, prolonged treatment with KCl induced the expression of SIK1 (salt-inducible kinase 1), which phosphorylated TORC1 and exported TORC1 from the nucleus to the cytoplasm (Li et al., 2009). In liver cells, SIK1 is also a key regulator of TORC2 activity during glucose metabolism (Koo et al., 2005). It is possible that prolonged activity of RGCs may induce the expression of SIK1, which may consequently export TORC1 from the nucleus and thus terminate CRE-target gene transcription.

Activity-dependent regulation of the CREB signaling pathway has been implicated in the development and disease conditions of the nervous system (Lonze et al., 2002). In the visual system, dynamic regulation of intracellular cAMP level and CRE-target gene transcription has also been implicated in the refinement of retinogeniculate axons (Stellwagen et al., 1999; Pham et al., 2001; Kumar et al., 2004). TORC1 signal pathways and its downstream gene transcription have also been implicated in brain ischemia (Kitagawa et al., 2007; Takemori et al., 2007). Recent findings indicated that nuclear accumulation of TORC1 is critical for BDNF gene transcription (Zhou et al., 2007; Kovacs et al., 2007). In retinal injury condition, expression of BDNF has been shown to confer a protective role for RGCs (Unoki et al., 1994). Overexpression of CREB-target genes can promote the regeneration of optic nerve fibers (Kitagawa et al., 2007; Yu et al., 2006). It is likely that increasing the nuclear accumulation of TORC1 may confer protective effects to RGCs during injury conditions. Recent elegant viral or electroporation-mediated gene delivery strategies have been developed to examine the role of genes and signal pathways in protecting RGCs under disease conditions (Polo et al., 1998; Matsuda et al., 2004, 2007). It would be interesting to investigate the causal relationship between TORC1 translocation and the survival of RGCs after ischemia.

CONCLUSION

In summary, this study found the enriched expression of TORC1 in RGCs, and increasing intracellular Ca^{2+} and cAMP level lead to accumulation of TORC1 in RGCs. Transient nuclear translocation of TORC1 was observed in RGCs in an acute retinal ischemic injury model, suggesting that manipulation of TORC1 activity may promote the survival of RGCs in retinal disease conditions.

Acknowledgments—This work was supported by grants from Project of Science and Technology of Guangdong Province (2008B060600061) to J.D.; Guangdong Natural Science Foundation Key Project (7117359), National Natural Science Foundation of China (30872831) and the opening project of State Key Laboratory of Neuroscience, Chinese Academy of Sciences (SKLN-2008A03) to X.Z.; National Basic Research Project (973 Project, 2007CB512200) to J.G.; National Nature Science Foundation of China (30800318) to Y.Z.

REFERENCES


The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature 437:1109–1111.


