TRP mechanism for store-operated Ca^{2+} entry. First, there is no similarity between Orai and TRP channels. There is also a lack of evidence that Ca^{2+} store depletion activates TRP channels when intracellular Ca^{2+} is buffered. Lastly, there is no effect on Ca^{2+} store repletion in mice lacking TRPC3 and in triple-knockout mice lacking TRPC1, TRPC4, and TRPC6 (Hartmann et al., 2008).

The two papers address an important detail in the mechanism of store-operated Ca^{2+} entry and cast doubt on the hypothesis that STIM links Orai dimers to form active tetrameric channels (Penna et al., 2008). The fact that Orai is a self-contained Ca^{2+} channel raises the possibility that there are other Orai-activating mechanisms. On a practical level, given the essential role of CRAC channels in T cell activation, it is possible that a membrane-permeant peptide (such as TAT-CAD) could be a useful means of activating T cells, or, conversely, a CAD blocking peptide might be used to prevent their activation. If store-operated Ca^{2+} entry proves important in other cellular processes, such as apoptosis resulting from prolonged ER stress, then CAD reagents will find additional uses.

REFERENCES

Feske, S. (2007). Nat. Rev. Immunol. *7*, 690–702.

Hartmann, J., Dragicevic, E., Adelsberger, H., Henning, H.A., Sumser, M., Abramowitz, J., Blum, R., Dietrich, A., Freichel, M., Flockerzi, V., et al. (2008). Neuron *59*, 392–398.

Luik, R.M., Wang, B., Prakriya, M., Wu, M.M., and Lewis, R.S. (2008). Nature *454*, 538–542.

Parekh, A.B., and Putney, J.W., Jr. (2005). Physiol. Rev. *85*, 757–810.

Park, C.Y., Hoover, P.J., Mullins, F.M., Bachhawat, P., Covington, E.D., Raunser, S., Walz, T., Garcia, K.C., Dolmetsch, R.E., and Lewis, R.S. (2009). Cell, this issue.

Penna, A., Demuro, A., Yeromin, A.V., Zhang, S.L., Safrina, O., Parker, I., and Cahalan, M.D. (2008). Nature *456*, 116–120.

Roos, J., Digregorio, P.J., Yeromin, A.V., Ohlsen, K., Lioudyno, M., Zhang, S., Safrina, O., Kozak, J.A., Wagner, S.L., Cahalan, M.D., et al. (2005). J. Cell Biol. *169*, 435–445.

Stathopulos, P.B., Zheng, L., Li, G.Y., Plevin, M.J., and Ikura, M. (2008). Cell *135*, 110–122.

Wu, M.M., Luik, R.M., and Lewis, R.S. (2007). Cell Calcium *42*, 163–172.

Yuan, J.P., Zeng, W., Dorwart, M.R., Choi, Y.J., Worley, P.F., and Muallem, S. (2009). Nat. Cell Biol. Published online February 1, 2009. 10.1038/ncb1842.

PPTR-1 CounterAkts Insulin Signaling

Reuben Shaw[1,*] and Andrew Dillin[1,2,*]
[1]Molecular and Cell Biology Laboratory
[2]The Howard Hughes Medical Institute
The Salk Institute for Biological Studies, La Jolla, CA 92037, USA
*Correspondence: shaw@salk.edu (R.S.), dillin@salk.edu (A.D.)

DOI 10.1016/j.cell.2009.02.029

The serine/threonine kinase Akt is a focal point in signaling pathways that control cell tumorigenesis and insulin resistance. In this issue, Padmanabhan et al. (2009) identify a phosphatase regulatory subunit PPTR-1 that regulates the insulin/insulin-like growth factor 1 pathway by counteracting Akt activity in worms and mammalian cells.

The serine/threonine protein kinase Akt is a central effector of the insulin/insulin-like growth factor 1 signaling (IIS) pathway that is conserved from worm to man. The IIS pathway regulates growth, development, reproduction, stress responsiveness, and longevity. Akt is commonly hyperactivated in a large number of human cancers, often because of activating mutations in its key upstream regulator phosphoinositol 3-kinase (PI3K) or inactivating mutations in the opposing lipid phosphatase, the tumor suppressor PTEN. In contrast to tumorigenesis, loss of AKT activity accompanies insulin resistance in peripheral metabolic tissues in diabetic patients, and loss of Akt-2 in mice results in a type 2 diabetes-like phenotype. Therefore, gaining insight into the key positive and negative regulatory modules controlling AKT activity is central to our basic understanding of two common diseases. In this issue, Padmanabhan et al. (2009) identify the phosphatase regulatory subunit PPTR-1 in the worm *Caenorhabditis elegans*, which regulates the IIS pathway by counteracting Akt activity. This regulation controls longevity and has implications for the study of cancer and diabetes.

Two different phosphorylation events are known to be involved in Akt activation, both of which appear to be dependent on PI3K and are conserved across all metazoans (Figure 1). Phosphorylation of Akt at threonine 308 (Thr350 in worm) in the activation loop by the phosphoinositide-dependent kinase 1 (PDK1) (Figure 1) is absolutely required for Akt activity. In contrast, phosphorylation at a hydrophobic motif serine site (human Ser473/worm Ser517) by the TORC2 (TOR-rictor) kinase complex is important, but appears not to be essential for Akt activation in all settings (Polak and Hall, 2006). In stark contrast to our detailed

understanding of the circuitry of kinases activating Akt, very little is known about the counteracting phosphatases.

Padmanabhan et al. performed an RNA interference (RNAi)-based screen in *C. elegans* to identify critical phosphatases that function within the IIS pathway. Fifty-seven of the 60 catalytic phosphatase genes and six regulatory phosphatase subunits were screened for their ability to suppress the dauer developmental arrest phenotype induced by a temperature-sensitive allele of *daf-2*, the sole insulin/insulin-like growth factor 1 receptor in worms. Strikingly, of these 63 genes, RNAi directed against only two genes, *fem-2* and *pptr-1*, strongly suppresses the *daf-2* phenotype to the same degree as RNAi against *daf-18*, the homolog of PTEN. However, *fem-2* RNAi did not suppress all *daf-2* mutant alleles and was not studied further, leaving *pptr-1* as the focus for additional characterization.

The *pptr-1* gene encodes a member of the B56 family of regulatory subunits for phosphatase 2A (PP2A). RNAi against *pptr-1* also specifically suppresses the extension of life span and fat accumulation observed in IIS pathway mutants, but has no effect on a mutant strain deficient in transforming growth factor β (TGF-β) signaling. RNAi against *pptr-1* also suppresses transcription that is dependent on DAF-16. DAF-16 is a key Akt substrate that modulates life span and stress resistance; overexpression of PPTR-1 extends life span in a DAF-16 dependent manner. Epistasis experiments with multiple components of the IIS pathway suggest that *pptr-1* acts at the level of *akt-1*, but interestingly does not affect the closely related family members *akt-2* or *sgk-1*. Moreover, an examination of the expression pattern of *pptr-1* reveals a distinct overlap with *akt-1*, but not *akt-2* or *sgk-1*. Similarly, the PPTR-1 protein coimmunoprecipitates with Akt-1 but not Akt-2. An examination of the in vivo phosphorylation of Akt-1 in worms with phospho-specific antibodies reveals that overexpression of *pptr-1* decreases phosphorylation of the PDK1 target Thr308, but not phosphorylation of the hydrophobic Ser473 site.

Importantly, the investigators provide evidence that regulation of Akt Thr308 dephosphorylation by *pptr-1* orthologs is conserved in mammalian cells. RNAi directed against B56β, one of the five genes encoding B56 regulatory subunits of PP2A in mammals, results in Akt hyperphosphorylation at Thr308 in insulin-treated adipocytes. These findings are also consistent with a recent overexpression screen in the fruit fly *Drosophila*, which reported that the closest homolog of *pptr-1*, *widerborst* (*wdb*), regulates PI3K/Akt signaling to promote FOXO activation (Vereshchagina et al., 2008). Furthermore, pharmacological inhibitors of PP2A, such as okadaic acid, have long been known to activate Akt.

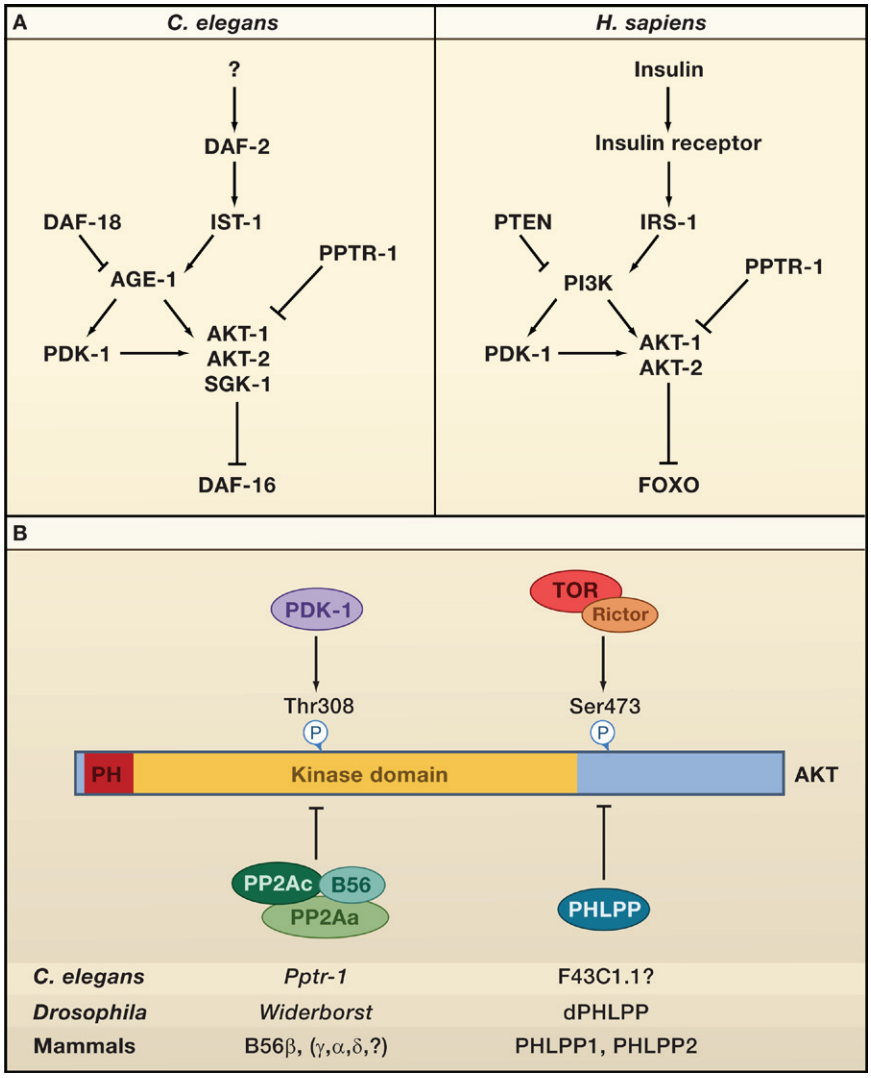

Figure 1. Akt and Insulin/Insulin-like Growth Factor Signaling

(A) Wiring of the insulin/insulin-like growth factor signaling pathway in *C. elegans* and humans. In worms, the insulin receptor DAF-2 activates an IRS-1 homolog, IST-1, which activates a phosphoinositide 3-kinase (PI3K) AGE-1. The lipid phosphatidylinositol-3-phosphate (PIP3) generated by PI3K activates the two worm Akt homologs, Akt-1 and Akt-2, via their recruitment to the upstream phosphoinositide-dependent kinase 1 (PDK1). PDK1 phosphorylates a key residue in the activation loop of Akt to stimulate its kinase activity. Activated Akt then phosphorylates a number of downstream substrates, key among them being the conserved forkhead transcription factor DAF-16, resulting in its inactivation by preventing its translocation to the nucleus. Consistent with these molecules acting together to regulate insulin signaling, loss-of-function mutations of *daf-2*, *age-1*, *pdk-1*, *akt-1*, and *akt-2* cause precocious entry into diapause, a developmentally arrested larval stage, and increased longevity. Loss-of-function mutations of *daf-16* completely suppress these phenotypes, indicating that it is a key downstream target of insulin signaling controlling longevity in *C. elegans*.

(B) Regulation of two critical evolutionarily conserved phosphorylation sites in Akt. PDK1 phosphorylates threonine 308 (Thr308) in the activation loop of Akt family members, promoting their kinase activity. This site is dephosphorylated by PP2A bound to B56 regulatory subunits. The serine 473 (Ser473) hydrophobic motif (PDK2) site is phosphorylated by the TORC2 kinase complex composed of TOR and rictor, and this site is dephosphorylated by the PHLPP family of phosphatases. Names of gene homologs are provided.

The specificity of *pptr-1* and B56 isoforms for Akt Thr308 but not Ser473 dephosphorylation is paralleled by recent discoveries of a PHLPP family of conserved phosphatases that target Ser473 but not Thr308 (Gao et al., 2005). As observed here for *pptr-1* in worms, different Akt family members are targeted by distinct PHLPP family members in mammalian cells (Brognard et al., 2007). Similarly, one might anticipate that multiple mammalian B56 isoforms would play a role in regulating Akt isoforms. Indeed, B56γ and B56β have previously been shown to modulate Akt signaling in different contexts (Chen et al. 2005; Rocher et al., 2007).

Notably, B56 and PHLPP isoforms are implicated in cancer and diabetes. B56γ is inactivated by SV40 small T antigen in cellular transformation (Chen et al., 2004), and B56γ is also downregulated in some tumors. Somatic mutations that result in loss of B56 binding have been reported in A subunits of PP2A, and the resulting haploinsufficiency promotes cancer (Westermarck and Hahn, 2008). Conversely, the upregulation of PHLPP1 in skeletal muscle from diabetic patients correlates with the loss of Akt-2 phosphorylation at Ser473 (Cozzone et al., 2008). Importantly, it is unlikely that Akt will be the only target of a particular B56 isoform, as p53 and Wnt signaling are both regulated by B56 isoforms. Similarly, protein kinase C signaling has been well established as a target for PHLPP beyond Akt.

Given the aforementioned complexities of different phosphatase isoforms regulating different Akt family members, it is likely that the tissue expression patterns of different mammalian B56 and PHLPP isoforms will play a major role in dictating the level of responsiveness of the PI3K-Akt pathway. Hence, inactivation of a given isoform of B56 or PHLPP might result in a restoration of insulin signaling in metabolic tissues from diabetic patients, but could trigger unrestrained proliferation in epithelial tissues. Understanding the intricacies of Akt regulation in different mammalian tissues awaits further work, but B56 subunits are now clearly front and center in the investigation of aging and disease.

REFERENCES

Brognard, J., Sierecki, E., Gao, T., and Newton, A.C. (2007). Mol. Cell *25*, 917–931.

Chen, W., Possemato, R., Campbell, K.T., Plattner, C.A., Pallas, D.C., and Hahn, W.C. (2004). Cancer Cell *5*, 127–136.

Chen, W., Arroyo, J.D., Timmons, J.C., Possemato, R., and Hahn, W.C. (2005). Cancer Res. *65*, 8183–8192.

Cozzone, D., Frojdo, S., Disse, E., Debard, C., Laville, M., Pirola, L., and Vidal, H. (2008). Diabetologia *51*, 512–521.

Gao, T., Furnari, F., and Newton, A.C. (2005). Mol. Cell *18*, 13–24.

Padmanabhan, S., Mukhopadhyay, A., Narasimhan, S.D., Tesz, G., Czech, M.P., and Tissenbaum, H.A. (2009). Cell, this issue.

Polak, P., and Hall, M.N. (2006). Dev. Cell *11*, 433–434.

Rocher, G., Letourneux, C., Lenormand, P., and Porteu, F. (2007). J. Biol. Chem. *282*, 5468–5477.

Vereshchagina, N., Ramel, M.C., Bitoun, E., and Wilson, C. (2008). J. Cell Sci. *121*, 3383–3392.

Westermarck, J., and Hahn, W.C. (2008). Trends Mol. Med. *14*, 152–160.

TRIM-NHL Proteins Take on miRNA Regulation

Inga Loedige[1,*] and Witold Filipowicz[1,*]
[1]Friedrich Miescher Institute for Biomedical Research, 4002 Basel, Switzerland
*Correspondence: inga.loedige@fmi.ch (I.L.), witold.filipowicz@fmi.ch (W.F.)

DOI 10.1016/j.cell.2009.02.030

The TRIM-NHL family of proteins is conserved among metazoans and has been shown to regulate cell proliferation and development. In this issue, Hammell et al. (2009) and Schwamborn et al. (2009) identify two members of this protein family, NHL-2 in worms and TRIM32 in mice, as positive regulators of microRNA function.

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression posttranscriptionally. In animals, they typically base pair imperfectly with sequences in the 3′ untranslated regions (3′UTRs) of mRNAs to either inhibit translation or accelerate mRNA decay. Following excision from longer precursors, miRNAs are loaded into ribonucleoprotein particles called miRNPs. Argonaute (AGO) and GW182 family proteins, the best-characterized components of miRNPs, act as key effectors in miRNA function. Although as many as 50% of all genes might be regulated by the hundreds of miRNAs identified in mammals, the exact mechanism of this regulation is not known. The effects of miRNAs are diverse—some targeted mRNAs undergo destabilization whereas others are mainly translationally inhibited, with the degree of repression varying for different mRNA-miRNA combinations. Consistent with this, protein cofactors