STRUCTURE WATCH

Complex construction

There are more than 20 isoforms of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) — an oligomeric serine/threonine kinase that is activated by Ca^{2+}-bound calmodulin. CaMKII responds to changes in Ca^{2+} levels in a complex manner that is presently unclear, but is known to depend on the architecture of this oligomeric assembly. The CaMKII oligomer was thought to be dodecameric (12 subunits). However, the 2.6-Å resolution crystal structure of the association domain of the CaMKII α-isoform, which is described by Kuriyan and colleagues in *Molecular Cell*, now challenges this view.

The carboxy-terminal association domain of CaMKII is responsible for its oligomerization, and the authors found that CaMKIIα association domains come together to form a tetradecameric assembly. They form a circular hub, which has a central pore and is made up of two seven-membered rings stacked head-to-head. Each of the 14 protomers is wedge-shaped and has a long α-helix at its amino terminus that extends towards the equatorial plane of the circular structure. As this α-helix links the association domain to the CaMKII kinase domain, it is probable that the kinase domains form a second outer ring around the circular hub, and this complex construction is crucial to allow CaMKII to respond to Ca^{2+} levels in a complex way. Furthermore, the authors identified a potential peptide-binding pocket in each protomer, which faces the pore's centre and might function as a docking site for proteins that interact with CaMKII. However, the functional significance of this pocket needs to be clarified.

REFERENCE Hoelz, A. *et al.* Crystal structure of a tetradecameric assembly of the association domain of Ca^{2+}/calmodulin-dependent kinase II. *Mol. Cell* **11**, 1241–1251 (2003)

One way to recognize?

Monoubiquitylation functions as a regulatory modification in various cellular processes, and the monoubiquitin signal is transmitted when it interacts with ubiquitin-binding motifs that are found in eukaryotic proteins with varied functions. Although several ubiquitin-binding motifs have been characterized, high-resolution information regarding monoubiquitin-signal recognition is lacking. Now, though, in *Cell*, Radhakrishnan and co-workers provide new insights.

CUE domains are involved in monoubiquitin and polyubiquitin recognition, and they also facilitate intramolecular monoubiquitylation. So, to understand further how CUE domains recognize ubiquitin, the authors determined the NMR solution structure of the CUE2-1 domain from the yeast Cue2 protein bound to yeast ubiquitin. This structure revealed interactions between CUE2-1 and ubiquitin that involve hydrophobic patches, such as the Leu8-Ile44-Val70 patch on ubiquitin. In addition, the interaction site encompasses Lys48 of ubiquitin — the site of polyubiquitin chain formation. This indicates that the CUE domain might stop polyubiquitin chains forming during monoubiquitin signalling. Comparative modelling showed that the UBA ('ubiquitin-associated') motif might also interact with ubiquitin in this way, so the authors believe that the CUE2-1–ubiquitin structure might "…serve as a paradigm for ubiquitin recognition and signaling by ubiquitin binding proteins".

REFERENCE Kang, R. S. *et al.* Solution structure of a CUE–ubiquitin complex reveals a conserved mode of ubiquitin binding. *Cell* **113**, 621–630 (2003)

SIGNALLING

A sugar fix for fasters

Normally, insulin binding to its receptor induces phosphorylation of the serine/threonine kinase Akt/protein kinase B (PKB). One of Akt/PKB's functions is to inhibit glucose output from the liver when glucose is already available from food. If this fails, hepatic glucose output increases. So, a report in *Science* showing that TRB3 is a negative modulator of Akt/PKB has widespread implications for type II diabetes.

Du *et al.* isolated TRB3 in a yeast two-hybrid screen for proteins that modulate Akt/PKB activity. Like its *Drosophila melanogaster* homologue tribbles, TRB3 has a truncated kinase domain and lacks detectable catalytic activity. However, further truncation of the kinase domain weakened the interaction of TRB3 with Akt/PKB.

The effect of TRB3 on Akt/PKB was to inhibit its phosphorylation at threonine (Thr) 308 and serine (Ser) 473, thereby reducing its kinase activity — there was no change in the protein levels of Akt/PKB. Perhaps unsurprisingly, therefore, when the authors disrupted endogenous TRB3 expression in hepatocytes using RNA interference, Akt/PKB phosphorylation was potentiated in response to growth-factor signalling. So, how does this happen?

Phosphorylation-defective and phosphorylation-mimicking Akt/PKB constructs in yeast two-hybrid assays indicated that TRB3 preferentially binds to unphosphorylated Akt/PKB. Further studies showed that this was probably through TRB3 binding to, and therefore blocking, the Akt/PKB Thr308 phosphorylation site.

The authors then looked at TRB3 expression in mouse liver under feeding or fasting conditions. Hepatic TRB3 messenger RNA and protein levels were 10–20 times higher in fasted animals than fed ones, and were also higher in livers from *db/db* diabetic mice. When Du *et al.* overexpressed TRB3 *in vivo*, they saw increased blood glucose levels in re-fed, but not fasted, mice, indicating that TRB3 might interfere with insulin-mediated hepatic glucose release. And in a glucose-output assay, insulin could hardly inhibit glucose release from TRB3-overexpressing cells, in contrast to wild-type cells. This might be partly due to TRB's ability to block phosphorylation of Akt/PKB substrates such as glycogen synthase kinase-3β.

Under fasting conditions, TRB3-mediated hepatic glucose output might be beneficial, but pathological TRB3 overexpression after feeding might contribute to insulin resistance and hyperglycaemia. So, TRB3 could be an attractive target for treating type II diabetes.

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References and links

ORIGINAL RESEARCH PAPER Du, K. *et al.* TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science* **300**, 1574–1577 (2003)