HOST-MICROBIAL INTERACTIONS

F-box for energy

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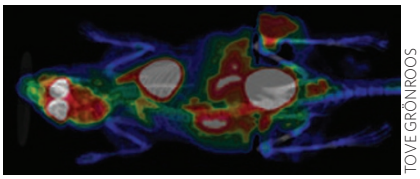
A type IV secretion system delivers *Legionella pneumophila* effectors into human host cells to promote bacterial proliferation within *Legionella*-containing vacuoles (LCVs). AnkB, an F-box protein effector that assembles polyubiquitinated (polyUb) proteins on the LCV, is required for bacterial proliferation. Price *et al.* demonstrate that disruption of ubiquitination via Lys48—the linkage associated with proteasomal degradation—decreased recruitment of polyUb proteins to LCVs and bacterial proliferation. Inhibition of the proteasome with small molecules decreased bacterial replication in host cells, and this phenotype could be rescued by amino acid supplementation, observations suggesting that degradation of polyUb proteins provides amino acids to support bacterial growth. Similarly, bacterial proliferation defects in host cells treated with small-molecule inhibitors of aminopeptidases—enzymes that degrade peptides—or in those overexpressing ubiquitin with a K48R substitution were also rescued by amino acid supplementation. MS revealed an increase in free amino acids in cells infected with *Legionella* (compared to uninfected cells) that was dependent upon AnkB expression. Supplementation with cysteine or serine alone was sufficient to rescue proliferation, suggesting that the bacteria may use amino acids as an energy source. Consistent with this idea, supplementation with citrate or pyruvate, intermediates in the tricarboxylic acid cycle, also rescued proliferation. Taken together, these data indicate that for replicating *Legionella*, amino acids produced by promoting the proteasomal degradation of host proteins are a source of energy, carbon or both. *AD*

METABOLISM

A Warburg shakeup

Proc. Natl. Acad. Sci. USA **108**, 18814–18819 (2011)



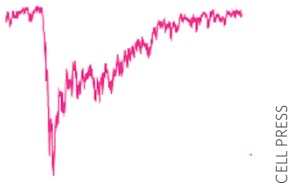
TOVE GRÖNROOS

The observation that cancer cells preferentially use glycolysis rather than oxidative phosphorylation—the Warburg effect—has substantially influenced thinking regarding cancer progression and potential therapeutics. However, a new study from Landor *et al.* demonstrates that increased glycolysis is not directly correlated with cancer progression and that metabolic flexibility is instead tied to aggressive cancer growth. The authors examined the relationship of Notch signaling to cellular metabolism by creating three engineered breast cancer cell lines with high, medium or low levels of Notch activity (N^{high}, N^{medium} and N^{low}, respectively). Implantation of these cells into mice caused initial tumor formation in all three lines, but after 8 weeks, N^{high} tumors had grown dramatically, whereas N^{low} tumors had regressed. Surprisingly, both N^{high} and N^{low} tumors showed increased glucose uptake and lactate production—hallmarks of increased glycolysis—compared to N^{medium} tumors. To explain these observations, the authors examined two cellular pathways that have previously been linked to glycolysis and mitochondrial function and discovered that Notch activation increased PI3K-AKT signaling, whereas decreased Notch activity inhibited mitochondrial function in a p53-dependent manner. The diminished mitochondrial function made N^{low} cells more susceptible to cell death upon glucose deprivation, whereas N^{high} cells were able to continue growing under the same circumstances. These results suggest that a deeper understanding of the link between metabolism and cellular signaling is needed to understand cancer development. *CG*

NEURONAL FUNCTION

A Doc uses calcium

Cell **147**, 666–677 (2011)



CELL PRESS

Evoked neurotransmitter release consists of two Ca^{2+}-regulated phases: a fast synchronous phase essential for rapid communication with downstream neurons and a slow asynchronous phase that has been implicated in neural network activity. Synaptotagmin 1 (syt1) is the Ca^{2+} sensor of the fast phase, but the sensor of the second phase was unknown. To identify this component, Yao *et al.* focused on the Doc2 family of cytosolic proteins, which interact with membrane phospholipids in presynaptic terminals in response to Ca^{2+}. The authors found that Doc2 isoforms α and β have several features of a Ca^{2+} sensor: binding to and functional dependence on SNARE fusion proteins, binding to membranes in a Ca^{2+}-dependent fashion, regulating Ca^{2+}-triggered membrane fusion *in vitro* and dependence on the lipid phosphatidylserine. Compared to syt1, Doc2α and Doc2β bind to membranes with slower kinetics and disassemble from the membrane with much longer timescales. Knockdown of Doc2α or mutation of its Ca^{2+} binding sites also support a role for Doc2α in asynchronous release in cultured neurons. Finally, the authors showed that Doc2 has a role in the induction and maintenance of persistent reverberatory activity in neural networks, in which asynchronous release is known to be important. These results suggest that Doc2 is a Ca^{2+} sensor kinetically tuned to regulate the slow component of transmitter release. *MB*

BIOSYNTHESIS

Sacrificial sulfur

Nature **478**, 542–546 (2011)

Thiamine pyrophosphate (TPP), an enzymatic cofactor, is assembled by a convergent strategy that joins a core thiazole scaffold with a pyrimidine side chain. The biosynthetic route to the thiazole component in prokaryotes is well understood, but, curiously, in eukaryotes only one enzyme (known in *Saccharomyces cerevisiae* as THI4p) is responsible for the multistep conversion of nicotinamide adenine dinucleotide (NAD) and glycine to the thiazole scaffold. Chatterjee *et al.* now demonstrate that the sulfur atom of TPP is derived from THI4p itself. Biochemical analysis showed that THI4p copurifies with three thiazole biosynthetic intermediates and that its activity is enhanced in the presence of Fe(II). MS characterization revealed that the molecular mass of expressed THI4p was 34 Da lower than predicted; reanalysis of earlier THI4p crystallographic data revealed an absence of sulfur electron density at Cys205 and evidence for a dehydroalanine residue at this position. Taken together, these observations suggested that during catalysis THI4p covalently captures biosynthetic intermediates at Cys205, and the linking sulfur atom is transferred into the substrate through β-elimination. The successful reconstitution of thiazole biosynthesis in yeast provides compelling evidence that THI4p is a stoichiometric cosubstrate that, in addition to its other roles, acts as a sacrificial sulfur donor in TPP biosynthesis. *TLS*