Nature Reviews Molecular Cell Biology | AOP, published online 18 January 2012; doi:10.1038/nrm3275

CELL CYCLE

AMPK moonlights in mitosis

> This study identifies new AMPKα2 substrates … and suggests that AMPK has a role in regulating mitosis.

AMP-activated protein kinase (AMPK) is well known for its ability to regulate cell metabolism, but emerging evidence suggests that it functions in additional cellular processes. Banko *et al*. have identified novel substrates of AMPKα2 (a catalytic subunit of AMPK) that have a role in mitosis, including protein phosphatase 1 regulatory subunit 12C (PPP1R12C).

First, the authors generated an ATP analogue-specific AMPKα2 (AS-AMPKα2). The phosphate transferred to targets from the ATP analogues used can be labelled to identify direct AMPKα2 substrates in cells. After confirming that AS-AMPKα2 phosphorylated known AMPKα2 substrates, they expressed AMPKα2 or AS-AMPKα2 in cells, activated AMPK in the presence of the ATP analogue and used tandem mass spectrometry to identify phosphorylated proteins. Of the phosphorylated proteins that were expressed more than 10-fold higher in AS-AMPKα2 cells than in AMPK cells, 28 were novel AMPK substrates. Many of these substrates have a known role in mitosis and chromosome segregation, so the authors postulated that AMPKα2 might help to coordinate mitosis.

The authors validated six novel substrates, including PPP1R12C and p21-activated kinase 2 (PAK2). The authors focused on these two proteins, as they can regulate the phosphorylation of myosin regulatory light chain (MRLC) at Ser19, which is crucial for mitotic progression. They identified Ser452 and Ser20 as the AMPK phosphorylation site in PPP1R12C and PAK2, respectively. Furthermore, AMPK indirectly regulated the phosphorylation state of MRLC, at least in part, by phosphorylating PPP1R12C and PAK2. Thus, AMPK directly phosphorylates PPP1R12C and PAK2, which indirectly increases MRLC phosphorylation at Ser19.

So, what might be the mechanism behind this? AMPK-mediated phosphorylation of PPP1R12C increased its association with the conserved regulatory protein 14-3-3ζ, which may prevent it from interacting with a catalytic phosphatase subunit and from promoting MRLC dephosphorylation. How the AMPK-mediated phosphorylation of PAK2 influences MRLC phosphorylation is less clear, as it did not alter the ability of PAK2 to phosphorylate MRLC *in vitro*.

Finally, the authors asked whether AMPK-mediated PPP1R12C and PAK2 phosphorylation alters mitotic progression. The phosphorylation of AMPK at Thr172 (which is indicative of its activation) and of PPP1R12C at Ser452, but not of PAK2 at Ser20, was high in early mitosis and decreased with mitotic progression. Furthermore, the inhibition or overstimulation of AMPK in cells synchronized in mitosis increased the number of multinucleated cells, as did the expression of PPP1R12C that cannot be phosphorylated at Ser452. These data suggest that, for proper mitotic progression and cytokinesis, AMPK activity must be finely tuned and that the AMPK-meditated phosphorylation of PPP1R12C, but not necessarily of PAK2, is important in this process.

This study identifies new AMPKα2 substrates using a chemical genetic approach and suggests that AMPK has a role in regulating mitosis. Characterization of the other AMPKα2 substrates with known roles in mitosis will further validate this role of AMPK.

Katharine H. Wrighton

ORIGINAL RESEARCH PAPER Banko, M. R. *et al.* Chemical genetic screen for AMPKα2 substrates uncovers a network of proteins involved in mitosis. *Mol. Cell* **44**, 878–892 (2011)