

POST-TRANSLATIONAL MODIFICATIONS

Considering conditions

J. Biol. Chem., published online 13 Aug 2013;
doi:10.1074/jbc.M113.486753

Lysine modifications such as acetylation and succinylation have a variety of biological roles. Although nuclear acetyltransferases have been identified, no such enzymes are known in mitochondria, where nearly 65% of proteins are acetylated. Wagner and Payne hypothesized that the unusual conditions of the matrix—a basic pH and relatively high concentrations of acetyl-CoA, an activated acetate donor—could enable nonenzymatic acetylation. To test this idea, the authors incubated liver or heart mitochondrial proteins or the nonmitochondrial protein BSA in a variety of conditions. Acetylation was observed at pH 8 with acetyl-CoA, but no modifications were observed at lower pH or if acetate was provided. Acetylation by acetyl-CoA also occurred in the presence of CoA, which was expected to serve as an inhibitor of a putative acetyltransferase. The same chemical conditions were also sufficient to induce succinylation of BSA when tested with succinyl-CoA, indicating that lysine acylation generally should be possible in the mitochondria. Finally, the authors confirmed that SIRT3 treatment was able to reduce overall acetylation and that neighboring positively charged residues correlate with the largest increases in acetylation. In tandem, the data provides strong support for lysine acylation as a nonenzymatic modification, as has already been established for lysine glycation and carbamylation.

CG

proteins in human cell lines and mouse tissues, frequently at catalytic or regulatory lysines in the active sites of metabolic enzymes. Exposing cells to high concentrations of glucose led to an increase in the amount of pgK-labeled proteins, suggesting that pgK modifications may help cells react to an increase in glycolytic flux. Additional work is needed to determine how the presence of this new PTM affects the physiological properties of other proteins *in vivo*.

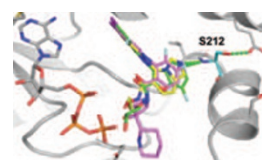
JMF

CANCER THERAPEUTICS

MEK it work

Nature, published online 11 August 2013;
doi:10.1038/nature12441

GEORGIA
HATZIVASSILIOU



Activating mutations of the MAPK signaling pathway—for example, in KRAS and BRAF—are detected in a large number of tumors. Inhibition of MAPK signaling by targeting the downstream kinase, MEK, was envisioned as the gold standard therapeutic to treat all upstream oncogenic mutations. However, inhibitors of MEK1 have shown varying degrees of effectiveness depending on the oncogenic genotype. Hatzivassiliou *et al.* set out to investigate why certain MEK inhibitors were more effective against cells with specific types of oncogenic mutations. MEK inhibitors with strong potency against KRAS-mediated tumors (GDC-0623 and G-573) had decreased phosphorylated MEK (pMEK), stabilized RAF-MEK interactions and decreased RAF plasma membrane localization. Molecular modeling experiments suggested that these compounds form a strong hydrogen bond with Ser212 in MEK, which prevented MEK phosphorylation by RAF. Loss of hydrogen bonding due to a mutation in Ser212 blocked the activity of GDC-0623 and G-573. Conversely, a different MEK inhibitor (GDC-0973) was most effective in BRAF-mediated tumors, which have elevated phosphorylated MEK (pMEK). The increased potency of GDC-0973 against BRAF-mutated tumors was due to a strong binding interaction with pMEK, whereas compounds with the reverse profile have decreased binding affinity for pMEK. These findings will better inform the treatment of particular MAPK tumor models based on their mechanism of activation.

GM

Written by Mirella Bucci, Amy Donner,
Joshua M. Finkelstein, Catherine Goodman,
Grant Miura & Terry L. Sheppard

TRANSLATION

Pack a PUNCH

Genes Dev. **27**, 1834–1844 (2013)

The ability to detect rapid changes in protein synthesis under different experimental conditions can provide insights into gene expression regulation. Techniques to identify newly synthesized proteins such as MS analysis of pulse-labeled amino acids (pSILAC) and RNA sequencing of ribosome-enriched transcripts (Ribo-seq) have advanced our understanding of the kinetics of mRNA translation. However, pSILAC is not applicable to monitoring rapid changes in protein synthesis, and large-scale Ribo-seq analysis is not yet economically feasible. Aviner *et al.* now report a new assay called puromycin-associated nascent chain proteomics (PUNCH-P) to monitor mRNA translation. This approach quickly labels nascent polypeptides with a biotinylated form of puromycin, and a streptavidin-mediated pulldown is then subjected to MS analysis. PUNCH-P was as effective as established techniques in terms of measuring protein synthesis, and although it lacked the high resolution of Ribo-seq, PUNCH-P identified large data sets of proteins in an economically efficient manner. As a test case, the authors used this technique to identify more than 5,000 proteins that are synthesized during particular stages of the cell cycle, including known components as well as proteins that were not previously implicated in the process. For example, CCRN4L, a circadian deadenylase and PCF11, a pre-mRNA 3' end-processing factor, were found to be upregulated during the S phase and M phase, respectively. Finally, the authors demonstrated the utility of the

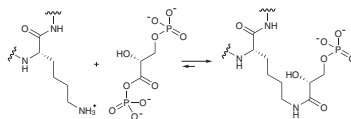
method to assay mRNA translation in tissues by successfully labeling newly synthesized proteins in a mouse brain. Taken together, PUNCH-P may be a cost-effective approach to measure rapid changes in global protein synthesis in cells and tissues.

GM

POST-TRANSLATIONAL MODIFICATIONS

Enzymes need not apply

Science **341**, 549–553 (2013)



The post-translational modification (PTM) of a protein—via the addition of chemical moieties such as acetate, phosphate or a carbohydrate—can dramatically alter the physiological behavior of that protein. The attachment and removal of most PTMs are catalyzed by enzymes; for example, kinases can phosphorylate a protein in a signaling cascade, and this PTM can be removed by a phosphatase. Moellering and Cravatt have identified a new PTM that seems to be added to target proteins in a nonenzymatic manner. The authors first showed that 1,3-bisphosphoglycerate, which is a product of GAPDH catalysis and contains an electrophilic acyl phosphate group, reacted with the amino groups of specific lysine side chains of GAPDH *in vitro*, forming 3-phosphoglyceroyl-lysine (pgK) linkages. pgK modification of GAPDH increased the K_m of the enzyme, suggesting that this PTM interfered with GAPDH's ability to interact with its substrate. The authors used proteomic profiling to show that pgK modifications were formed on diverse