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Proteomics and protein sciences

Phosphoproteome Exploration Reveals a Reformatting of Cellular Processes in Response to Low Sterol Biosynthetic Capacity in *Arabidopsis*

Journal of Proteome Research, **11**, 1228-1239 (2012)
Dimitri Heintz *et al.*

Tags
ZORBAX 300Extend-C18, proteomics & protein sciences, phosphoprotein analysis

Abstract
Sterols are membrane-bound isoprenoid lipids that are required for cell viability and growth. In plants, it is generally assumed that 3-hydroxy-3-methylglutaryl-CoA-reductase (HMGR) is a key element of their biosynthesis, but the molecular regulation of that pathway is largely unknown. In an attempt to identify regulators of the biosynthetic flux from acyl-CoA toward phytosterols, we compared the membrane phosphoproteome of wild-type *Arabidopsis thaliana* and of a mutant being deficient in HMGR1. We performed a N-terminal labeling of microsomal peptides with a trimethoxyphenyl phosphonium (TMPP) derivative, followed by a quantitative assessment of phosphopeptides with a spectral counting method. TMPP derivatization of peptides resulted in an improved LC–MS/MS detection due to increased hydrophobicity in chromatography and ionization efficiency in electrospray. The phosphoproteome coverage was 40% higher with this methodology. We further found that 31 proteins were in a different phosphorylation state in the hmgr1–1 mutant as compared with the wild-type. One-third of these proteins were identified based on novel phosphopeptides. This approach revealed that phosphorylation changes in the *Arabidopsis* membrane proteome targets major cellular processes such as transports, calcium homeostasis, photomorphogenesis, and carbohydrate synthesis. A reformatting of these processes appears to be a response of a genetically reduced sterol biosynthesis.

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