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

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Dimitri Heintz et al.

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. Reprinted with permission from Journal of Proteome Research. © 2012 American Chemical Society.