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Identification of New *in Vivo* Phosphorylation Sites of EBV-LMP1 by Mass Spectrometry

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Epstein-Barr virus (EBV) encoded latent membrane protein 1 (LMP1) is the key oncoprotein in EBV-associated carcinoma. The LMP1 protein mimics cellular receptor to modulate plenty cellular signal transductions through self-aggregation and phosphorylation. The phosphorylation of LMP1 can be detected in EBV-infected or LMP1-gene transfected cell lines. Two phosphorylation sites, S313 and T324, have been predicted by site-directed mutagenesis. However there are additional phosphorylation sites that may involve in regulating the turnover rate of LMP1 protein. By *in vitro* kinase assay, we have demonstrated that the protein casein kinase 2 (CK2) is a potent LMP1 kinase. For further understanding whether this phosphorylation can be detected *in vivo*, the LMP1 protein was immunoprecipitated from flag-LMP1 gene transfected HEK293T cell lysate and characterized by using MALDI-TOF/TOF and LC-ESI MS/MS spectrometry. We found that there are one to two phosphorylation residues located in the carboxyl-terminus activation domain 1 (CTAR1) of LMP1. S211 and S215 are the two candidate amino acids. Since S211 is located in consensus CK2 recognition sites, whether LMP1 can be phosphorylated by CK2 *in vivo* will be further studied.

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A Strategy for Phosphoproteomics That Integrates Various Gel- and MS-based Techniques

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Objective: Protein phosphorylations have an outstanding role in signal transduction pathways and are thus of significant importance in disease-related studies. Therefore, reliable methods are needed for the detection and characterisation of phosphorylation sites in large numbers of proteins. Because of its well characterised genome and the relatively high abundance of phosphorylations, Arabidopsis thaliana is a convenient model organism. So far, many phosphorylation studies deal with individual proteins that are purified in order to submit them to extensive analysis approaches. Lately, a commercial staining kit became available that allows to selectively stain phosphoproteins in a 2D gel. This allows to parallelise phosphoprotein analysis and thus identify a high number of phosphorylated proteins.

Methods: After homogenisation, leaves of A. thaliana were submitted to 2D-gel-electrophoresis. For phosphoprotein analysis, the gel was stained using the Pro-Q-Diamond-stain (MolecularProbes). After a comparison with a total-protein stain, spots of various intensities were picked and digested. After PMF identification (MALDI-TOF-MS), aliquots of the potentially phosphorylated spots were submitted to an in-depth LC-MS/MS analysis.

Results and Conclusion: When a gel of Arabidopsis leaf material is stained using the phosphoprotein-specific dye, surprisingly many spots appear. Part of them, by comparison to the total-protein image of a Sypro-Ruby-stained gel and also MS identification results, seem to be false positives. The application of a phospho-sensitive stain allows a stepwise approach: Candidates that do not seem phosphorylated are eliminated in the staining and/or the MS identification step. Thus, the workload for LC-MS/MS analytics is reduced. This strategy can be applied not only to plants but to various kinds of (sub-)proteomes.