

Evidence for the plastidial origin of the isoprenyl residue used for the geranylgeranylation of a chimeric green fluorescent protein in BY-2 cells: A test system for inhibitors.

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Isoprenylation of proteins is a fundamental process in eukaryotic cells and consists in the formation of a thioether bond between the cysteine residue of a carboxyterminal CaaX motif and a C15 (farnesyl) or a C20 (geranylgeranyl) isoprenyl group, followed by proteolytic cleavage of the aaX part and carboxylation of the isoprenylated cysteine residue. “aa” stands for two aliphatic amino acid residues. If “X” stands for L (leucine), the protein is recognized by geranylgeranyl transferase I, if X corresponds to M (methionine) or to a few other amino acids, it will be farnesylated by farnesyl transferase. The covalent modification serves as signal for membrane targeting of proteins and/or interaction with other proteins,¹ and their becoming functional in various regulatory processes, for instance in hormone signalling. Using a rice calmodulin (*Oriza sativa* CaM61) C-terminal fragment with a motif for geranylgeranylation, we developed an experimental system that allows visualizing the localization of isoprenylated proteins in transformed tobacco Bright Yellow-2 cells. The geranylgeranylated GFP fusion protein is predominantly associated with the plasma membrane. Inhibition of isoprenylation by inhibitors or mutations in the CaaX box triggers a change in the intracellular targeting of the chimeric proteins, leading to their accumulation in the nucleus instead of at the plasma membrane. By using specific inhibitors of both isoprenoid biosynthetic pathways occurring in higher plants (i.e. mevinolin, which blocks cytoplasmic mevalonate formation, or fosmidomycin and oxoclozoxime, preventing the formation of methylerythritol diphosphate [MEP] and thus of the isoprenoid precursors in plastids) and by chemical complementation assays, we provide evidence for the essential role played by the plastidial MEP pathway in the geranylgeranylation of proteins in tobacco cells and another proof of metabolic cross-talk between plastids and cytoplasm. Cell-free extracts from BY-2 cells could isoprenylate such GFP fusion proteins when expressed in *E. coli*. Furthermore, mass spectral analyses of GFP fusion proteins isolated from transformed plant cells and purified through SDS-PAGE confirmed the presence of a geranylgeranylated peptide and its absence following inhibitor treatment. This experimental system can be used to evaluate new herbicides and drugs that could interact with the MEP pathway or the geranylgeranylation of proteins after having entered the cells and after their potential conversion into an active compound by cellular metabolic processes. It might also be useful in demonstrating toxic effects of specific inhibitors and in measuring biosynthetic fluxes, in extension of our previous studies.²

1 Crowell, D.N. (2000) *Progr. Lipid Res.* **39**, 393-408

2 Hemmerlin, A., Hoeffler, J.-F., Meyer, O., Tritsch, D., Kagan, I.A., Grosdemange-Billiard, C., Rohmer, M., and Bach, T.J. (2003) *J. Biol. Chem.* **278**, 26666-26676