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Research paper

Farnesol-mediated shift in the metabolic origin of prenyl groups used for protein prenylation in plants

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ABSTRACT

Little is known about how plant cells regulate the exchange of prenyl diphosphates between the two compartmentalized isoprenoid biosynthesis pathways. Prenylation of proteins is a suitable model to study such interactions between the plastidial methylerythritol phosphate (MEP) and the cytosolic mevalonate (MVA) pathways because prenyl moieties used to modify proteins rely on both origins. Tobacco cells expressing a prenylatable GFP were treated with specific MEP and/or MVA pathways inhibitors to block the formation of prenyl diphosphates and therefore the possibility to modify the proteins. Chemical complementation assays using prenyl alcohol precursors restore the prenylation. Indeed, geranylgeraniol (C_{20} prenyl alcohol) and to a lesser but significant level C_{15}-farnesol restored the prenylation of a protein bearing a geranylgeranylation CaaX motif, which under standard conditions is modified by a MEP-derived prenyl group. However, the restoration takes place in different ways. While geranylgeraniol operates directly as a metabolic precursor, the C_{15}-prenyl alcohol functions indirectly as a signal that leads to shift the metabolic origin of prenyl groups in modified proteins, here from the plastidial MEP pathway in favor of the cytosolic MVA pathway. Furthermore, farnesol interferes negatively with the MEP pathway in an engineered *Escherichia coli* strain synthesizing isoprenoids either starting from MVA or from MEP. Following the cellular uptake of a fluorescent analog of farnesol, we showed its close interaction with tobacco plastids and modification of plastid homeostasis. As a consequence, in tobacco farnesol supposedly inhibits the plastidial MEP pathway and activates the cytosolic MVA pathway, leading to the shift in the metabolic origin and thereby acts as a potential regulator of crosstalk between the two pathways. Together, those results suggest a new role for farnesol (or a metabolite thereof) as a central molecule for the regulation of isoprenoid biosynthesis in plants.

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1. Introduction

In eukaryotes, proteins with C-terminal CaaX motifs are modified by a zinc cation-mediated sulfur alkylation catalyzed by specific protein prenyl transferases (PPTs), referred to as type-I protein prenylation [1]. Similar to other isoprenoid molecules, protein prenylation depends on the biosynthesis of cellular isopentenyl diphosphate (IPP), the common biosynthetic precursor of this family of compounds. The PPT enzymes use one of two prenyl diphosphate substrates: farnesyl diphosphate (C_{15}, FPP) or geranylgeranyl diphosphate (C_{20}, GGPP) and a protein-CaaX substrate. CaaX motifs share a common C-terminal pattern described as follows: Cys-aliphatic amino acid-aliphatic amino acid, and X as an amino acid providing prenylation specificity [1]. Along with geranyl

Abbreviations: DX, 1-deoxy-D-xylulose; FOS, fosmidomycin; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IPP, isopentenyl diphosphate; MEcPP, 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate; MEP, 2-*C*-methyl-D-erythritol 4-phosphate; MV, mevinolin; MVA, mevalonic acid; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; PPTs, protein prenyltransferases.

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diphosphate (C_{10}, GPP), FPP and GGPP are the major isoprenoid precursors required for the biosynthesis of sterols, side chains of ubiquinones, of carotenoids, and a large variety of secondary isoprenoid metabolites [2,3]. In plants the organization of isoprenoid biosynthesis is considerably more complex than in other eukaryotes. One of the reasons of this increased intricacy is the huge number of molecules plants synthesize simultaneously. As a consequence, isozymes with dedicated functions operate in parallel. In addition, IPP is synthesized in the cytosol from mevalonic acid (MVA) by the same pathway found in animals and fungi, but also in the plastidial compartment via the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway commonly found in bacteria [3]. Recently, we demonstrated that a C_{20}-geranylgeranyl moiety of plastidic origin and formed via the MEP pathway, was used for geranylgeranylation of a chimeric GFP-CVIL protein substrate [4]. The limited use of a MEP pathway-derived prenyl group to modify the protein is characterized by an inhibition of protein prenylation when cells are treated with inhibitors of PPTs but also more particularly of MEP pathway specific enzymes (Fig. 1). Conversely, blocking the MVA pathway does not apparently affect the geranylgeranylation of the protein.

Accordingly, we proved that plastids are involved in the regulatory mechanism for supplying isoprenyl diphosphate used for protein prenylation in the cytoplasm of higher plants. Inhibition of protein geranylgeranylation with inhibitors of the MEP pathway can be chemically complemented by isoprenols such as geranylgeraniol, but also by farnesol, which is five carbons shorter than geranylgeraniol [4] (Fig. 1). The goal of this new study was to understand why farnesol showed some capacity to reestablish prenylation of a protein bearing a geranylgeranyl motif.

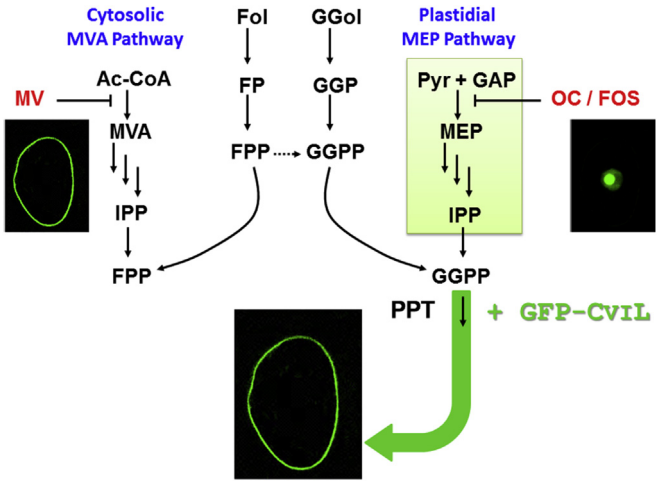

Fig. 1. Protein geranylgeranylation in tobacco BY-2 (TBY-2) cells involves the plastidial MEP pathway. The geranylgeranyl moiety used by the protein prenyltransferase (PPT) to modify a GFP-CVIL chimeric protein bearing a specific C-terminal geranylgeranylation CaaX motif (CVIL) is generated via the plastidial MEP pathway [4] and is characterized by a plasma membrane localized fluorescence of the GFP. In the presence of specific inhibitors of MEP pathway enzymes (OC: oxoclomazone; FOS: fosmidomycin) this plasma membrane protein is not further modified by a hydrophobic prenyl group and delocalizes into the nucleus. The absence of an isoprenoid pool synthesized from the MVA pathway (MV: mevinolin) has no effect on the prenylation of this protein. Complementation by exogenous prenyl alcohols such as farnesol (Fol) or geranylgeraniol (GGol) that are phosphorylated by endogenous kinases into the corresponding prenyl diphosphates (farnesyl diphosphate/FPP and geranylgeranyl diphosphate/GGPP) is indicated. They can virtually be utilized as prenyl diphosphates substrates by protein prenyltransferases. Ac-CoA: acetyl coenzyme A; IPP: isopentenyl diphosphate; Pyr: pyruvate; GAP: glyceraldehyde 3-phosphate.

2. Materials and methods

2.1. Chemicals

All-*trans*-farnesol, *trans*-geraniol, all-*trans*-geranylgeraniol, farnesyl acetate, dexamethasone, fosmidomycin and mevalonolactone were purchased from Sigma-Aldrich (Saint Quentin Fallavier). Mevinolin was a kind gift from Drs. M. Greenspan and A. W. Alberts (Merck Sharp and Dohme). Before use, lactone forms of mevinolin and mevalonolactone were converted into their free salt forms as described by Gerber et al. [4]. 1-deoxy-D-xylulose (DX) was obtained from AlsaChim (Illkirch Graffenstaden, France). The fluorescent analog to farnesol, NBD-geraniol (geranyl-*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) alcohol) was obtained from Prof. Herbert Waldmann (MPI, Dortmund) and utilized as described by Hemmerlin et al. [5].

2.2. Biological materials and growth conditions

The tobacco (*Nicotiana tabacum*) Bright Yellow-2 (TBY-2) cell line was cultured in MS-medium as described previously [4]. The TBY-2 cells expressing the chimeric GFP-CVIL prenylation substrate (TBY2::GFP-CVIL) [4] were cultured under identical conditions. For protein prenylation inhibition analyses, 7-day-old cells were diluted 5-fold into fresh MS-medium, treated for 3 h with chemicals, before protein expression was induced with dexamethasone (15 μM) and cells incubated for an additional 15 h. Working stock solutions of isoprenols were prepared in ethanol and used at a final 1:1000 (v:v) dilution in the culture medium. Controls were treated with the solvent alone. For transit expression of PT5-RFP, TBY-2 cells were transformed by tungsten particle shooting with an inflow gun as described [6], then incubated for 5 h before being recovered in liquid MS medium and cultured in the presence of farnesol (1–6 h) as indicated.

The engineered *dxs::KAN* disrupted *Escherichia coli* mutant strain expressing the *Lactobacillus plantarum* MVA metabolizing operon was obtained as described previously [7]. This strain grows at 28 °C on LB medium supplemented with kanamycin (15 μg/mL), ampicillin (100 μg/mL) and requires a source of isoprenoid precursors either in the form of MVA or DX (750 μM). The nature of the precursor used to grow defines the pathway that is activated and used to synthesize isoprenoids in this strain, either through the *Lactobacillus plantarum* MVA pathway when MVA is used, or the endogenous MEP pathway when DX is used to initiate cell growth.

2.3. Microscopy methods

As previously described in detail, the subcellular localization of a GFP fusion protein with a C-terminal prenylatable CaaX sequence was used to evaluate protein prenylation *in vivo* [4]. This technique has the added benefit of providing information about the metabolic origin of the prenyl groups used to modify a chimeric GFP-fusion protein bearing a geranylgeranylation motif (GFP-CVIL) by inhibiting specific enzymes within the isoprenoid biosynthesis pathways [8]. Confocal laser scanning microscopy (CLSM) was used to monitor the subcellular location of the modified GFP, which, if prenylated, integrates into the plasma membrane, otherwise dislocates to the nucleus. Protein prenylation was visualized with a Zeiss Confocal LSM700 microscope. Co-localization experiments were performed in multitrack mode. Excitation/emission wavelengths were set as follows: 488 nm/500–600 nm for GFP and 555 nm/585–615 nm for RFP. Images were acquired using Carl Zeiss ZEN software and processed using Adobe Photoshop.

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