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Characterization of the Arabidopsis *clb6* Mutant Illustrates the Importance of Posttranscriptional Regulation of the Methyl-D-Erythritol 4-Phosphate Pathway [W]

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The biosynthesis of isopentenyl diphosphate and dimethylallyl diphosphate, the two building blocks for isoprenoid biosynthesis, occurs by two independent pathways in plants. The mevalonic pathway operates in the cytoplasm, and the methyl-D-erythritol 4-phosphate (MEP) pathway operates in plastids. Plastidic isoprenoids play essential roles in plant growth and development. Plants must regulate the biosynthesis of isoprenoids to fulfill metabolic requirements in specific tissues and developmental conditions. The regulatory events that modulate the plant MEP pathway are not well understood. In this article, we demonstrate that the *CHLOROPLAST BIOGENESIS6* (*CLB6*) gene, previously shown to be required for chloroplast development, encodes 1-hydroxy-2-methyl-butenyl 4-diphosphate reductase, the last-acting enzyme of the MEP pathway. Comparative analysis of the expression levels of all MEP pathway gene transcripts and proteins in the *clb6-1* mutant background revealed that posttranscriptional control modulates the levels of different proteins in this central pathway. Posttranscriptional regulation was also found during seedling development and during fosmidomycin inhibition of the pathway. Our results show that the first enzyme of the pathway, 1-deoxy-D-xylulose 5-phosphate synthase, is feedback regulated in response to the interruption of the flow of metabolites through the MEP pathway.

INTRODUCTION

Like all living organisms, plants synthesize an enormous variety of isoprenoids that serve as growth regulators, pigments, and structural components of membranes. Additionally, many isoprenoids are of biotechnological importance (Chappell, 2002). All isoprenoids are derived from two basic five-carbon precursors, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). Depending on the specific isoprenoid, these five carbon units undergo consecutive condensations and ulterior chemical modifications to produce the enormous variety of isoprenic compounds (Sacchettini and Poulter, 1997; Croteau et al., 2000).

In higher plants, two pathways are used for the synthesis of the basic isoprenoid units. The mevalonic (MVA) pathway occurs in the cytoplasm where sesquiterpenes (C_{15}) and triterpenes (C_{30}), such as phytosterols, dolichols, and farnesyl residues, for protein prenylation are produced (Bach et al., 1999; Lichtenthaler, 1999; Eisenreich et al., 2001). By contrast, the methyl-D-erythritol 4-phosphate (MEP) pathway operates in plastids and produces IPP and DMAPP (Figure 1) for the synthesis of isoprenoids, such as isoprene, carotenoids, plastoquinones, phytol conjugates (such as chlorophylls and tocopherols), and hormones (gibberellins and abscisic acid) (Schwender et al., 1996; Zeidler et al., 1997; Eisenreich et al., 1998; Lichtenthaler, 1999; Rohmer, 1999). In spite of this compartmentalization, evidence indicates that cross talk between both pathways exists (Kasahara et al., 2002; Bick and Lange, 2003; Hemmerlin et al., 2003; Laule et al., 2003), although the biological implications of this communication are not fully understood.

Through the valuable contributions of many laboratories, the elucidation of the entire MEP pathway has been accomplished in an impressively short time (Rohmer et al., 1996; Lichtenthaler, 1999; Eisenreich et al., 2001, 2004; Rodríguez-Concepción and Boronat, 2002). In the initial step of the pathway (Figure 1), the precursors pyruvate and glyceraldehyde 3-phosphate are converted to 1-deoxy-D-xylulose-5-phosphate (DXP) by the enzyme 1-deoxy-D-xylulose 5-phosphate synthase (DXS) (Rohmer et al., 1996; Sprenger et al., 1997; Lois et al., 1998). The DXP produced by this reaction is not used exclusively in the MEP pathway because it is also required for the production of the vitamins thiamin and pyridoxal (Julliard and Douce, 1991; Julliard, 1992). In the second step, DXP is converted to MEP by the enzyme DXP reductoisomerase (DXR). This represents the first committed step of the pathway, from which its name is derived (Kuzuyama et al., 1998; Takahashi et al., 1998; Schwender et al., 1999; Miller et al., 2000). The following steps involve five consecutive reactions (Figure 1) that culminate with the production of IPP

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