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Breaking new ground in the regulation of the early steps of plant isoprenoid biosynthesis

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The common metabolic precursors used for the production of all isoprenoid compounds are synthesized by two unrelated pathways in plants. The methylerythritol 4-phosphate (MEP) pathway produces these precursors in the plastid, whereas the biosynthesis of non-plastidial isoprenoids relies on the operation of the mevalonic acid (MVA) pathway. Despite the physical separation of the two pathways, some interaction exists at molecular and metabolic levels. Recent results have provided strong evidence that a high degree of control over each individual pathway takes place at the post-translational level. In particular, new mechanisms regulating the levels and activity of rate-determining enzymes have been unveiled. Current challenges include the study of the subcellular operation of the MEP and MVA pathways and their coordination with upstream and downstream pathways that supply their substrates and consume their products.

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Occurrence and functions of isoprenoids

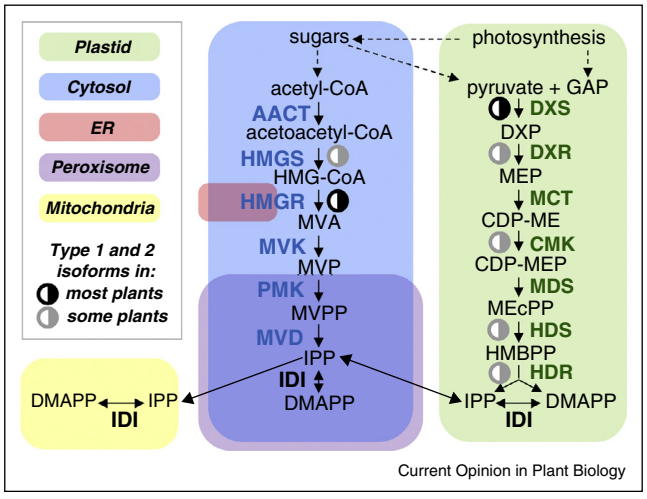
Isoprenoids are an astoundingly diverse group of metabolites that include compounds essential for cell functions and interactions with the environment [1]. While isoprenoids are produced in all free-living organisms, their abundance and variety in plants is unparalleled. Plant isoprenoids include tens of thousands of compounds. Some isoprenoids can be considered as 'primary' metabolites because they are essential for normal plant function. These essential isoprenoids participate in membrane architecture (sterols), respiration (ubiquinone), photosynthesis (chlorophylls, carotenoids, prenylquinones), and regulation of growth and development (cytokinins, brassinosteroids, gibberellins, abscisic acid, strigolactones), and therefore they are common to most if not all plant species. However, most plant isoprenoids are 'secondary' metabolites, i.e. non-essential compounds with specialized functions in allelopathic and biotic interactions and they are usually produced by particular plant families, species, or/and organs. They include many monoterpenes, diterpenes, triterpenes, sesquiterpenes and polyterpenes. A large number of secondary isoprenoids (also known as terpenoids) have economic relevance as flavours, pigments, polymers, or drugs [1,2].

All types of isoprenoids derive from the same five-carbon (C_5) precursors, i.e. isoprene units: isopentenyl diphosphate (IPP) and its double-bond isomer dimethylallyl diphosphate (DMAPP) (Figure 1). Condensation of these units generates prenyl diphosphate molecules of defined chain length (C_{10}, C_{15}, C_{20},... C_{5n}) which serve as the starting points for the production of all the variety of isoprenoids [2]. Until the mid-1990s, it was generally believed that IPP was synthesized from acetyl-CoA via mevalonic acid (MVA) and then isomerized to DMAPP in all living organisms. However, biochemical and genetic evidence demonstrated the existence of a completely novel pathway in bacteria and plastids for the simultaneous production of both IPP and DMAPP from pyruvate and GAP (Figure 1). By 2002 all the steps of the new pathway, currently known as the methylerythritol 4-phosphate (MEP) pathway, had been identified [3–5] (Figure 1).

Isoprenoid precursor biosynthesis in plant cells: two pathways in different compartments

While most organisms only have one of the two pathways for the production of isoprene units, plants and some algae use both MVA and MEP pathways to produce their isoprenoids, albeit in different cell compartments [4•,5] (Figure 1). Algae retained the MEP pathway once acquired but lost the MVA pathway in various (sub)groups [5]. In plants, however, isoprene units used in the cytosol, ER and mitochondria for the production of sesquiterpenes, triterpenes, sterols, brassinosteroids, and ubiquinone are typically generated by the MVA pathway, whereas the plastidial precursors for the biosynthesis of isoprene, monoterpenes, diterpenes, carotenoids, abscisic acid, strigolactones, gibberellins, and the side chain of chlorophylls and prenylquinones (tocopherols, phylloquinones, and plastoquinone) are mostly provided by the MEP pathway. However, labelling experiments suggest that some isoprenoids can be produced from precursors

Figure 1



The MVA and MEP pathways in plants. MVA pathway enzymes are shown in blue, and MEP pathway enzymes in green. The subcellular localization of these enzymes is indicated with colours. The existence of differentially expressed isoforms with a reported primary (type 1) or specialized (type 2) role is also indicated (see box). Dashed arrows mark multiple steps, and open arrows represent transport of metabolites between cell compartments. DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; MVA, mevalonic acid; MVP, 5-phosphomevalonate; MVPP, 5-diphosphomevalonate; GAP, glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-*C*-methyl-D-erythritol 4-phosphate; CDP-ME, 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol; CDP-MEP, CDP-ME 2-phosphate; MEcPP, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate; HBMPP, 1-hydroxy-2-methyl-2-butenyl 4-diphosphate. Enzymes are indicated in bold: IDI, IPP isomerase; AACT, acetoacetyl-CoA thiolase; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; MVK, MVA kinase; PMK, MVP kinase; MVD, MVPP decarboxylase; DXS, DXP synthase; DXR, DXP reductoisomerase; MCT, MEP cytidyltransferase; CMK, CDP-ME kinase; MDS, MEcPP synthase; HDS, HMBPP synthase; HDR, HMBPP reductase.

produced by both pathways [4•]. Although a limited cross-flow of IPP and prenyl diphosphates (C_5 to C_{15}) can actually take place between the plastids and the cytosol, the exchange rate under normal growth conditions is not high enough to rescue the pharmalogical or genetic block of one of the pathways with the products of the other pathway [6–13]. The advantages for plants of retaining two pathways (MVA and MEP) in separate compartments are not fully understood [4•]. It is likely, however, that the physical separation of the pathways facilitates the optimal supply of the metabolic precursors required in each cell compartment. For example, many plastidial isoprenoids are required for photosynthesis and therefore a chloroplast-based control of their production appears to be more effective.

Overall regulation of the supply of isoprenoid precursors in plant cells

The analysis of global gene co-expression networks in *Arabidopsis thaliana* has shown very little interaction between the MVA and MEP pathways [14,15]. Moreover, the few connections between the pathways are negative, i.e. transcriptional activation of genes in one pathway is correlated with the repression of genes in the other pathway. For example, MEP pathway genes are up-regulated by light during seedling deetiolation, when an active production of plastidial isoprenoids is required to support photosynthetic development [16–19]. By contrast, illumination of dark-grown seedlings results in down-regulated levels of transcripts for MVA pathway enzymes and a reduced accumulation of sterols [16,17]. MVA and MEP pathway genes are also regulated antagonistically by the circadian clock, which further controls the expression of other genes involved in downstream pathways [20]. Interestingly, an Arabidopsis mutant defective in the first step of the MEP pathway was isolated in a screening for plants showing an enhanced resistance to the pharmacological block of the MVA pathway [21], highlighting the existence of mechanisms that coordinate the operation of these pathways. The isolation and characterization of mutants that survive otherwise lethal concentrations of inhibitors specific for the MVA pathway (including mevinolin or lovastatin) or the MEP pathway (clomazone and fosmidomycin) also unveiled a number of unexpected factors co-regulating both the MVA and MEP pathways, including mitochondrial respiration [22,23], carbon (sugar) availability [12], and light signalling [9]. In many cases, these factors act at the post-translational level, modulating the levels and activity of particular biosynthetic enzymes. Our current understanding is that transcriptional regulation of genes encoding biosynthetic enzymes exerts a coarse control of the MVA and MEP pathways, whereas post-transcriptional and post-translational regulation of enzyme levels and activity contributes to the fine-tuning of their metabolic flux [4•,15,17]. In the next sections we will review some of the latest findings on the post-translational mechanisms that control the activity of the main rate-determining enzymes of the MVA and MEP pathways. The enzyme acronyms correspond to those in Figure 1.

Regulation of HMGR and the MVA pathway

It is well accepted that HMGR is a key enzyme of the MVA pathway, but the regulatory role of other MVA pathway enzymes has been little explored so far [4•,24]. In plants, HMGR is encoded by gene families. While some HMGR isoforms participate in the synthesis of housekeeping isoprenoids, others are known to play a role in the production of secondary isoprenoid metabolites [2]. Recent reports are providing new insights on isoform-specific functional roles of the structural domains of the protein (Figure 2). The N-terminal region of HMGR has been involved in the differential targeting of enzyme isoforms to either the ER or to vesicular structures that may represent ER-derived subcompartments for isoprenoid biosynthesis [25,26]. The specific interaction of this region with PROTEIN PHOSPHATASE 2A (PP2A) subunits has been reported to mediate