Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants

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Abstract

The methyl-D-erythritol 4-phosphate pathway is responsible for the biosynthesis of a substantial number of natural compounds of biological and biotechnological importance. In recent years, this pathway has become an obvious target to develop new herbicides and antimicrobial drugs. In addition, the production of a variety of compounds of medical and agricultural interest may be possible through the genetic manipulation of this pathway. To this end, a complete understanding of the molecular mechanisms that regulate this pathway is of tremendous importance. Recent data have accumulated that show some of the multiple mechanisms that regulate the methyl-D-erythritol 4-phosphate pathway in plants. In this review we will describe some of these and discuss their implications. It has been demonstrated that 1-deoxy-D-xylulose-5-phosphate synthase (DXS), the first enzyme of this route, plays a major role in the overall regulation of the pathway. A small gene family codes for this enzyme in most of the plants which have been analysed so far, and the members of these gene families belong to different phylogenetic groups. Each of these genes exhibits a distinct expression pattern, suggesting unique functions. One of the most interesting regulatory mechanisms recently described for this pathway is the post-transcriptional regulation of the level of DXS and DXR proteins. In the case of DXS, this regulation appears conserved among plants, supporting its importance. The evidence accumulated suggests that this regulation might link the activity of this pathway with the plant’s physiological conditions and the metabolic demand for the final products of this route.

Key words: DXS enzyme, isoprenoids, MEP pathway, plastids.

Introduction

Isoprenoids constitute one of the most diverse families of natural products. The total number of these compounds is in the order of 20 000–35 000 and their structures and functions are overwhelmingly varied (Chappell, 1995, 2002; Chasan, 1995; McGarvey and Croteau, 1995; Sacchettini and Poulter, 1997; Eisenreich et al., 2004; Hunter, 2007). These compounds are present in all living organisms, and several of them have essential functions in overall growth and development. All isoprenoid compounds are produced by two universal 5-carbon precursors; isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Through evolution, two non-related biosynthetic routes have been selected for the synthesis of these two basic building blocks which use different precursors (Bouvier et al., 2005). One is the mevalonate pathway where IPP and DMAPP are synthesized from mevalonic acid. The other, known as the methyl-D-erythritol 4-phosphate (MEP) pathway, uses pyruvate and D-glyceraldehyde 3-phosphate for IPP and DMAPP synthesis (Fig. 1). Although the existence of the mevalonic pathway has been known for many years and has been the subject of intense studies, it has been little more than 15 years since the MEP pathway has been shown to be an alternative route for the biosynthesis of IPP and DMAPP (Rohmer et al., 1993; Sprenger et al., 1997; Eisenreich et al., 1998). In the years since its discovery remarkable advances have been made, including the identification of each of the biosynthetic steps of the MEP pathway and, more recently, the understanding...
The MEP pathway is present in many eubacteria, apicomplexa parasites, and photosynthetic eukaryotes, but is absent from other eukaryotes, including humans (Lange et al., 2000). Thus, the genes and enzymes of this pathway are attractive targets for the development of new antibacterial and antiparasitic drugs and herbicides (Zeidler et al., 2000; Rodriguez-Concepción et al., 2004; Rohdich et al., 2005). A number of compounds produced by this pathway also have nutritional or medical value, and are important targets for biotechnological manipulation. Among these are essential antioxidant molecules such as carotenoids and tocopherols as well as anti-oncogenic drugs (Dubey et al., 2003; DellaPenna and Pogson, 2006). Finally, and no less important, several major plants hormones are produced through this pathway from direct biosynthesis (gibberellins) or as cleavage derivatives, such as abscisic acid and the recently discovered hormone strigolactone (Gomez-Roldan et al., 2008; Umehara et al., 2008). Due to the central role this pathway plays in the growth and development of plants and for human health and nutrition, the identification of the major regulatory steps is important.

Recent work has centred on the identification of the signals that regulate the expression of the genes and proteins of the MEP pathway as well as determining the rate-limiting enzymes in this biosynthetic route. The aim of this review is to highlight some of the recent findings of the regulatory mechanisms that operate in this pathway in plants. We think that these findings are essential for future manipulation of this route and will shed light on unprecedented regulatory mechanisms that modulate this metabolic route.

**DXS; a critical enzyme of the MEP pathway**

The MEP pathway operates through the participation of eight consecutive enzymes to produce the IPP and DMAPP universal basic blocks of isoprenoid compounds from the precursors pyruvate and d-glyceraldehyde 3-phosphate (Fig. 1). Despite their prokaryotic origin during endosymbiosis, all the genes coding for the enzymes of this pathway in plants are encoded in the nucleus. The first step of the MEP pathway is catalysed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS, EC 2.2.1.7), converting the precursors pyruvate and glyceraldehyde 3-phosphate into 1-deoxy-D-xylulose 5-phosphate (DXP) (Sprenger et al., 1997; Lange et al., 1998; Lois et al., 1998). Historically DXS was the first gene identified from this pathway and a considerable amount of data has accumulated on its regulation and function. In *Escherichia coli*, this enzyme is also required for the synthesis of the non-isoprenoid vitamins thiamine and pyridoxal (Julliard and Douce, 1991). Consequently, this initial biosynthetic step is not specific to the MEP pathway in this organism. However, this feature appears different in other organisms, as a DXS-independent pathway for the synthesis of pyridoxal (B<sub>6</sub>) has been found in plants and several bacteria (Tambasco-Studart et al., 2005; Fitzpatrick et al., 2007). In fact, recent data suggest that the B<sub>6</sub>
DXP-independent pathway is the original pathway for the biosynthesis of vitamin B6 in most organisms and it has been lost during the evolution of some bacterial lineages, including that of E. coli (Fitzpatrick et al., 2007).

Diverse experimental evidence demonstrates that DXS plays a critical role in the synthesis of IPP and DMAPP. Analysis of Arabidopsis transgenic lines that express higher (over-expressors) or lower (antisense) DXS levels showed that the level of various isoprenoid final products including chlorophyll, carotenoids, tocopherols, and ABA are increased or decreased in the corresponding transgenic plants (Estévez et al., 2001). Similar results have been obtained in other plants including tomato (Lois et al., 2000; Enfissi et al., 2005), potato (Morris et al., 2006) and Ginkgo biloba (Gong et al., 2006). The impact on the level of some final products of the pathway (between 2–7-fold) reported in these analyses support that, in plants, this enzyme catalyses a rate-limiting step for the synthesis of the IPP and DMAPP building blocks and, also, for the accumulation of various isoprenoid final products. The rate-limiting function of this enzyme has also been found in eubacteria (Harker and Bramley, 1999; Kuzuyama et al., 2000; Matthews and Wurtzel, 2000).

In addition to DXS, experimental evidence in plants suggest that the second and the seventh enzymes of this pathway; DXP reductoisomerase (DXR, EC 1.1.7.12) and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR, EC 1.1.7.12) also have rate-limiting roles in IPP and DMAPP synthesis (Veau et al., 2000; Walter et al., 2000; Mahmoud and Croteau, 2001; Botella-Pavía et al., 2004; Carretero-Paulet et al., 2006). However, the rate-limiting role for each of these two enzymes appears to vary among plants and in different conditions. Additional studies are required to determine the precise conditions in which these enzymes are rate-limiting steps for the pathway flow. Thus, at present, DXS represents the most obvious target for the manipulation of this metabolic route. An example of DXS manipulation of food crops through biotechnology has been demonstrated in tomato (Enfissi et al., 2005) and potato tubers (Morris et al., 2006). In the transgenic tomato lines presented in this study, the content of nutritional isoprenoid compounds, particularly phytolene and β-carotene, was increased more than 2-fold. In potato tubers up to 7-fold increase in phytoene was reported. The importance of such manipulations of the MEP pathway and the experimentation that has led to improved crops has been reviewed recently (Botella-Pavía and Rodríguez-Concepción, 2006). An interesting alternative to explore in the future is the manipulation of this enzyme in conjunction with other downstream enzymes specific for particular isoprenoid biosynthesis.

Some MEP pathway enzymes are encoded by small gene families in plants

Initial molecular characterization suggested that most genes of the MEP pathway exist as single copy genes in the genomes of plants. This contrasts with the mevalonic pathway where most enzymes are encoded by gene families and in which the specific expression of each gene is an important mode of regulation. However, after the full Arabidopsis genome sequence was obtained, the existence of three DXS-like genes was clear, based on sequence similarity (Araki et al., 2000). The presence of multiple genes that potentially encode DXS appears to be widespread in plant species, including conifers, dicots, and monocots (Table 1). Based on the number of isoprenoid compounds synthesized from this pathway, a tempting speculation is that each gene member of this family might be involved in the synthesis of particular products and, consequently, subject to independent regulation.

Multiple DXS genes have been found in Zea mays, Medicago truncatula, Oryza sativa, Picea abies, G. biloba, and Pinus densiflora (Walter et al., 2000, 2002a; Kim et al., 2005, 2006, 2009; Phillips et al., 2007). In all these plants, two or three potential DXS genes have been reported and phylogenetic analysis shows that these genes clustered into independent clades that apparently originated before the diversification of monocotyledonous and dicotyledonous plants (Krushkal et al., 2003; Kim et al., 2005, 2009). The fact that representative genes belonging to each of these clades are maintained during evolution in several species supports a particular function for them. Exceptions are Arabidopsis and P. abies in which, although three different DXS-like genes exist, two of them cluster into the same clade (Kim et al., 2005; Phillips et al., 2007).

Several reports have shown that, in different plants, each of the DXS-like genes displays a particular expression pattern. For example, the transcripts from one of the DXS-like genes from maize, Medicago, tomato, and tobacco (referred as DXS type-1) accumulates at high levels in photosynthetical tissues, displaying a similar pattern to DXS/CLAI from Arabidopsis (Walter et al., 2002a). These DXS genes group into the same phylogenetic clade which suggests that they may have similar functions in the synthesis of the various isoprenoids required for the photosynthetic process. By contrast, the transcript of a second DXS gene-type (DXS type-2) accumulates in maize, rice, barley, and Medicago roots upon colonization by mycorrhizal fungi, concurrent with the increase in apocarotenoid compounds (Walter et al., 2000, 2002a). Specific expression patterns of the DXS type-2 genes have been reported in G. biloba (Kim et al., 2006), P. abies (Phillips et al., 2007), and P. densiflora (Kim et al., 2009). In each of these cases the synthesis of particular isoprenoid compounds has been correlated with the expression of the DXS type-2 genes, leading to speculation that these genes might be involved in the synthesis of specific isoprenoid-derived secondary metabolites.

Finally, in some species a third DXS-like gene referred to as DXS type-3 has been identified that clusters into an independent clade with DXS genes from other plants. The expression pattern of the rice ddx3 gene is restricted to specific tissues (Kim et al., 2005), which suggests a particular function for these types of DXS genes, but the role of this
Table 1. MEP pathway gene families

The nomenclature used for each gene follows the name given in the literature. The extension ‘like’ is used as suggested by Phillips et al., 2008 in those genes whose function has not been demonstrated. The number in parenthesis indicates the proposed class in which the DXS protein belongs.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Plant species</th>
<th>Gene</th>
<th>Protein (type*)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS</td>
<td>Arabidopsis thaliana</td>
<td>DXS</td>
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<td>Estevez et al., 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DXT1</td>
<td>DXS2 (1)</td>
<td>Araki et al., 2000</td>
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<tr>
<td></td>
<td></td>
<td>DXT2</td>
<td>DXS3 (3)</td>
<td>Araki et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Picea abies</td>
<td>PaDXS1</td>
<td>PaDXS1 (1)</td>
<td>Phillips et al., 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PaDXS2A</td>
<td>PaDXS2A (2)</td>
<td>Phillips et al., 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PaDXS2B</td>
<td>PaDXS2B (2)</td>
<td>Phillips et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Medicago truncatula</td>
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<td>MDTX1 (1)</td>
<td>Walter et al., 2002a</td>
</tr>
<tr>
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<td></td>
<td>MDTX2-like</td>
<td>MDTX2 (2)</td>
<td>Walter et al., 2002a</td>
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<tr>
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<td>Ginkgo biloba</td>
<td>GbDXS1</td>
<td>GbDXS1 (1)</td>
<td>Kim et al., 2006</td>
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<tr>
<td></td>
<td></td>
<td>GbDXS2</td>
<td>GbDXS2 (2)</td>
<td>Kim et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Zea mays</td>
<td>dxs1-like</td>
<td>OsDXS1 (1)</td>
<td>Kim et al., 2005</td>
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<tr>
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<td>OsDXS2 (3)</td>
<td>Kim et al., 2005</td>
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<td>dxs3</td>
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<td>Kim et al., 2005</td>
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<td></td>
<td>Hevea brasiliensis</td>
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<td>HbDXR1</td>
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<td>GbHDR1</td>
<td>Kim et al., 2008</td>
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<td>GbIDS2</td>
<td>GbHDR2</td>
<td>Kim et al., 2008</td>
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<tr>
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<td>Pinus taeda</td>
<td>PtIDS1</td>
<td>PtHDR1</td>
<td>Kim et al., 2008</td>
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<tr>
<td></td>
<td></td>
<td>PtIDS2</td>
<td>PtHDR2</td>
<td>Kim et al., 2008</td>
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gene-type is not sufficiently clear and requires further analysis.

In contrast to DXS, the following six enzymes of the MEP pathway are encoded by single genes in Arabidopsis. This seems also to be true for some other plants whose genomes have been fully sequenced, including rice, Populus trichocarpa, and M. truncatula. However, recent reports have shown that, in some plants, multiple genes do exist for other downstream pathway proteins. An example of this is the enzyme DXR, which catalyses the second step of the pathway. A recent report shows that this enzyme is encoded by at least two genes in the angiosperm Hevea brasiliensis (Seetang-Nun et al., 2008). Two different genes that code for HDR, which catalyses the penultimate step in the pathway, have also been identified in gymnosperms (Kim et al., 2008). These results open the possibility that tissue-specific expression might contribute to the regulation of the MEP pathway in plants. Finally, in the Arabidopsis genome, two genes encoding isopentenyl diphosphate isomerase (IDI) exist. This gene catalyses the interconversion between IPP and DMAPP and it is also required for the mevalonic pathway. It has been shown that HDR has the capacity to synthesize a mixture of IPP and DMAPP, implying that IDI might not be essential in the MEP pathway (Hoeffler et al., 2002; Rohdich et al., 2002). However, later studies have suggested that IDI plays a critical role in this pathway (Page et al., 2004; Okada et al., 2008; Phillips et al., 2008a), but the precise contribution of each of the genes encoding this enzyme is not yet fully understood.

A complex scenario has started to emerge in which the existence of multicopy genes for some enzymes of the MEP pathway allows for the possibility of differential control and expression of each particular copy. These different gene family members may be responsible for the synthesis of particular isoprenoid products in some plants.

Functional analysis of some MEP pathway genes

To date the enzymatic activity of only some of the multiple DXS genes has been experimentally demonstrated. In Arabidopsis, enzymatic activity and function as a DXP synthase has been shown for DXS/CLA1 (Estévez et al., 2000). Knockout mutations in DXS gene (accession number U27099 initially reported as CLA1) result in lethal albino plants (Fig. 2), with severely affected chloroplasts that are unable to synthesize photosynthetic pigments (Mandel et al., 1996). These data show that the other DXS-like genes (DXL genes) present in the Arabidopsis genome, if functional, do not have completely redundant activities with DXS sufficient to support photosynthetic pigment (chlorophyll and carotenoid) biosynthesis. In Arabidopsis, the functionality of the DXL1 and DXL2 genes remains unclear, and a recent report suggests that both of these genes appear functionally inactive (Phillips et al., 2008b). Activity for other DXS type-1 enzymes has been shown in other species including tomato, P. abies, and P. densiflora (Lois.
Enzymatic activity of some of the DXS genes that belong to other clades, and that are involved in the synthesis of particular isoprenoids, has been demonstrated in *M. truncatula*, *G. biloba*, *P. abies*, and *P. densiflora* (Kim et al., 2006, 2009; Phillips et al., 2007; Floss et al., 2008). The isolation and characterization of mutants in MEP pathway genes are important in order to corroborate the functionality of some genes in this pathway (references in Phillips et al., 2008a). Knock-out mutations for several genes that constitute the pathway in *Arabidopsis*, display an albino phenotype (Fig. 2) with profound alterations to chloroplast development (Mandel et al., 1996; Gutiérrez-Nava et al., 2004). An exception to this is mutants in the IPP isomerase genes (IDI1 and IDI2), which appear to be non-essential for the functioning of the pathway (Okada et al., 2008; Phillips et al., 2008a). However, neither the phenotypes of mutants in these two genes nor their subcellular localization is entirely clear and requires future analysis.

An interesting observation, derived from the phenotypic characterization of MEP pathway mutants affecting the first (DXS) and the last two steps in the pathway (HDS; encodes for 4-hydroxy-3-methylbut-2-enyl diphosphate synthase EC 1.17.4.3, and HDR) is that these genes act in a non-cell autonomous way during embryogenesis. In the developing embryos of these mutants, partial complementation of chlorophyll accumulation is observed under microscopic observation (Gutiérrez-Nava et al., 2004). This is attributed to the import of an as yet unknown precursor of maternal origin into the developing embryo. This finding is itself notable and it will be of interest to identify the compound or compounds capable of moving between cells to give such complementation.

### The MEP pathway is subject to multiple levels of regulation

Mutants that affect the MEP pathway have also been useful in identifying regulatory signals that modulate this pathway at both the transcript and protein levels (Guevara-Garcia et al., 2005; Sauret-Güeto et al., 2006). In mutants that affect chloroplast development (including mutants in the MEP pathway) the accumulation of the different MEP pathway transcripts is reduced compared with wild-type plants (Guevara-Garcia et al., 2005). A similar response has been observed with the carotenoid biosynthesis inhibitor norflurazon that promotes oxidative damage in the chloroplasts and which results in retrograde signalling (Jarvis, 2003; Pogson et al., 2008). These results suggest that the expression of all of the genes in this pathway are regulated by retrograde chloroplast to nuclear signalling in response to the arrested chloroplast development. Although the transcript level in all MEP genes is reduced in these conditions, some genes appear particularly sensitive to this regulatory signal, which remains to be studied in more detail.

Comparative expression analysis of the MEP pathway genes under various growing conditions shows that transcript accumulation of these genes is also modulated by multiple external signals, and in a co-ordinated manner in plants. One of the signals that has a major impact on the transcript accumulation of several genes in the MEP pathways is light. Expression analyses in *Arabidopsis* showed that the transcript levels of all genes from the pathway accumulate upon exposure to light (Fig. 3), and in seedlings during the development of the first true leaves (Carretero-Paulet et al., 2002; Guevara-Garcia et al., 2005; Hsieh et al., 2008). Light regulation for some of the MEP pathway genes has also been reported in other plants (Hans et al., 2004; Kim et al., 2005). Positive regulation of all the genes of the pathway by light provides an advantage during early seedling development, during which there is an elevated demand for the photosynthetic pigments derived from this pathway. This regulation may not be a universal plant feature, in particular for those proteins encoded by multiple genes with specific expression patterns. For example, a recent report shows that the expression of the DXR gene in rubber trees is not induced by light (Seetang-Nun et al., 2008).

Circadian regulation of all the genes of the pathway has also been observed in *Arabidopsis*. Transcripts for all MEP pathway genes fluctuate following the same 24 h phase,
reaching their highest transcript levels just before dawn (Fig. 4). Similar results have been published by Hsieh and Goodman (2005), however, in this report, the synchronized response of all the different genes is not as obvious. In rice, an additional external signal that was reported to regulate the expression of one of the $\text{DXS}$ genes (referred as $\text{dxs3}$) is UV light (Kim et al., 2005). It will be interesting to determine if this regulation is present in other genes of the pathway and in different plants, or if it is a particular regulation of this member of the $\text{DXS}$ family. A recent report has shown that the transcript accumulation of $\text{DXS}$, $\text{DXR}$, and $\text{HDR}$ genes is regulated by mechanical wounding and fungal elicitors. Interestingly, this regulation only affects one of the $\text{DXS}$ gene-types, which supports a particular need for some of these genes under specific conditions (Phillips et al., 2007).

It is not only environmental signals that regulate the expression of multiple MEP pathway genes in a co-ordinated manner, but also nutritional cues. It has been reported that sugars (sucrose) have the capacity to increase the accumulation of several of the MEP pathway genes transcripts in dark-grown plants (Hsieh and Goodman, 2005). Transcript regulation by sugar availability has also been observed by our group (C San Román, unpublished results). Since the two precursor molecules of the MEP pathway (d-glyceraldehyde 3-phosphate and pyruvate) are derived directly from photosynthesis or glycolysis, it is not surprising that sugar levels regulate this pathway by altering the expression of these enzymes.

The expression analysis of the MEP pathway in different conditions has shown co-ordinated regulation between most genes of the pathway. This prompted speculation that there

Fig. 3. Light regulation of the MEP pathway genes. RNA blot analysis of $\text{DXS}$, $\text{DXR}$, $\text{MCT}$, $\text{CMK}$, $\text{MDS}$, $\text{HDS}$, and $\text{HDR}$ transcript accumulation in 8-d-old $\text{Arabidopsis}$ seedlings grown in the dark for 5 d and exposed to light for 0 h or 6 h. Five $\mu$g of total RNA was loaded in each lane and hybridized with the corresponding probe. The $\text{CAB1}$ gene was used as a positive control for the light induction treatment. The ethidium bromide (EtBr) gel is shown as a loading control. The changes in expression in the Northern blots have been quantified and the corresponding graph for each gene at 0 h (black bars) or 6 h (grey bars) is also included.
may be common regulatory factors responsible for these responses. A future area of interest will be to identify those regulatory factors involved in the common responses observed for the genes in this pathway.

The accumulation of the MEP pathway enzymes is regulated by novel post-transcriptional mechanisms

In order to understand any additional regulatory events that modulate the MEP pathway, recent work has focused on the accumulation of the proteins in this pathway in different developmental and environmental conditions. Western blot analysis of individual enzymes revealed that the low transcript levels of the MEP pathway genes observed in mutants affected in any particular step of the pathway correlates with the low accumulation of the corresponding proteins, except for the DXS and HDR proteins. DXS protein accumulates to high levels in all of the MEP pathway mutants that are compromised in different biosynthetic steps of the route, despite its transcript being dramatically reduced, strongly suggesting a post-translational regulatory mechanism. In these mutant plants, DXS and HDR protein levels are considerably higher than those present in wild-type seedlings at a similar developmental stage. This response is not observed in unrelated pigment mutants (clb5 and chl1). These mutants affect the development of chloroplasts in earlier (clb5) or later (chl1) stages of development, displaying an albino or pale green phenotype. The expression of several chloroplast genes is also affected in both of these mutants, similar to mutants in the MEP pathway. These results confirm that this protein accumulation is particular to mutants in the MEP pathway, not a more general result of chloroplast arrest (Guevara-Garcia et al., 2005). Further confirmation of this was obtained using a specific inhibitor of the MEP pathway. Wild-type plants treated with fosmidomycin, which blocks the second enzymatic step of the MEP pathway, also accumulate high levels of the DXS protein. An interesting possibility that has been proposed is that this regulation represents a feedback mechanism of the flow of the pathway in response to the fluctuating demand for the final pathway products, IPP and DMAPP (Guevara-Garcia et al., 2005). Taken together, these studies have demonstrated an important post-transcriptional control mechanism that modulates the levels of DXS, a critical enzyme of the MEP pathway which has a rate-limiting role in this biosynthetic pathway.

Post-transcriptional regulation of some enzymes of the MEP pathway has also been detected in fosmidomycin-resistant mutants (rif) (Sauret-Güeto et al., 2006; Flores-Perez et al., 2008). Interestingly, the rif1 and rif10 mutants display defects in plastid RNA processing, protein accumulation, and protein degradation. RIF10 encodes a plastid-targeted exoribonuclease polyribonucleotide phosphorylase (PNPase), implicated in the processing of a variety of plastid transcripts (Walter et al., 2002b). Similar to mutants in the MEP pathway, this mutant accumulates higher levels of DXS, DXR, and HDR enzymes. Although the exact function of RIF1 is still unclear, its mutation also affects the expression of plastid-encoded genes (Flores-Perez et al., 2008). This defect apparently results in the alteration of the activity of the major plastid stromal proteases ClpP (Koussevitzky et al., 2007), which might be the cause of the high accumulation of the DXS and DXR proteins. A tempting hypothesis is that DXS and DXR proteins are targets of this degradation pathway; however, that remains to be directly addressed by, for example, demonstrating direct interaction of these proteins with these proteases under specific conditions.

Although the signal(s) responsible for the post-transcriptional regulation of some of the enzymes of the MEP
pathway have not yet been identified, the above-mentioned studies did provide the first evidence for the existence of a novel level of regulation of this pathway. The exact molecular mechanism by which these proteins are regulated is the subject of investigation of several groups. Further, the implications of this regulation on overall pathway functionality need to be analysed in detail.

**Conclusion and perspectives**

The diverse isoprenoid products synthesized through the MEP pathway play central roles as primary and secondary metabolites essential for plant survival and human health. Today, the MEP pathway represents one of the most promising targets to develop new herbicides and anti-parasitic drugs as well as targets to improve the nutritional value of crop plants. To achieve these goals, elucidation of the mechanisms that regulate this biosynthetic route is critical as is the further biochemical characterization of the enzymes that participate in this pathway. In the past several years diverse genetic, molecular, and biochemical approaches have been used to get a better understanding of the regulation of the MEP pathway in higher plants. These studies have shown that expression of the MEP pathway genes is regulated in response to a variety of developmental as well as environmental signals. Future challenges in this field will be to identify the trans-acting factors as well as the cis-acting elements involved in each particular regulatory response.

Probably one of the most interesting findings over the past few years has been the post-transcriptional regulation of the DXS, DXR, and HDR enzymes. Interestingly, in some cases, this regulation operates inversely to its corresponding transcript accumulation. This example highlights the need to be alert and to be suspicious of the all too common practice of extrapolating protein levels based on transcript accumulation. Although other examples like this have been reported in the literature, and it is likely that more will follow, this regulation is unusual.

Previous work has demonstrated that DXS and DXR have rate-limiting roles for the pathway flow in several plants. Thus, the regulation of the level of these enzymes could have an important influence on overall pathway efficiency, in particular, for rapid responses due to environmental fluctuations. The molecular mechanisms of this regulation are still unknown, but some clues have started to emerge such as the participation of the plastid protein degradation process. A detailed understanding of this mechanism will provide a promising target for future manipulations of this pathway and will certainly be explored in the near future. Finally, and no less important, is analysis of the pathway regulation at the enzymatic level for each of the enzymes, as well as an understanding of the mechanisms that adapt their activity. To analyse the impact that each of the regulations described here have in the final enzymatic activity is a critical aspect that remains to be demonstrated. This area of study has yet to be fully addressed and is one of the most important topics for the future.

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