REVIEW

Plant Signaling & Behavior 6:8, 1127-1131; August 2011; ©2011 Landes Bioscience

# Modulation of plant HMG-CoA reductase by protein phosphatase 2A

## Positive and negative control at a key node of metabolism

Meritxell Antolín-Llovera,[1,2,†] Pablo Leivar,[1,2] Montserrat Arró,[1,3] Albert Ferrer,[1,3] Albert Boronat[1,2] and Narciso Campos[1,2,*]

[1]Department of Molecular Genetics; Center for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB); Campus Universitat Autònoma de Barcelona; Bellaterra (Cerdanyola del Vallès); [2]Departament de Bioquímica i Biologia Molecular; Facultat de Biologia; [3]Departament de Bioquímica i Biologia Molecular; Facultat de Farmàcia; Universitat de Barcelona; Barcelona, Spain

[†]Current Address: Genetics; Department of Biology; University of Munich (LMU); Munich-Martinsried, Germany

**Key words:** HMG-CoA reductase, HMGR, mevalonate pathway, isoprenoid biosynthesis, protein phosphatase 2A, PP2A, salt stress

**Abbreviations:** ABA, abscisic acid; ACS, 1-aminocyclopropane 1-carboxylate synthase; BR, brassinosteroid; ER, endoplasmic reticulum; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; MVA, mevalonate; PP2A, protein phosphatase 2A; SnRK1, SNF1-related kinase

The enzyme HMG-CoA reductase (HMGR) has a key regulatory role in the mevalonate pathway for isoprenoid biosynthesis, critical not only for normal plant development, but also for the adaptation to demanding environmental conditions. Consistent with this notion, plant HMGR is modulated by many diverse endogenous signals and external stimuli. Protein phosphatase 2A (PP2A) is involved in auxin, abscisic acid, ethylene and brassinosteroid signaling and now emerges as a positive and negative multilevel regulator of plant HMGR, both during normal growth and in response to a variety of stress conditions. The interaction with HMGR is mediated by B″ regulatory subunits of PP2A, which are also calcium binding proteins. The new discoveries uncover the potential of PP2A to integrate developmental and calcium-mediated environmental signals in the control of plant HMGR.

Plant 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase or HMGR) was detected for the first time long ago[1] and is modulated by many diverse endogenous signals and environmental factors. However, no protein factor interacting with and controlling plant HMGR in vivo had been described until recently.[2] The finding that HMGR is under multilevel control by protein phosphatase 2A (PP2A)[2] raises interesting hypotheses concerning the modulation of HMGR and, therefore, isoprenoid biosynthesis via the signal transduction network. In the present review we discuss these hypotheses, in connection the current knowledge on plant HMGR and PP2A. Other interesting reviews are available for a more detailed background on plant HMGR[3,4] or PP2A.[5-8]

*Correspondence to: Narciso Campos; Email: ncampos@ub.edu
Submitted: 05/06/11; Accepted: 05/07/11
DOI: 10.4161/psb.6.8.16363

## Plant HMG-CoA Reductase

The enzyme HMG-CoA reductase catalyzes the first committed step of the mevalonate (MVA) pathway for isoprenoid biosynthesis. In plants, this pathway provides precursors for a wide variety of isoprenoid products that are required for diverse functions including membrane biogenesis, respiration, control of growth and development, defense and protein prenylation and glycosylation.[9,10] In addition, many isoprenoids have economical interest as drugs, nutraceuticals, flavors, fragrances, pigments, agrochemicals or desinfectants.[11] Plant HMGR has a key regulatory role in the MVA pathway and is modulated by a myriad of endogenous signals and external stimuli such as phytohormones, calcium, calmodulin, light, chemical challenge, wounding, elicitor treatment and pathogen attack.[3,4] It has been proposed that the major changes of HMGR activity would be determined at the transcriptional level, whereas post-translational mechanisms would allow a finer and faster adjustment.[10] Transcriptional modulation of HMGR has been demonstrated in many plant systems, but evidence of post-translational control is still scarce.[3] *A. thaliana* HMGR responds post-translationally to compensate the enhancement or depletion of the metabolic flux through the MVA pathway.[12] Reversible phosphorylation at a conserved site of the catalytic domain,[13] inhibition by calcium[14] and protein degradation[15] have been proposed as post-translational mechanisms regulating plant HMGR (**Fig. 1**).

In all plant species studied so far, HMGR is encoded by a multigenic family composed of a variable number of genes. For instance, two HMGR-encoding genes (*HMG1* and *HMG2*) are present in *Arabidopsis thaliana*,[16,17] four in *Solanum lycopersicum*[18] and at least seven in *Medicago truncatula*.[19] Alternative splicing or transcription start could generate an even higher number of HMGR isoforms. In *A. thaliana*, HMGR1L and HMGR1S derive from the *HMG1* gene and HMGR2 derives from *HMG2*.[17,20] Gene expression and mutant analyses indicate a

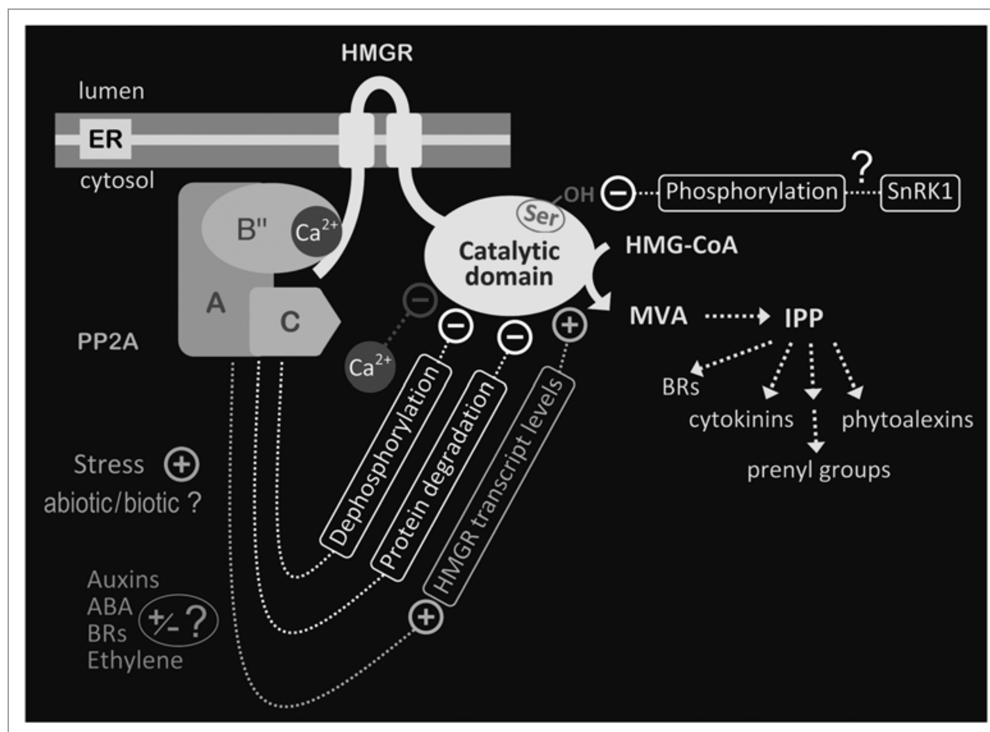


**Figure 1.** Working model on the modulation of plant HMGR by PP2A. Plant HMGR is a transmembrane protein of the ER, having the N-terminal region and catalytic domain in the cytosol. HMGR is a regulatory enzyme of the cytokinin, brassinosteroid (BR) and prenyl moiety biosynthetic pathway and can thus play a role in the signaling of these molecules. HMGR also regulates the synthesis of MVA-derived phytoalexins. PP2A is an heterotrimeric enzyme that binds the N-terminal region of HMGR through its B'' regulatory subunit, which is a calcium-binding protein. Calcium can inhibit plant HMGR post-translationally. In response to salt stress, HMGR is submitted to post-translational negative control by PP2A through dephosphorylation and protein degradation mechanisms. Dephosphorylation of HMGR by PP2A does not occur at the conserved serine residue of the catalytic domain previously proposed to be phosphorylated by the SNF1-related kinase (SnRK1). In the response to salt stress, PP2A also mediates the increase of HMGR transcript levels. Control of HMGR by PP2A could be modulated by environmental or developmental calcium- and phytohormone-mediated signals. Some of these signals could derive from the isoprenoid biosynthetic pathway, thus allowing feedback regulation on HMGR.

housekeeping role for HMGR1S and a more specialized function for HMGR1L and HMGR2.[17,20-23] All known plant HMGR variants are targeted primarily to the endoplasmic reticulum (ER),[24] but they may have specific locations in the endomembrane system.[25] The diverged N-terminal region and the conserved catalytic domain of HMGR are located in the cytosol, whereas only a short stretch of amino acids connecting the two transmembrane segments is in the lumen (**Fig. 1**).[24] This is consistent with the cytosol being the only site for MVA biosynthesis in plant cells. It was proposed that different HMGR variants might be integrated in specific arrays of enzymes or channels devoted to the synthesis of particular isoprenoid products.[10] Each of these complexes could contain its own set of regulatory proteins.

## Protein Phosphatase 2A

Protein phosphatase 2A (PP2A) is a structurally conserved heterotrimeric enzyme composed of a scaffolding A subunit, a regulatory B-type subunit and a catalytic C subunit (**Fig. 1**).[6,26] Many studies in animals[27-29] but still few in plants[2,30] indicate that the B-type subunit determines the intracellular location and substrate specificity of the PP2A holoenzyme. So far, three distinct B-type protein families, named B, B' and B'', have been confirmed as components of PP2A holoenzymes.[5,6] PP2A is a major phosphatase that accounts for about 25% of the total protein phosphatase activity in crude homogenates from several plants.[31] It is found in most plant tissues and in diverse subcellular locations including the nucleus, cytosol, membranes and insoluble fractions.[8]

PP2A has been proposed to modulate several metabolic processes in vascular plants, including sucrose synthesis,[32] quinate metabolism,[33] nitrogen assimilation,[34] nocturnal $CO_2$ fixation[35] and more recently, isoprenoid biosynthesis (see below).[2] Cumulative evidence indicates that PP2A participates in the signaling of diverse stress conditions.[7] On the one hand, the transcript levels of particular PP2A subunits increase or decrease in response to biotic[36-39] or abiotic[39-47] challenges. On the other, PP2A activity or particular PP2A subunits participate as positive or negative transducers in the signaling of abscisic acid (ABA),[48-50] and ethylene,[51,52] in the response to cold[53] or drought,[45] or to ionic, osmotic and oxidative stress,[54] and in the defense against biotic challenge.[37] The broad distribution and functional diversity likely correspond to a multiplicity of PP2A holoenzymes (each formed by one A, one B-type and one C subunit), denoted at the genomic level. In *A. thaliana*, for instance, 3 genes code for A subunits, 17 for B-type subunits and 5 for C subunits, which together could theoretically form up to 255 different PP2A

heterotrimers.[55] The role of these *A. thaliana* PP2A subunits has been uncovered only in few cases: RCN1 (subunit A), involved in the targeting of auxin transport proteins,[56,57] responsiveness to ABA,[48] ethylene biosynthesis[52] and seedling phototropism;[58] B'α and B'β, involved in brassinosteroid (BR) signaling;[30] B"α and B"β, involved in isoprenoid biosynthesis;[2] and TONNEAU2 (subunit B"), involved in the organization of the cortical cytoskeleton.[59] It was proposed that RCN1 might contribute to the crosstalk between auxin and ABA signaling pathways, which would be in turn modulated by accompanying regulatory and catalytic PP2A subunits.[48] The identification of phosphorylated protein targets for PP2A in these processes is being pursued very actively at present. These studies confirm the involvement of PP2A in auxin signaling through the PIN auxin transport proteins as targets,[57] seedling phototropism through the blue light sensors phot1 and phot2,[58] brassinosteroid signaling through the BZR1 transcription factor,[30] isoprenoid biosynthesis through the regulatory enzyme HMGR[2] and ethylene biosynthesis through the regulatory enzyme 1-aminocyclopropane 1-carboxylate synthase (ACS).[52]

## The B" PP2A Subunit Family

The *A. thaliana* genome encodes five closely related B" PP2A subunits (B"α, B"β, B"γ, B"δ and B"ε)[2] and a diverged B" variant named TONNEAU 2.[59] In other plant species, the B" PP2A subunit is also encoded by a multigenic family with two members in rice *Oryza sativa* (rapdb.dna.affrc.go.jp/), at least two members in *Solanum lycopersicum* (solgenomics.net/) and at least three members in *Medicago truncatula* (www.medicagohapmap.org/?genome). *A. thaliana* B"α and B"β where identified as proteins that interact specifically with HMGR1S and HMGR1L.[2] They do not interact with HMGR2, the other member of the *A. thaliana* HMGR family.[2] Whereas B"γ is nearly identical to B"β, with only two conservative changes in 536 amino acid residues, B"α and B"β are 67.4% identical (76.9% similar), i.e., nearly as divergent as the most distant members B"δ and B"ε (64.0% identical, 74.7% similar). Therefore, it would no be surprising that all five *A. thaliana* B" isoforms bind HMGR1.[2] The *B"*α (34% of the EST entries of *A. thaliana* B" subunits), *B"*β (29%) and *B"*γ (27%) transcripts are much more abundant than those encoding B"δ (4%) and B"ε (6%). These observations indicate that the interaction with HMGR, contributed at least by B"α, B"β and B"γ, is a major role of the *A. thaliana* B" protein family.

The ATH1 22k microarray data accessible at the Genevestigator facility (www.genevestigator.com/gv/index.jsp) suggest that the abundance of the *B"*α transcript increases in response to salt, ABA or mildew and decreases in response to potyvirus infection. Under salt stress, B"α is a negative regulator of root growth, whereas B"β does not affect this process.[2] Interestingly, B"α and B"β are calcium binding proteins.[2] They have a typical tandem of EF-hand calcium motifs and their binding to HMGR is stimulated by the cation.[2] Calcium is a secondary messenger essential in the adaptation of plants to the environment. It mediates the signaling of many challenges such as heat- or cold-shock, slow cooling, UV radiation, red or blue light, oxidative stress, anoxia, drought/hyper-osmotic stress, salinity, hypo-osmotic stress, mechanical stimulation, heavy metals, pathogens and elicitors[60,61] and might thus, be involved in the modulation of HMGR by PP2A under diverse stress conditions.

## Control of HMG-CoA Reductase by PP2A

It was recently shown that PP2A exerts a multilevel control on HMGR through the B" protein family in *A. thaliana* (**Fig. 1**).[2] PP2A is not only a post-translational negative regulator of HMGR activity and protein levels, but also a positive regulator of *HMG1* transcript levels. The post-translational control by PP2A involves HMGR dephosphorylation and degradation.[2] Dephosphorylation of HMGR by PP2A should occur at a position different to the conserved phosphorylation site of the catalytic domain.[2] Whereas *B''*β plays a role in the post-transcriptional repression of HMGR in unchallenged seedlings, *B''*α modulates HMGR transcript, protein and activity levels in response to salt challenge. The knock-out of the *A. thaliana B''*α gene completely abolished the modulation of HMGR by salt stress, indicating that the B'' PP2A subunits have specific roles and can not substitute for each other in the response to this challenge.[2] When seedlings were transferred to salt-containing medium, B''α and PP2A mediated the decrease and subsequent increase of HMGR activity, which resulted from a steady rise of HMGR1-encoding transcript levels and an initial sharper reduction of HMGR protein level.[2] A similar biphasic profile of HMGR activity was previously observed when BY-2 tobacco cells were subjected to a block of isoprenoid biosynthesis with mevinolin[62] or when potato tubers were subjected to wounding,[63] suggesting that PP2A and B'' subunits might also participate in these responses. HMGR transcript, protein and activity levels were also altered by the mere transfer of the seedlings to fresh plates that did not contain additional salt.[2] PP2A and B"α were required for this HMGR response, likely induced by short-time desiccation or soft mechanical stimulus.[2] Altogether, the data indicate that PP2A controls HMGR transcript and activity levels in different plant systems, during normal development and in response to a variety of challenging conditions. Since PP2A is involved ABA and auxin signaling, it is tempting to speculate that the effect of these phytohormones on HMGR activity observed in the past[64,65] could be mediated by PP2A complexes.[2]

HMGR is a key regulatory enzyme of the isoprenoid biosynthetic pathway and its modulation by PP2A may be considered as an end point of the signal transduction network. PP2A might be at the heart of the control of HMGR, integrating phytohormone signals and environmental calcium-mediated stimuli (**Fig. 1**). The complexity of the PP2A-HMGR system and, in particular, the fact that HMGR is subjected to both positive and negative control by the same regulatory factor may account for the lengthy failure in identifying HMGR regulatory partners. Interestingly, positive and negative control by PP2A on the same target is not exclusive of HMGR. It was recently shown that *A. thaliana* ACS is subjected both to positive and negative regulation by PP2A.[52] PP2A mediates the degradation of Type I ACS isoforms (ACS2

and ACS6) by the 26S proteasome and the stabilization of Type II ACS isoform (ACS5).[52] Both HMGR and ACS catalyze key regulatory steps of biosynthetic pathways that generate phytohormone or prenyl moiety signals (cytokinins, brassinosteroids and prenyl groups for the former and ethylene for the later). PP2A connects these key nodes of metabolism with the signal transduction network, allowing modulation by environmental stimuli and, maybe, feedback regulation by the phytohormone or prenyl moiety products.

## Concluding Remarks and Future Perspectives

Plant HMGR is a key regulatory enzyme of the MVA pathway and is modulated by many diverse endogenous signals and environmental factors, but the mechanisms behind this control have remained largely unknown. The recent finding of a complex HMGR control by PP2A under particular stress conditions opens new perspectives in the research of both HMGR and PP2A. It will be interesting to analyze whether PP2A is involved in the response of HMGR to other environmental challenges and to define the role of phytohormone and calcium signals to this respect. The study could uncover additional connections of HMGR with the signal transduction network. The specialization of HMGR isoforms in the synthesis of particular defense products will be relevant to this respect. The observation that PP2A can exert both positive and negative control on HMGR is intriguing. From a conceptual point of view, it will be appealing to determine whether such a behavior may result from the integration of different signals in the PP2A regulatory center.

### Acknowledgments

This work was supported by grants of the Spanish Ministerio de Ciencia e Innovación (BMC2003-03450 and BFU2006-14655 to N.C., BFU2006-00544 and BIO2009-06984 to A.F. and BIO2009-09523 to A.B., all of them including FEDER funds), the Spanish Consolider-Ingenio 2010 Program (CSD2007-00036 Centre for Research in Agrigenomics) and the Generalitat de Catalunya (2009SGR0026). M.A. and P.L. were recipients of fellowships, respectively, from the Spanish Ministerio de Educación Cultura y Deporte and the Comissió Interdepartamental de Recerca i Innovació Tecnològica.

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