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Article Addendum

Albinism and cell viability in cycloartenol synthase deficient Arabidopsis

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Phenotypes of *Arabidopsis thaliana* that carry mutations in *CYCLOARTENOL SYNTHASE 1 (CAS1)* which is required in sterol biosynthesis have been described. Knockout mutant alleles are responsible of a male-specific transmission defect. Plants carrying a weak mutant allele *cas1-1* accumulate 2,3-oxidosqualene, the substrate of CAS1, in all analyzed organs. Mutant *cas1-1* plants develop albino inflorescence shoots that contain low amount of carotenoids and chlorophylls. The extent of this albinism, which affects Arabidopsis stems late in development, may be modulated by the light/dark regime. The fact that chloroplast differentiation and pigment accumulation in inflorescence shoots are associated with a low *CAS1* expression could suggest the involvement of 2,3-oxidosqualene in a yet unknown regulatory mechanism linking the sterol biosynthetic segment, located in the cytoplasm, and the chlorophyll and carotenoid biosynthetic segments, located in the plastids, in the highly complex terpenoid network. CAS1 loss of function in a mosaic analysis of seedlings further demonstrated that leaf albinism associated with an accumulation of 2,3-oxidosqualene is a novel phenotype for plant sterol deficient mutant.

A Genetic Approach to Investigate Sterol Functions

Sterol-mediated signaling and molecular regulation of sterol synthesis, transport, metabolism and cell homeostasis is described with many details in mammals[1] but is not fully understood in plants. The sequence of biosynthetic steps that leads from acetyl-CoA to the C5 building blocks isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) via mevalonate and then generates the C30 precursor squalene further converted into 24-alkyl-Δ^5-sterols (Fig. 1) is similar in the model species *Arabidopsis thaliana* and in cells of mammals, although CAS1 and sterol-C24-methyltransferases are plant-specific. A genetic approach to address the question of the role of sterols was quite informative in recent years. Mutants affected in various steps of sterol biosynthesis have a modified composition of sterol to which are associated pleiotropic macroscopic phenotypes. Arabidopsis mutants deficient in biosynthetic steps that convert cycloartenol into Δ^7-sterol intermediates (episterol, Δ^7-avenasterol) are embryo or seedling lethals.[2-4] It has been shown recently that a cyclopropylsterol isomerase (*CPI1*) mutant is affected in post-cytokinetic acquisition of an auxin transporter by endocytosis, which in turn alters root gravitropism.[5] Arabidopsis mutants deficient in biosynthetic steps that convert the Δ^7-sterol intermediates into campesterol and sitosterol are characterized by a typical dwarf phenotype due to lack of the bioactive brassinosteroids synthesized from campesterol.[6-9] Modulation of the expression of genes implicated in the relative composition of the 24-alkyl-Δ^5-sterols (cholesterol, campesterol, sitosterol, stigmasterol) has been shown to affect growth, branching and fertility,[10] vascular patterning[11] or endoreduplication.[12] However, the biological role of sterols at the molecular level (i.e., mechanism of action) is scarcely described.[13]

Cell Viability and Plastid Biogenesis in *Cycloartenol Synthase 1* Mutants

Two biosynthetic routes have been described that generate tetracyclic steroidal end-products from 2,3-oxidosqualene. Mammals and fungi cyclize it into lanosterol, a tetracyclic triterpenol, whereas plants cyclize it into the pentacyclic steroidal intermediate cycloartenol, a 9β,19-cyclopropyl derivative[14(for review)] (Fig. 1). Cycloartenol-derived sterols are then produce through the mandatory cyclopropyl sterol isomerase.[15] The reason why plants synthesize their sterols according to a major cycloartenol route and do not favor the lanosterol one[16,17] is not understood. A series of allelic mutants deficient in the expression of the Arabidopsis cycloartenol synthase (*CAS1*) has been reported recently.[18] In this work, albinism is described as a novel phenotype for a plant sterol mutant. A weak *cas1-1* allele is characterized by an organ-dependent albinism late in development. Cortical cells of the part of stems that carry flowers and siliques contain plastids with a morphology reminiscent of photooxidation, in full accordance with a severe reduction in total carotenoids and chlorophylls in these white tissues[18] (Fig. 2). This cellular phenotype correlates with the accumulation of 2,3-oxidosqualene, the

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substrate of CAS1. Loss of function of cycloartenol synthase occurs also in green tissues of *cas1-1* but to a lesser extent than in the albino parts. Interestingly, a set of transgenic lines with mutant phenotypes that can be explained by co-suppression of the *CAS1* gene were obtained.[18] The most common of these phenotypes affecting co-suppressed lines was albinism of upper stems, as it is the case of *cas1-1*. Less lines had the entire length of the stem affected, even less lines had albino leaf petiole. Although it is shown that *CAS1* is ubiquitously expressed in the wild-type,[18] the chemical phenotype observed in green versus non-green tissues might indicate different sterol requirements in different organs. Strong, most probably null, *cas1-2* and *cas1-3* alleles used in genetic crosses show that knockout mutations in *CAS1* are causing a male-specific transmission defect. It is therefore assumed that cycloartenol-derived metabolites play major roles for all cell types and developmental stages. A knockout mutant was used in a mosaic analysis approach[18] which further demonstrated that progressive depletion of CAS1 in young growing leaves caused an accumulation of 2,3-oxidosqualene associated with albinism. Consequently, the onset of albinism in Arabidopsis is an immediate consequence of a cycloartenol synthase deficiency.

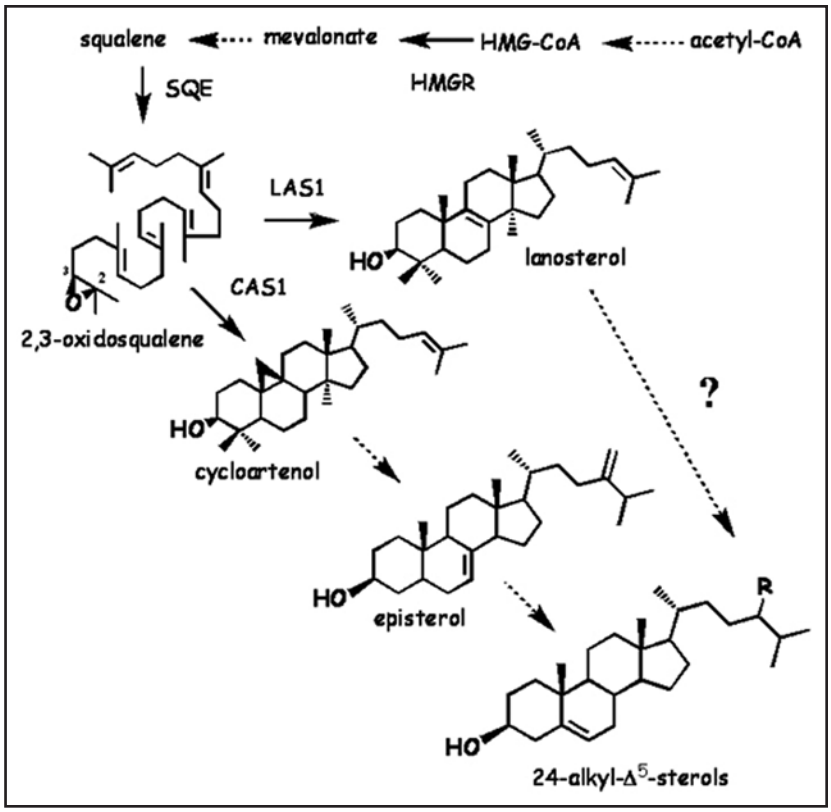

Figure 1. Sterol biosynthetic pathway emphasizing cyclization of 2,3-oxidosqualene. HMG-CoA, 3-hydroxy-3-methylglutarylcoenzyme A; HMGR, HMG-coA reductase; SQE, squalene epoxydase; LAS, lanosterol synthase; CAS, cycloartenol synthase.

Light Influences the Albino Phenotype in a *Cycloartenol Synthase* Mutant

In spite of the *CAS1* defect in *cas1-1* hypomorphic allele, it is shown that 24-alkyl-Δ^5sterols are synthesized in amounts similar to those of the wild-type.[18] This observation could suggest the existence of a positive feed-back regulatory interaction between sterol biosynthetic intermediates and upstream enzymes of the pathway. Previous studies have shown that HMGR enzymatic activity is limiting for the accumulation of sterols in plants.[19,20] In the case of *cas1-1* flowering stems, we detected a 30% increase of HMGR activity compared with the wild-type.[18] Regulatory aspects of HMGR and more generally of the mevalonate pathway indicate that light is implicated in its control. Indeed, HMGR in Arabidopsis is downregulated by light[21] and this process was shown to occur via photoreceptors: a phytochrome B (PHY B) knockout mutant was isolated in a screening for resistance to inhibition of mevalonate biosynthesis by mevinolin, a potent inhibitor of HMGR.[22] Likewise, mutants affected in *PHY* genes displayed elevated enzyme activity for HMGR.[22]

In a simple approach to test whether light influences the onset of the albino phenotype in the *cas1-1* mutant, we compared the development of plants grown under different day length conditions. Wild-type and *cas1-1* plants were grown in 12-hour light conditions until they reached the rosette stage 6.00,[23] then transferred (or not) in long day conditions (16-hour light). After four weeks of growth, relevant morphological parameters were recorded (Table 1). Interestingly, we noted a reduction of 25% of the total length of *cas1-1* white stems in plants grown in long day conditions, as compared to the length of white stems in plants grown in 12-hour light conditions. Likewise, the ratio of green to white stem lengths showed an increase of 76% for plants grown in long day conditions. We also

Figure 2. Stem albinism of a hypomorphic cycloartenol synthase mutant (left) compared to the wild-type.

Table 1 **Phenotype of wild-type and *cas1-1* plants grown under different light conditions[a]**

	12 h light/12 h dark		16 h light/8 h dark	
	wt	***cas1-1***	**wt**	***cas1-1***
Height above ground in cm	38.5 ± 3.5	28.6 ± 5.4	44.1 ± 2.2	32.6 ± 2.7
Number of branches per main stem	8.4 ± 2.0	7.2 ± 1.5	9.5 ± 1.4	7.9 ± 1.2
Number of white branches	0	6.7 ± 2.3	0	7.8 ± 1.3
Length of white stems per plant in cm	0	39.5 ± 15.6	0	29.6 ± 9.9
Ratio of green to white stem lengths		1.95		3.44
Number of siliques[b] per plant	25.4 ± 4.6	25.3 ± 4.5	35.1 ± 2.9	25.5 ± 4.4
Rank[c] of the first silique on white stem		5.2 ± 2.3		15.0 ± 5.5

[a]experiments included at least 15 plants per genotype and were done three times. One representative experiment is shown. [b]siliques over 0.5 cm were counted. [c]position number from bottom to top.

recorded the rank from bottom to top of the first silique attached to the white part of the stem. It is striking to observe that the bleaching of *cas1-1* stems started at silique 5 under 12-hour light conditions whereas it started at silique 15 under long-day conditions (Table 1). This experiment showed clearly that the bleaching of *cas1-1* developing stems can be influenced by light and points out that complex regulatory processes are at play in the molecular regulation of terpenoid biosynthetic networks.[24]

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