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Current Opinion in Plant Biology

Illuminating colors: regulation of carotenoid biosynthesis and accumulation by light

Briardo Llorente[1], Jaime F Martinez-Garcia[1,2], Claudia Stange[3] and Manuel Rodriguez-Concepcion[1]

Light stimulates the biosynthesis of carotenoids and regulates the development of plastid structures to accommodate these photoprotective pigments. Work with Arabidopsis revealed molecular factors coordinating carotenoid biosynthesis and storage with photosynthetic development during deetiolation, when underground seedlings emerge to the light. Some of these factors also adjust carotenoid biosynthesis in response to plant proximity (*i.e.*, shade), a mechanism that was readapted in tomato to monitor fruit ripening progression. While light positively impacts carotenoid production and accumulation in most cases, total carotenoid levels decrease in roots of colored carrot cultivars when illuminated. The recent discovery that such cultivars might be photomorphogenic mutants provides an explanation for this striking phenotype.

Addresses
[1] Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Campus UAB Bellaterra, 08193 Barcelona, Spain
[2] Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain
[3] Plant Molecular Biology Laboratory, Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile

Corresponding author: Rodriguez-Concepcion, Manuel (manuel.rodriguez@cragenomica.es)

Current Opinion in Plant Biology 2017, **37**:49–55

This review comes from a themed issue on **Physiology and metabolism**

Edited by **Krishna K Niyogi**

For a complete overview see the Issue and the Editorial

Available online 12th April 2017

http://dx.doi.org/10.1016/j.pbi.2017.03.011

Introduction

As sessile organisms that rely on sunlight as the main source of energy, plants have developed sophisticated systems to sense and respond to light cues. Light regulates plant development right after germination. When seeds from angiosperm plants germinate in the dark, seedlings develop skotomorphogenetically (*i.e.*, with long hypocotyls and closed cotyledons harboring etioplasts with no chlorophylls and very little carotenoid contents). Upon illumination, however, a completely different developmental program unfolds: photomorphogenesis. The deetiolation process involves the inhibition of hypocotyl growth, the expansion of cotyledons, and the differentiation of etioplasts into chloroplasts with the concomitant production of high levels of chlorophylls and carotenoids. The main role of carotenoids in chloroplasts is to protect the photosynthetic apparatus against photooxidative damage caused by excessive illumination [1••,2]. Oxidative cleavage of carotenoids can produce retrograde signals such as β-cyclocitral to regulate the expression of nuclear genes in response to photooxidative stress, whereas their enzymatic cleavage generates hormones (abscisic acid and strigolactones) and other signaling molecules whose identity is still unknown [3]. Carotenoids also function as pigments, accumulating at massive levels in specialized plastids called chromoplasts [2]. Chromoplast carotenoids are hence responsible for the yellow color of corn, the orange color of carrots, and the red color of tomatoes. Humans cannot produce carotenoids but take them in the diet as an essential source of retinoids (including vitamin A) and other health-related biologically-active metabolites [2–4].

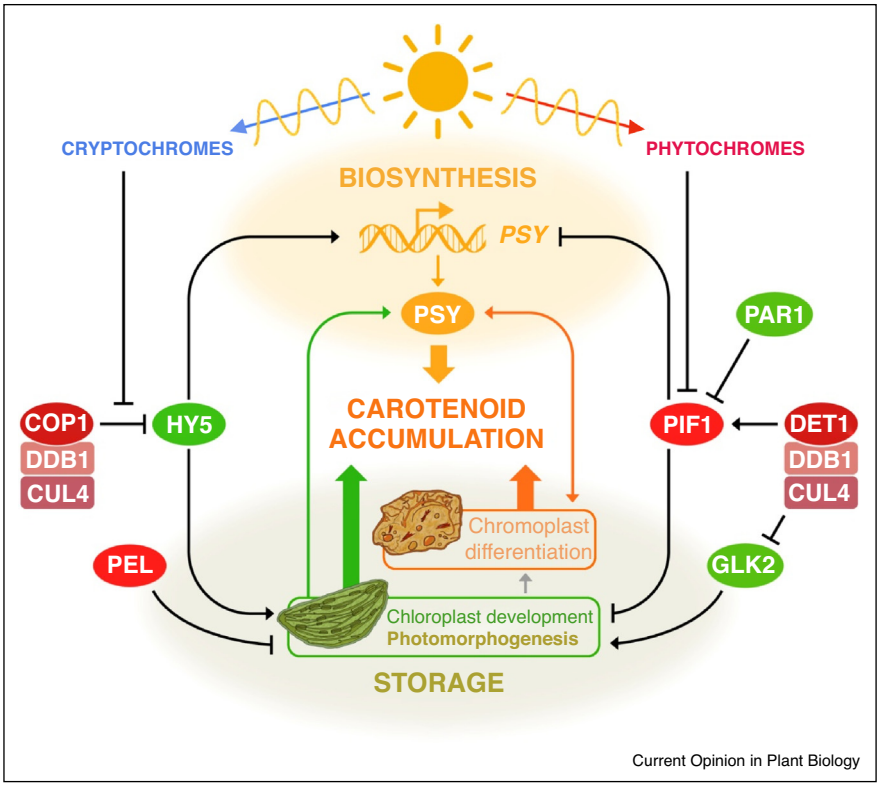
Despite the relevance of carotenoids for agriculture and health, we still have a limited knowledge on how plants regulate their synthesis and accumulation. Molecular mechanisms have been described to control (1) expression of genes involved in carotenoid biosynthesis and degradation, (2) activity of the corresponding enzymes, and (3) development of carotenoid storage structures in plastids. All three mechanisms are tightly coordinated by both developmental (internal) and environmental (external) signals, with light having a major role [2,4–8]. In this article we will focus on the molecular mechanisms by which light signals are transduced to regulate carotenoid biosynthesis at the level of phytoene synthase (PSY), the first and main rate-determining enzyme of the carotenoid pathway. We will also revise the impact of light signaling on carotenoid storage capacity through the regulation of plastid development and differentiation. In particular, we will cover three case studies involving different scenarios of light-regulated carotenoid accumulation: *Arabidopsis thaliana* deetiolating and shade-exposed seedlings, tomato (*Solanum lycopersicum*) ripening fruits, and carrot (*Daucus carota*) roots.

Case-study 1: Arabidopsis seedlings. Emerging to light

During deetiolation, the production of carotenoids is boosted at the biosynthetic gene expression and enzyme

activity levels [4,9]. At the same time, etioplasts differentiate into chloroplasts, which have a much higher capacity to accommodate the newly produced carotenoids. The molecular factors directly coordinating carotenoid biosynthesis and storage (*i.e.*, chloroplast differentiation) were discovered mainly using Arabidopsis deetiolation as a model system. Perception of light by photoreceptors such as cryptochromes (receptors of blue light) and phytochromes (receptors of red (R) and far-red (FR) light) is transduced by transcription factors to regulate photomorphogenesis (Figure 1). The expression of the only Arabidopsis gene encoding PSY is under the direct control of two of these transcription factors: PHYTOCHROME INTERACTING FACTOR 1 (PIF1) and LONG HYPOCOTYL 5 (HY5) [10•,11••]. PIF1 and other members of the so-called PIF quartet (formed by PIF1, PIF3, PIF4 and PIF5, collectively referred to as PIFq) belong to a bHLH subfamily of phytochrome-regulated transcription factors that typically accumulate in the dark and degrade in the light [12]. By contrast, the bZIP transcription factor HY5 is degraded in the dark but accumulates in the light [13]. PIFq and HY5 act antagonistically for a broad set of photomorphogenic responses. In the case of photosynthetic development, PIFq proteins are repressors and HY5 is an activator (Figure 1). High levels of PIFq and low levels of HY5 in etiolated seedlings block photosynthetic development (including carotenoid biosynthesis and chloroplast development). During deetiolation, light-mediated degradation of PIF1 (a direct repressor of *PSY* expression) and stabilization of HY5 (an

Figure 1



Schematic representation of light-related molecular pathways impacting carotenoid biosynthesis and storage in plants. Sunlight is perceived by photoreceptors such as cryptochromes (receptors of blue light) and phytochromes (receptors of R and FR light). Photoreceptors then transduce the light signal by regulating the stability of transcription factors such as PIF1 (a repressor of photomorphogenesis degraded in the light upon interaction with photoactivated phytochromes) and HY5 (an activator of photomorphogenesis degraded in the dark upon interaction with COP1). COP1 acts as a substrate receptor of the CUL4-DDB1 E3 ligase, which mediates the ubiquitination of substrates for degradation by the 26S proteasome (revised in Ref. [13]). Besides HY5, another COP1 client appears to be PAR1, a transcription cofactor that prevents PIF1 binding to the promoters of target genes, including *PSY* [16•,18]. The photomorphogenesis repressor DET1 can also bind to the CUL4-DDB1 complex and directly interact with Arabidopsis PIF1 to stabilize it [46,47] and with tomato GLK2 (a promoter of chloroplast development in the fruit) to target it to proteasome-mediated degradation [34]. Photomorphogenesis is additionally repressed by PEL [43••]. In carrot roots and tomato fruits, but not in Arabidopsis seedlings, chloroplasts can differentiate into chromoplasts. Development of chloroplasts and, to a higher level, chromoplasts increases deposition sink capacity for carotenoids and improves PSY activity. PSY activity is also regulated at the gene expression level. PIF1 proteins directly repress the expression of PSY-encoding genes in Arabidopsis and tomato fruit, whereas HY5 is a direct activator of the Arabidopsis *PSY* gene [10•,11••,31••]. Together, these positive (in green) and negative (in red) regulatory factors coordinate carotenoid biosynthesis and storage in a highly interconnected fashion.

activator that binds to the same G-box motif in the *PSY* promoter) stimulates the expression of *PSY* and the eventual production of carotenoids in coordination with chlorophyll biosynthesis and chloroplast development (Figure 1). This dynamic repression-activation transcriptional module also functions during day and night cycles in deetiolated plants, providing robustness in response to light but also temperature cues [10•,11••].

Photosynthesis and growth of deetiolated plants can be heavily compromised by the shading of nearby plants. Changes in light quality associated with crowded (*i.e.*, high density) plant environments are actually perceived by photoreceptors and rapidly transduced into changes in gene expression aimed to adapt to eventual shading, for example, by decreasing the production of chlorophylls and carotenoids to readjust photosynthetic metabolism [14,15]. Specifically, phytochromes sense and transduce shade signals generated when the preferential absorption of R light by chlorophylls present in nearby leaves and stems decreases the R to FR ratio (R/FR) of the resulting light. PIF1 and related PIFq proteins were recently found to repress Arabidopsis *PSY* expression and carotenoid biosynthesis in response to shade (*i.e.*, low R/FR) signals [16•]. While HY5 does not appear to be required for this process, other known PIF antagonists were found to contribute to the early response of the carotenoid pathway to shade. In particular, PHYTOCHROME-RAPIDLY REGULATED 1 (PAR1), a transcriptional co-factor of the bHLH superfamily that prevents binding of true transcription factors to their target promoters [17], was found to directly induce *PSY* expression by interacting with PIF1 [16•] (Figure 1). Similar to HY5, PAR1 was found to be degraded in the dark and to enhance seedling deetiolation through multiple photoreceptor signaling pathways [18]. These results suggest that different antagonistic modules of repressors (PIFq) and activators (HY5, PAR1) ensure a fine regulation of carotenoid biosynthesis in response to light signals in Arabidopsis.

Beyond the control of *PSY* transcript accumulation, light also controls PSY protein and activity levels post-transcriptionally [19,20,21••]. Arabidopsis PSY enzyme levels were shown to be feedback-regulated by translational control elements located in the 5′-UTR of *PSY* mRNAs transcribed during deetiolation [21••]. Translation inhibition requires a hairpin loop whose removal in shorter alternative splice variants results in a translation-permissive 5′-UTR. These shorter transcripts appear to quickly provide PSY activity following immediate carotenoid biosynthetic requirements caused by environmental stress, for example, to replace carotenoids consumed by photooxidation after a high light episode [21••]. During light-triggered deetiolation, PSY is enzymatically activated upon relocation to thylakoid membranes of developing chloroplasts [19]. Genetic derepression of deetiolation in the dark was found to be sufficient to increase PSY activity and carotenoid accumulation [20], likely because chloroplast differentiation (but not chlorophyll biosynthesis) was partially unblocked. Further supporting a close link between plastid ultrastructure and PSY levels, cochaperone-like ORANGE (OR) proteins promote both the stability of the PSY protein and the differentiation of chromoplasts [22••,23,24].

Case-study 2: tomatoes. Ripe for new functions

In tomato and many other fleshy fruits, ripening is associated with the differentiation of green fruit chloroplasts into ripe fruit chromoplasts [8,25]. The characteristic orange and red colors of ripe tomatoes actually result from the degradation of chlorophylls and the accumulation of the health-promoting carotenoids β-carotene (provitamin A) and lycopene, respectively. The relevance of fruit-localized photosensory pathways as important players in the regulation of fruit ripening in general and carotenoid biosynthesis in particular is well established [5,8]. Cryptochrome (CRY2) overexpression in tomato increased the levels of carotenoids in the fruit [26], whereas phytochrome-deficient mutants produce pigment-devoid white fruits similar to those obtained when fruit development occurs in complete darkness [27]. Several downstream factors identified in Arabidopsis as regulators of photomorphogenesis have been shown to also modulate fruit carotenoid accumulation in tomato (Figure 1). They include DAMAGED DNA BINDING PROTEIN 1/HIGH PIGMENT 1 (DDB1/HP1), DEETIOLATED 1/HIGH PIGMENT 2 (DET1/HP2), CULLIN 4 (CUL4), CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), HY5 and PIF1a (the tomato fruit homologue of Arabidopsis PIF1) [5,28–30,31••]. To date, only PIF1a has been shown to directly regulate the transcription of carotenoid biosynthetic genes. Specifically, PIF1a binds to a PBE-box located in the promoter of the tomato *PSY1* gene (encoding the PSY isoform that controls the flux to the carotenoid pathway during fruit ripening) to repress its transcription [31••].

While the mechanism for the PIF1-mediated control of PSY and carotenoid levels is conserved between Arabidopsis and tomato, the tomato PIF1a protein appears to play a completely different physiological function that is not directly related to responding to an environmental (*i.e.*, light) signal. The occurrence of chlorophyll in chloroplasts of green fruit progressively decreases the R/FR of the light as it penetrates through the fruit pericarp [31••,32]. As a consequence of this reduction in R/FR (a sort of 'self-shading'), PIF1a protein levels accumulate and repress *PSY1* expression. Developmentally-controlled degradation of chlorophylls gradually reduces the self-shading effect as ripening progresses, hence allowing the phytochrome-mediated degradation of PIF1a and subsequently the activation of *PSY1*

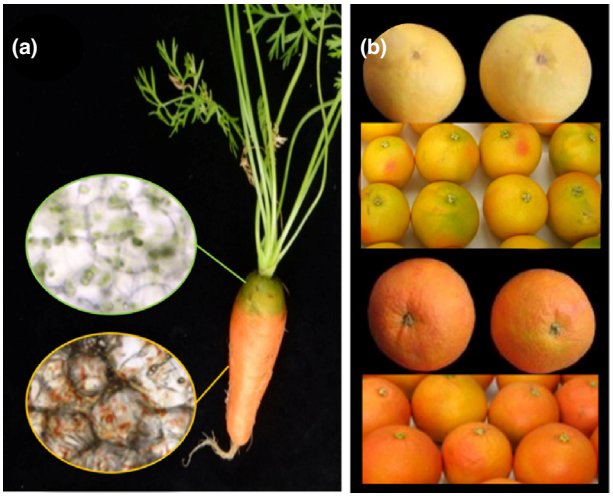
expression and carotenoid biosynthesis [31••]. This mechanism is proposed to adjust the genetic control of tomato *PSY1* expression to the actual ripening stage of the fruit sensed through the chlorophyll-dependent changes in the R/FR of the light that reaches the carotenoid-producing pericarp cells. Given the ubiquitous distribution of phytochromes and PIFs in plants, it is most likely that the self-shading mechanism reported in tomato might also be operative in fruits that lose chlorophylls and accumulate carotenoids during ripening as a visual signal to inform seed-dispersing animals that the fruit is ripe and tasty [8,25].

In many cases, the increased accumulation of tomato fruit carotenoids observed upon the manipulation of light signaling components does not involve direct regulation of carotenoid biosynthetic genes but is mainly coupled to carotenoid storage capacity. In particular, mutants defective in DDB1/HP1, DET1/HP2, or CUL4 (Figure 1) show an increase in plastid number and size [5,28–30]. A positive effect of increasing chloroplast and chromoplast number or size on fruit carotenoid accumulation has also been observed in tomato mutants for genes not directly related to light, such as *UNIFORM* (*u*, defective in the Golden 2-like transcription factor GLK2, targeted to degradation by the DDB1-DET1-CUL4 complex) [33,34] and *HIGH PIGMENT 3* (*hp3*, defective in abscisic acid synthesis) [35]. This effect might be mediated in part by carotenoid-associated proteins that assist in the sequestration and stabilization of carotenoids [36]. In all the cases, however, an efficient conversion of chloroplasts to chromoplasts is required to permit massive accumulation of the newly synthesized carotenoids. Based on the reported role of OR proteins on increasing PSY activity and promoting the differentiation of chromoplasts in several plant systems [22••,23,24], it might be expected that manipulating OR levels could further contribute to boost the carotenoid content of ripe tomatoes.

Case-study 3: carrots. Underground colors unearthed

Unlike most plants, which contain very low levels of carotenoids in their roots, most commercial varieties of carrot develop roots that accumulate massive amounts of carotenoids [6,37]. Wild carrot roots are white (*i.e.*, they lack carotenoids) but domestication led to the selection of carotenoid-rich varieties. The popular orange carrots mostly accumulate β-carotene and α-carotene, reaching highest levels just before completing secondary growth. Analysis of carotenoid gene expression in orange carrot roots either exposed or not to light has shown that both transcriptional control and plastid identity determine their carotenoid content [38•,39]. When grown underground (*i.e.*, in the dark), carotenoids accumulate in chromoplasts that develop from carotenoid-devoid leucoplasts [40,41]. Strikingly, when root sections are exposed to light they become dark green due to the differentiation of chloroplasts (Figure 2a). Illuminated root segments have a carotenoid profile typical of photosynthetic tissues and lower contents of total carotenoids compared to dark-grown roots [38•]. When light-exposed roots are transferred back to darkness (*e.g.*, covered with soil), their chloroplasts differentiate into chromoplasts and their carotenoid content changes accordingly, eventually reaching the levels found in segments never exposed to light [38•,42]. Interestingly, a recent work has shown that the accumulation of carotenoids in carrot roots is genetically associated to a homologue of the Arabidopsis PSEUDO-ETIOLATION IN LIGHT (PEL) protein [43••]. PEL presumably acts as a repressor of photomorphogenesis (Figure 1). Only carrot varieties with a loss-of-function allele of the *PEL* gene accumulate carotenoids in the root, suggesting that their high pigment contents might result from a derepressed development of carotenoid-accumulating plastids (*i.e.*, chloroplasts in the light but chromoplasts in the dark). This possibility requires further experimental support [7]. If confirmed, however, it would provide an explanation to why carotenoid accumulation does not improve after illumination in some systems. For example, similar to that observed in carrot roots, the accumulation of lycopene in some

Figure 2



Examples of negative correlations between light and carotenoid levels. **(a)** Carrot root. A plant with root segments developed underground (orange) or exposed to light (green) is shown. Insets show microscopy images of both segments. Illuminated root sections develop chloroplasts with a carotenoid profile typical of photosynthetic tissues, whereas dark-grown roots show chromoplasts with much higher levels of carotenes. **(b)** Red grapefruit (Star Ruby) fruits. Pictures show representative fruits developed in the outer part of the tree canopy (upper panels) or protected from light (lower panels). The stronger red color of covered fruits is indicative of higher carotenoid contents in their peel. Note that some uncovered fruits display green areas (which correspond to the parts of the peel most directly exposed to sunlight) and red sectors (in areas that were covered by leaves or other fruits).

cultivars of red grapefruits (*Citrus paradisi*) is higher when fruits are kept in the dark or shaded [44] (Figure 2b). This increment is not mirrored by enhanced expression of carotenoid biosynthetic genes but correlates with an accelerated differentiation of chloroplasts into chromoplasts. If photomorphogenesis is enhanced in these grapefruit cultivars, it might be expected that light would promote the differentiation of chloroplasts, hence interfering with the developmentally-controlled differentiation of lycopene-accumulating chromoplasts during ripening.

As described above, the ultrastructure of plastids not only impacts carotenoid profile and storage capacity but can also influence biosynthetic capacity by modulating the activity of PSY [4,19]. Levels of PSY-encoding transcripts in white carrot varieties are similar to those in colored carrots [39,43••], but PSY activity is only limiting in white carrots [45]. It is therefore likely that PSY enzyme activity increases when leucoplasts differentiate into chloroplasts (in the light) or chromoplasts (in the dark). On the other hand, increasing PSY activity is sufficient to promote the differentiation of chromoplasts from leucoplasts in roots of white carrot varieties but also in Arabidopsis calli grown in the dark [45], confirming that both PSY activity and chromoplast differentiation might be mutually influenced (Figure 1). Together, it appears that the carotenoid contents of carrot roots are essentially controlled by the same basic mechanisms described in other plant systems, with light modulating an intricate network of signals connecting carotenoid biosynthesis (via gene expression and enzyme activities) and storage (via plastid ultrastructure).

Conclusions

The paramount influence of light on the accumulation of plant carotenoids was known for a long time. However, we are just starting to characterize the basic mechanisms by which light signals are transduced to eventually modulate the biosynthesis of these important photoprotective pigments. Besides PSY, other steps of the pathway are known to be relevant to determine the carotenoid contents of different plant tissues. Yet the light signaling pathways regulating the expression of genes encoding the corresponding enzymes remains virtually unexplored. It is becoming clearer, however, that regulating carotenoid biosynthesis is only part of the story. Light also has a major impact on the development of plastids, the organelles where carotenoids are synthesized and stored. Rather than mere reservoirs of carotenoids, plastids have a central role in controlling the activity of PSY and other carotenoid biosynthetic enzymes as well as the qualitative and quantitative profile of carotenoids. Light actually modulates the developmental programs determining plastid identity. Thus, illumination of etiolated seedlings triggers the differentiation of etioplasts into chloroplasts (which accumulate much higher levels of carotenoids to protect the photosynthetic apparatus from excess light). Differentiation of chromoplasts (*i.e.*, plastids specialized in the storage of massive amounts of carotenoids as pigments) is developmentally regulated in most cases, but it can be induced by increasing PSY (or OR) activity. Whereas light normally stimulates the differentiation of chromoplasts as it induces carotenoid biosynthesis (*e.g.*, during tomato fruit ripening), it can also have an opposite role (*e.g.*, in carrot roots). In the examples shown in Figure 2, it is likely that light prevents the differentiation of chromoplasts by overly promoting photomorphogenesis (*i.e.*, photosynthetic development and differentiation of chloroplasts instead of chromoplasts). Unveiling the molecular mechanisms underlying plastid identity is expected to be a major breakthrough to manipulate carotenoid contents in plants. To date, only OR proteins have been demonstrated to trigger the differentiation of chromoplasts. Searching for downstream targets of transcription factors known to regulate both carotenoid biosynthesis and plastid development (Figure 1) might be a productive strategy to obtain new molecular tools to improve the photoprotection of crops or to enrich plant-derived products in health-promoting carotenoids.

Acknowledgements

We thank Ernesto Llamas (CRAG, Barcelona) for contributing to the figures with digital drawings and Lorenzo Zacarías and Maria Jesus Rodrigo (IATA-CSIC, Valencia) for providing the grapefruit pictures. Work at the authors' groups is supported by grants from the Spanish Ministry of Economy and Competitiveness (MINECO grants BIO2015-71703-REDT, BIO2014-59092-P, BIO2014-59895-P, and FPDI-2013-018882); Generalitat de Catalunya (2014SGR-1434 and 2014-SGR-447); and Chilean Comisión Nacional de Investigación Científica y Tecnológica (grant FONDECYT 1130245). We also acknowledge the financial support of the MINECO Severo Ochoa Programme for Centres of Excellence in R&D 2016–2019 (SEV-2015-0533) and the Generalitat de Catalunya CERCA Programme to CRAG.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Kromdijk J, Glowacka K, Leonelli L, Gabilly ST, Iwai M, Niyogi KK, Long SP: **Improving photosynthesis and crop productivity by accelerating recovery from photoprotection**. *Science* 2016, **354**:857-861.

A neat example of how manipulating carotenoid-dependent photoprotection mechanisms can improve the plant response to natural shading events and increase crop productivity.

2. Nisar N, Li L, Lu S, Khin NC, Pogson BJ: **Carotenoid metabolism in plants**. *Mol. Plant* 2015, **8**:68-82.

3. Hou X, Rivers J, Leon P, McQuinn RP, Pogson BJ: **Synthesis and function of apocarotenoid signals in plants**. *Trends Plant Sci.* 2016, **21**:792-803.

4. Ruiz-Sola MA, Rodriguez-Concepcion M: **Carotenoid biosynthesis in Arabidopsis: a colorful pathway**. *Arabidopsis Book* 2012, **10**:e0158.

5. Azari R, Tadmor Y, Meir A, Reuveni M, Evenor D, Nahon S, Shlomo H, Chen L, Levin I: **Light signaling genes and their manipulation towards modulation of phytonutrient content in tomato fruits**. *Biotechnol. Adv.* 2010, **28**:108-118.

6. Rodriguez-Concepcion M, Stange C: **Biosynthesis of carotenoids in carrot: an underground story comes to light**. *Arch. Biochem. Biophys.* 2013, **539**:110-116.

7. Garcia-Mas J, Rodriguez-Concepcion M: **The carrot genome sequence brings colors out of the dark**. *Nat. Genet.* 2016, **48**:589-590.

8. Llorente B, D'Andrea L, Rodriguez-Concepcion M: **Evolutionary recycling of light signaling components in fleshy fruits: new insights on the role of pigments to monitor ripening**. *Front. Plant Sci.* 2016, **7**:263.

9. Meier S, Tzfadia O, Vallabhaneni R, Gehring C, Wurtzel ET: **A transcriptional analysis of carotenoid, chlorophyll and plastidial isoprenoid biosynthesis genes during development and osmotic stress responses in *Arabidopsis thaliana***. *BMC Syst. Biol.* 2011, **5**:77.

10. • Toledo-Ortiz G, Huq E, Rodriguez-Concepcion M: **Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors**. *Proc. Natl. Acad. Sci. U. S. A.* 2010, **107**:11626-11631.
This article reports the first example of a light-regulated transcription factor directly controlling the expression of a carotenoid biosynthesis gene in coordination with photosynthetic development.

11. •• Toledo-Ortiz G, Johansson H, Lee KP, Bou-Torrent J, Stewart K, Steel G, Rodriguez-Concepcion M, Halliday KJ: **The HY5-PIF regulatory module coordinates light and temperature control of photosynthetic gene transcription**. *PLoS Genet.* 2014, **10**: e1004416.
The relevance of antagonistic modules for the control of carotenoid biosynthesis and accumulation in *Arabidopsis* was nicely described in this work. They also show that the HY5-PIF module can integrate light and temperature cues.

12. Leivar P, Monte E: **PIFs: systems integrators in plant development**. *Plant Cell* 2014, **26**:56-78.

13. Lau OS, Deng XW: **The photomorphogenic repressors COP1 and DET1: 20 years later**. *Trends Plant Sci.* 2012, **17**:584-593.

14. Casal JJ: **Photoreceptor signaling networks in plant responses to shade**. *Annu. Rev. Plant Biol.* 2013, **64**:403-427.

15. Roig-Villanova I, Bou-Torrent J, Galstyan A, Carretero-Paulet L, Portoles S, Rodriguez-Concepcion M, Martinez-Garcia JF: **Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins**. *EMBO J.* 2007, **26**:4756-4767.

16. • Bou-Torrent J, Toledo-Ortiz G, Ortiz-Alcaide M, Cifuentes-Esquivel N, Halliday KJ, Martinez-Garcia JF, Rodriguez-Concepcion M: **Regulation of carotenoid biosynthesis by shade relies on specific subsets of antagonistic transcription factors and cofactors**. *Plant Physiol.* 2015, **169**:1584-1594.
Analysis of the role of PIFs on the regulation of carotenoid biosynthesis by shade unveils new antagonistic modules in *Arabidopsis*.

17. Galstyan A, Cifuentes-Esquivel N, Bou-Torrent J, Martinez-Garcia JF: **The shade avoidance syndrome in Arabidopsis: a fundamental role for atypical basic helix-loop-helix proteins as transcriptional cofactors**. *Plant J.* 2011, **66**:258-267.

18. Zhou P, Song M, Yang Q, Su L, Hou P, Guo L, Zheng X, Xi Y, Meng F, Xiao Y *et al.*: **Both PHYTOCHROME RAPIDLY REGULATED1 (PAR1) and PAR2 promote seedling photomorphogenesis in multiple light signaling pathways**. *Plant Physiol.* 2014, **164**:841-852.

19. Welsch R, Beyer P, Hugueney P, Kleinig H, von Lintig J: **Regulation and activation of phytoene synthase, a key enzyme in carotenoid biosynthesis, during photomorphogenesis**. *Planta* 2000, **211**:846-854.

20. Rodriguez-Villalon A, Gas E, Rodriguez-Concepcion M: **Phytoene synthase activity controls the biosynthesis of carotenoids and the supply of their metabolic precursors in dark-grown Arabidopsis seedlings**. *Plant J.* 2009, **60**:424-435.

21. •• Alvarez D, Voss B, Maass D, Wust F, Schaub P, Beyer P, Welsch R: **Carotenogenesis is regulated by 5′UTR-mediated translation of phytoene synthase splice variants**. *Plant Physiol.* 2016, **172**:2314-2326.
This is a very elegant work and one of the very few examples of post-transcriptional control of carotenoid gene expression to meet regular and extraordinary carotenoid needs.

22. •• Zhou X, Welsch R, Yang Y, Alvarez D, Riediger M, Yuan H, Fish T, Liu J, Thannhauser TW, Li L: **Arabidopsis OR proteins are the major posttranscriptional regulators of phytoene synthase in controlling carotenoid biosynthesis**. *Proc. Natl. Acad. Sci. U. S. A.* 2015, **112**:3558-3563.
A landmark work showing that OR proteins, previously known to promote carotenoid accumulation by triggering the differentiation of chromoplasts, also regulate carotenoid biosynthesis by direct interaction with PSY to post-translationally increase the amount of active enzyme.

23. Park S, Kim HS, Jung YJ, Kim SH, Ji CY, Wang Z, Jeong JC, Lee HS, Lee SY, Kwak SS: **Orange protein has a role in phytoene synthase stabilization in sweetpotato**. *Sci. Rep.* 2016, **6**:33563.

24. Chayut N, Yuan H, Ohali S, Meir A, Saar U, Tzuri G, Zheng Y, Mazourek M, Gepstein S, Zhou X *et al.*: **Distinct mechanisms of the ORANGE protein in controlling carotenoid flux**. *Plant Physiol.* 2017, **173**:376-389.

25. Klee HJ, Giovannoni JJ: **Genetics and control of tomato fruit ripening and quality attributes**. *Annu. Rev. Genet.* 2011, **45**:41-59.

26. Giliberto L, Perrotta G, Pallara P, Weller JL, Fraser PD, Bramley PM, Fiore A, Tavazza M, Giuliano G: **Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content**. *Plant Physiol.* 2005, **137**:199-208.

27. Weller JL, Schreuder ME, Smith H, Koornneef M, Kendrick RE: **Physiological interactions of phytochromes A, B1 and B2 in the control of development in tomato**. *Plant J.* 2000, **24**:345-356.

28. Davuluri GR, van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J *et al.*: **Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes**. *Nat. Biotechnol.* 2005, **23**:890-895.

29. Liu Y, Roof S, Ye Z, Barry C, van Tuinen A, Vrebalov J, Bowler C, Giovannoni J: **Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato**. *Proc. Natl. Acad. Sci. U. S. A.* 2004, **101**:9897-9902.

30. Wang S, Liu J, Feng Y, Niu X, Giovannoni J, Liu Y: **Altered plastid levels and potential for improved fruit nutrient content by downregulation of the tomato DDB1-interacting protein CUL4**. *Plant J.* 2008, **55**:89-103.

31. •• Llorente B, D'Andrea L, Ruiz-Sola MA, Botterweg E, Pulido P, Andilla J, Loza-Alvarez P, Rodriguez-Concepcion M: **Tomato fruit carotenoid biosynthesis is adjusted to actual ripening progression by a light-dependent mechanism**. *Plant J.* 2016, **85**:107-119.
This paper describes the adaptation of a shade-regulated PIF1-dependent pathway to monitor ripening progression in tomato fruits.

32. Alba R, Cordonnier-Pratt MM, Pratt LH: **Fruit-localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato**. *Plant Physiol.* 2000, **123**:363-370.

33. Powell AL, Nguyen CV, Hill T, Cheng KL, Figueroa-Balderas R, Aktas H, Ashrafi H, Pons C, Fernandez-Munoz R, Vicente A *et al.*: **Uniform ripening encodes a Golden 2-like transcription factor regulating tomato fruit chloroplast development**. *Science* 2012, **336**:1711-1715.

34. Tang X, Miao M, Niu X, Zhang D, Cao X, Jin X, Zhu Y, Fan Y, Wang H, Liu Y *et al.*: **Ubiquitin-conjugated degradation of golden 2-like transcription factor is mediated by CUL4-DDB1-based E3 ligase complex in tomato**. *New Phytol.* 2016, **209**:1028-1039.

35. Galpaz N, Wang Q, Menda N, Zamir D, Hirschberg J: **Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content**. *Plant J.* 2008, **53**:717-730.

36. Kilambi HV, Kumar R, Sharma R, Sreelakshmi Y: **Chromoplast-specific carotenoid-associated protein appears to be**

important for enhanced accumulation of carotenoids in hp1 tomato fruits. *Plant Physiol.* 2013, **161**:2085-2101.

37. Simpson K, Cerda A, Stange C: **Carotenoid biosynthesis in *Daucus carota***. *Subcell. Biochem.* 2016, **79**:199-217.

38. • Fuentes P, Pizarro L, Moreno JC, Handford M, Rodriguez-Concepcion M, Stange C: **Light-dependent changes in plastid differentiation influence carotenoid gene expression and accumulation in carrot roots**. *Plant Mol. Biol.* 2012, **79**:47-59.
The central relevance of light-dependent plastid development for carotenoid accumulation in carrot roots was first described in this paper.

39. Clotault J, Peltier D, Berruyer R, Thomas M, Briard M, Geoffriau E: **Expression of carotenoid biosynthesis genes during carrot root development**. *J. Exp. Bot.* 2008, **59**:3563-3573.

40. Baranska M, Baranski R, Schulz H, Nothnagel T: **Tissue-specific accumulation of carotenoids in carrot roots**. *Planta* 2006, **224**:1028-1037.

41. Kim JE, Rensing KH, Douglas CJ, Cheng KM: **Chromoplasts ultrastructure and estimated carotene content in root secondary phloem of different carrot varieties**. *Planta* 2010, **231**:549-558.

42. Stange C, Fuentes P, Handford M, Pizarro L: ***Daucus carota* as a novel model to evaluate the effect of light on carotenogenic gene expression**. *Biol. Res.* 2008, **41**:289-301.

43. •• Iorizzo M, Ellison S, Senalik D, Zeng P, Satapoomin P, Huang J, Bowman M, Iovene M, Sanseverino W, Cavagnaro P *et al.*: **A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution**. *Nat. Genet.* 2016, **48**:657-666.
This article provides interesting evidence that the carotenoid cultivars selected by their high carotenoid contents might actually be photomorphogenic mutants.

44. Lado J, Cronje P, Alquezar B, Page A, Manzi M, Gomez-Cadenas A, Stead AD, Zacarias L, Rodrigo MJ: **Fruit shading enhances peel color, carotenes accumulation and chromoplast differentiation in red grapefruit**. *Physiol. Plant.* 2015, **154**:469-484.

45. Maass D, Arango J, Wust F, Beyer P, Welsch R: **Carotenoid crystal formation in Arabidopsis and carrot roots caused by increased phytoene synthase protein levels**. *PLoS One* 2009, **4**: e6373.

46. Shi H, Wang X, Mo X, Tang C, Zhong S, Deng XW: **Arabidopsis DET1 degrades HFR1 but stabilizes PIF1 to precisely regulate seed germination**. *Proc. Natl. Acad. Sci. U. S. A.* 2015, **112**:3817-3822.

47. Dong J, Tang D, Gao Z, Yu R, Li K, He H, Terzaghi W, Deng XW, Chen H: **Arabidopsis DE-ETIOLATED1 represses photomorphogenesis by positively regulating phytochrome-interacting factors in the dark**. *Plant Cell* 2014, **26**:3630-3645.