

Plant colours come to light

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The colours of plant leaves, flowers and fruits are provided by different pigments. Among them, carotenoids are health-promoting antioxidants produced in virtually all plant organs. In leaves, they participate in photosynthesis and photoprotection. In flowers, they colour petals to attract pollinators. And in fruits, they accumulate at the ripe stage to provide not only distinctive pigments, but also nutrients for animals to eat them and disperse the seeds. Light normally stimulates the biosynthesis of carotenoids and regulates the development of storage structures to accommodate these lipophilic pigments. Thus, carotenoid levels decrease when plants become shaded in high-density environments, and they are usually very low in dark-grown organs such as roots or in seedlings that germinate underground. Work with the model plant *Arabidopsis thaliana* has revealed the molecular factors that transduce light signals to boost carotenoid biosynthesis and storage in coordination with photosynthetic development when seedlings emerge from the soil and expose their leaves to sunlight. The same factors appear to control leaf carotenoid contents when light conditions change in day/night cycles or in response to shade. Strikingly, recent discoveries suggest that light-related factors have been recruited during evolution to promote carotenoid accumulation in tomatoes (fruits) and carrots (roots).

Carotenoids are healthy antioxidant pigments

Most land plants are green because their stems and leaves accumulate chlorophylls, the pigments that collect light for photosynthesis. But non-photosynthetic plant organs often display non-green colours due to the presence of other pigments such as water-soluble anthocyanins and betalains and lipid-soluble carotenoids. Anthocyanin colours range from orange/red to violet/blue, and they are responsible for the reddish colour of strawberries or the dark purple skin colour of some grapes. Betalains provide colours from yellow to violet to plants of the order Caryophyllales, including beets and bougainvillea. Carotenoids confer yellow-to-red colours to corn, bananas, oranges, pumpkins, tomatoes, carrots and marigold flowers. These pigments typically function as visible signals to attract insects, birds and other animals. Carotenoids are particularly interesting for several reasons. Carotenoids are synthesized by all plants, but they are also produced by non-plant photosynthetic organisms and some non-photosynthetic bacteria and fungi. Only a few species of insects and mites produce carotenoids using fungal genes that were naturally incorporated to their genomes during evolution, in a typical example of horizontal or lateral gene transfer. The vast majority of animals take carotenoids from their food, and some use them for pigmentation purposes. For example, the pink colour of flamingoes and salmon derives from dietary carotenoids. Carotenoids are the main source of vitamin A and provide multiple health benefits. High carotenoid intake in humans has been associated with enhanced immune system and cognitive

functions and with lower risk of developing degenerative chronic diseases, such as age-related macular degeneration, type 2 diabetes, obesity, and certain types of cancer (breast, cervical, ovarian and colorectal) and cardiovascular diseases. Additionally, carotenoids are skin protectants and are used in the nutraceuticals area. Their major economic relevance, however, is as natural pigments in the agrofood industry. Enhancing the carotenoid content of plants is, therefore, an attractive goal from both industrial and nutritional perspectives. Generating sustainable biofactories of natural pigments or biofortified crops is now feasible, thanks to our increasing understanding of how plants regulate their carotenoid contents.

Plant carotenoids are produced in plastids, organelles of bacterial origin that distinguish plants from other eukaryotic organisms. Plastids are found in almost every plant cell and they adopt many shapes and forms, but carotenoids are most abundant in chloroplasts and chromoplasts. Chloroplasts are found in green tissues and they are the site of photosynthesis. Chloroplast carotenoids help chlorophylls to harvest light, contribute to the assembly of the photosynthetic apparatus, release excess light energy as heat and protect from oxidative damage. The colours of carotenoids are hidden in photosynthetic (green) tissues, but become obvious after chlorophylls degrade when leaves senesce (hence providing the characteristic colours of some trees in autumn) or fruits ripe (changing their colour to signal that they are ready to be consumed and the seeds can be dispersed). During senescence, chloroplasts transform into a different plastid type named gerontoplasts. During ripening of green fruits, however, chloroplasts differentiate into chromoplasts, which are plastids

specialized in the storage of carotenoids in membrane structures and lipophilic vesicles called plastoglobules. Different types of chromoplasts are present in organs that accumulate different types of carotenoids, resulting in different colours. Carotenoids are produced at much lower levels in other plastid types such as the etioplasts of dark-grown seedlings or the leucoplasts of non-coloured roots and petals. Besides their functions in photosynthesis, photoprotection and pigmentation, carotenoids act as precursors of biologically active molecules that regulate plant development and environmental interactions (including plant hormones such as abscisic acid and strigolactones) and inform on the physiological status of the chloroplast.

Light activates carotenoid production and storage in green tissues

Light is a major regulator of carotenoid accumulation in plants, in agreement with its central relevance for plant development, photosynthesis and pigmentation. Light coordinately controls the expression of genes involved in carotenoid biosynthesis and degradation, the catalytic activity of the corresponding enzymes and the differentiation of carotenoid storage structures in plastids. While the influence of light on these areas has been known for a long time, we are just starting to understand the molecular basis. Most of our knowledge of the light-mediated control of carotenogenesis comes from work in the model plant *Arabidopsis thaliana*. Transcription factors transducing light signals such as Phytochrome-Interacting Factors (PIFs) and Elongated Hypocotyl 5 (HY5) have been found to directly bind

to the promoters of thousands of genes (including carotenoid-related genes) in response to changing light conditions. PIFs repress and HY5 activates carotenoid biosynthesis in particular and photosynthetic development in general, hence linking the production of carotenoids with their storage in chloroplasts (Figure 1). In seedlings germinating in the dark, PIFs accumulate and HY5 degrades, causing a block in carotenogenesis. But when seedlings emerge from the soil and are exposed to sunlight, PIFs degrade and HY5 accumulates, collectively boosting the production of carotenoids while promoting the differentiation of etioplasts into chloroplasts (Figure 1). Chloroplast development involves the assembly of the photosynthetic apparatus and thylakoid membranes, which promote the catalytic activity of carotenoid biosynthetic enzymes (most of which are membrane associated) and have a much higher capacity to accommodate the newly produced lipid-soluble carotenoids. This antagonistic repression-activation system also functions during day and night cycles and when plants are shaded. Photosynthesis and growth can actually be dramatically compromised by the shading of nearby plants. Changes in light quality associated with crowded (i.e., high density) plant environments are perceived by plant photoreceptors and rapidly transduced into changes in gene expression aimed to adapt to eventual shading, e.g., by decreasing the production of chlorophylls and carotenoids to readjust their photosynthetic metabolism to the expected decrease in light quantity. PIFs accumulate under shade and down-regulate carotenoid levels soon after the light signal is perceived, whereas HY5 participates in a recently discovered mechanism by which chloroplasts

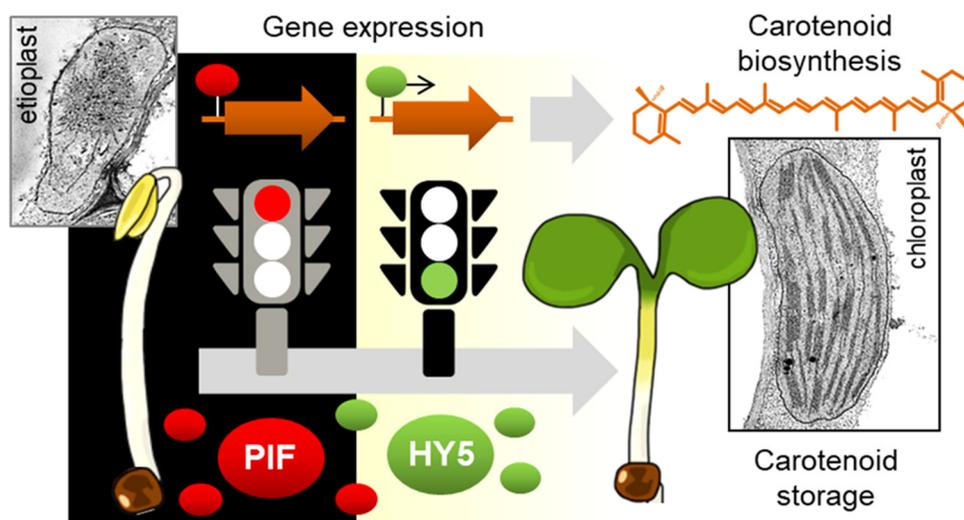


Figure 1. Light-related factors such as PIFs and HY5 antagonistically regulate seedling greening and chloroplast differentiation in coordination with carotenoid synthesis and storage. PIFs are repressors that accumulate in the dark and degrade in the light, whereas HY₅ is an activator that accumulates upon illumination.

repress the plant response to shade when photosynthetic activity decreases too much. This mechanism, which involves carotenoid-derived signals such as abscisic acid, allows chloroplasts to directly sense proximity shade conditions and activate a brake on the response as a safety mechanism, so that the plant does not compromise too much in case shading does not eventually occur.

Light response factors also control fruit colour change during ripening

The colours of ripe tomatoes result from the degradation of chlorophylls and the accumulation of health-promoting carotenoids such as orange β -carotene (pro-vitamin A) and red lycopene. In tomato and many other fleshy fruits (but not in those from *Arabidopsis*), this process involves the differentiation of green fruit chloroplasts into ripe fruit chromoplasts. Fruit-localized photosensory pathways are important players in the regulation of fruit ripening in general and carotenoid biosynthesis in particular. Analysis of mutants has shown that genetically enhanced light signalling improves carotenoid storage capacity by increasing chromoplast number and size. We still know very little on how chloroplasts differentiate into chromoplasts, but the analysis of light signalling components in tomato has provided very interesting insights on how these factors were recruited to regulate carotenoid accumulation during ripening. PIFs, HY5 and other transducers of light responses regulate fruit carotenoid contents as reported for green tissues. Thus, PIFs repress and HY5 activates carotenogenesis in tomatoes and other fleshy fruits. In the case of PIFs, however, it has been shown that their light-dependent degradation is not directly related to responding to environmental (external), but to developmental (internal) signals. The presence of chlorophylls in green tomato fruit changes the quality of the light that reaches the inner fruit tissues and results in a 'self-shade' effect that causes the accumulation of PIFs. Developmentally controlled degradation of chlorophylls gradually reduces the self-shading effect as ripening progresses, hence allowing degradation of PIFs and the subsequent de-repression of carotenoid biosynthesis. Given the ubiquitous distribution of PIFs in plants, it is most likely that this self-shading mechanism adjusts carotenoid biosynthesis to the actual progression of ripening in other fruits that lose chlorophylls and accumulate carotenoid pigments during ripening as a visual signal to inform seed-dispersing animals that the fruit is ripe and tasty.

In some cases, carotenoid levels are higher in the dark

In citrus trees, light can have opposite effects on fruit carotenoid contents depending on the species or cultivars.

Light promotes carotenoid accumulation in sweet orange and mandarin fruits, and those shaded by leaves or by other fruits display a paler peel pigmentation. In some cultivars of grapefruit, however, a higher accumulation of carotenoids causing an intense red colouration takes place when fruits are bagged or shaded. This phenotype is not linked to enhanced expression of carotenoid biosynthetic genes, but to accelerated differentiation of chloroplasts into chromoplasts. The molecular mechanism remains unknown.

Unlike most plant organs, roots develop underground in the dark. The roots of most plant species contain plastids with very low levels of carotenoids. A notable exception is carrot, the plant that gave carotenoids their name. Wild carrot roots are white due to very low carotenoid contents, but commercial carotenoid-rich varieties are now most appreciated by consumers. Studies carried out mostly using orange-coloured cultivars have shown that light has a strong negative impact on carrot carotenoid contents. When grown underground in the dark, high levels of β -carotene and other carotenoids accumulate in chromoplasts that develop from carotenoid-devoid leucoplasts. By contrast, when underground orange roots are exposed to light, they become dark green due to the differentiation of leucoplasts into chloroplasts with a carotenoid profile typical of photosynthetic tissues. Transferring of light-exposed roots back to darkness causes chloroplasts to differentiate into chromoplasts and a concomitant change in carotenoid levels and composition to eventually match those of roots that were never exposed to light. It has been proposed that carotenoid-rich carrots are mutants defective in the PEL protein, which functions as a repressor of photosynthetic development similar to PIFs (Figure 2). Loss of PEL function in roots would result in chloroplast development when illuminated. In the dark, however, chloroplasts cannot differentiate because chlorophyll biosynthesis requires actual light. Therefore, de-repressed carotenoid biosynthesis in PEL-defective underground roots might result in chromoplast differentiation, boosting both carotenoid production and sequestration (Figure 2). A similar mechanism might explain the enhanced differentiation of chromoplasts and carotenoid-based pigmentation of light-deprived grapefruits. Our recent results demonstrate that keeping an active carotenoid biosynthetic pathway in photosynthetically weak chloroplasts is sufficient to convert them into carotenoid-overaccumulating chromoplasts even in plant species that do not naturally develop chromoplasts. Based on this, the differentiation of chromoplasts, and hence the carotenoid overaccumulation phenotype in non-illuminated carrots and grapefruits might well be simply explained by an alteration of light signalling components that could maintain an active carotenoid biosynthesis

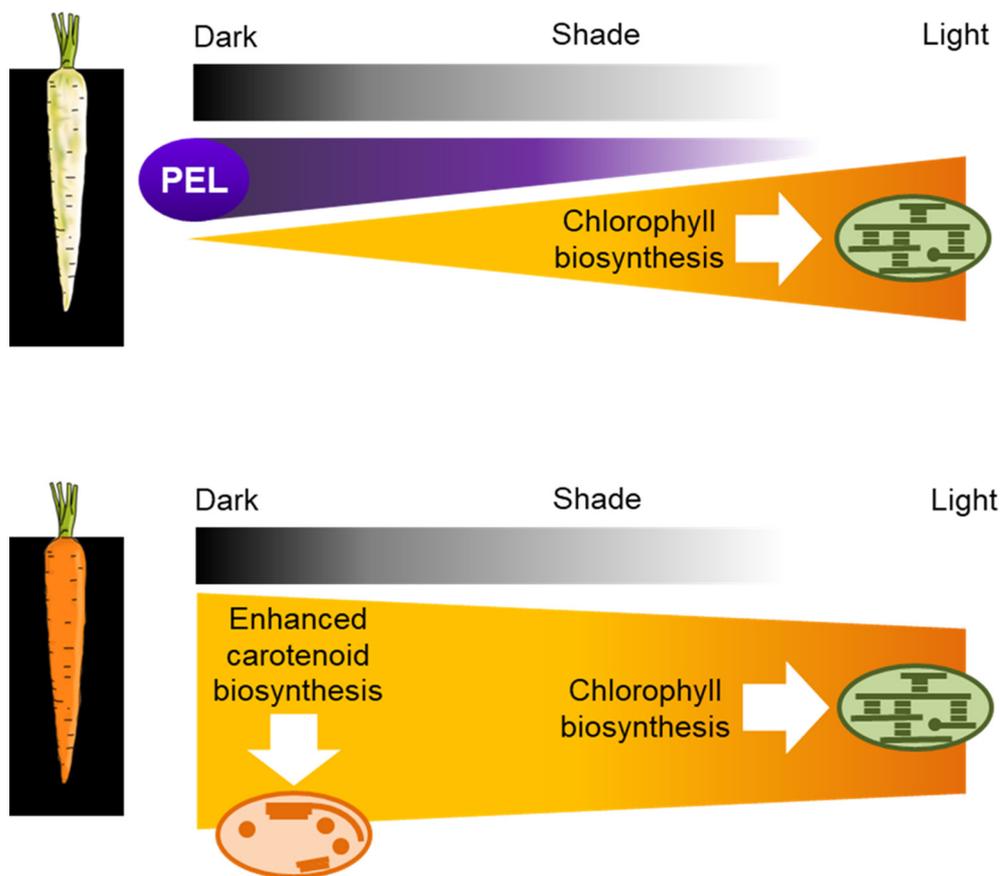


Figure 2. Carotenoid-rich orange carrots are defective in PEL, a protein that represses carotenoid biosynthesis in the dark. When PEL is not functional, carotenoids are produced in underground roots and promote the differentiation of chromoplasts, which in turn boost carotenoid contents by improving the activity of carotenoid-producing enzymes and the storage capacity.

under conditions in which fully functional chloroplasts cannot be formed.

Conclusions

The pivotal role of light for the control of plant carotenoid contents was known for a long time, but recent discoveries are providing new insights on the molecular mechanisms by which light signals modulate the accumulation of these important antioxidant pigments. Besides regulating the expression of genes involved in carotenoid biosynthesis, light influences the development of plastids, the organelles where carotenoids are synthesized and stored. Light triggers the differentiation of etioplasts into chloroplasts (which accumulate high carotenoid levels for photosynthesis and photoprotection) and

normally promotes the differentiation of chromoplasts (which sequester massive amounts of carotenoids as pigments) as it induces carotenoid biosynthesis (e.g., during tomato fruit ripening). Chromoplasts can also develop when altered light signalling keeps an active carotenoid production under conditions that prevent photosynthetic development (e.g., in dark-grown carrot roots). In summary, it is likely that the same basic light-dependent networks regulate carotenoid biosynthesis and accumulation in all plant systems. A better understanding of such basic mechanisms and how plants manipulate them to adapt carotenogenesis to environmental but also developmental cues (e.g., the 'self-shading' effect described in tomato) should provide new insights and molecular tools to improve crop productivity and biofortification of plant-derived foods. ■

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