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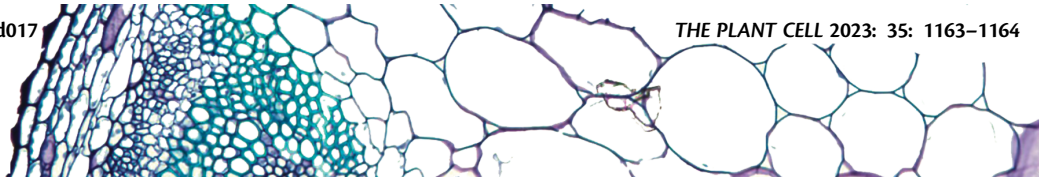
https://doi.org/10.1093/plcell/koad017 THE PLANT CELL 2023: 35: 1163–1164



Tanned but not burned: A negative feedback loop controls *Citrus* fruit coloration

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In Brief

Fruits are essential for the reproductive success of angiosperms by promoting seed maturation and dispersal. The attraction of seed dispersers is influenced by fruit scent and coloration, and the biosynthesis of carotenoids is central for the production of volatile precursors and pigments (Rodríguez et al., 2013). In specific orange varieties such as *Citrus sinensis*, the accumulation of the apocarotenoid β-citraurin (β-C) in the fruit peel confers a deep orange color. These specific varieties possess a distinct miniature inverted-repeat transposable element allele in the promoter of the gene encoding the last step of β-C biosynthesis, creating a basic helix-loop-helix protein-binding element (E-box) associated with increased gene expression in orange peels (Zheng et al., 2019).

The phytohormone jasmonic acid (JA) promotes carotenoid accumulation in tomato (Liu et al., 2012), and the basic helix-loop-helix transcription factor (TF) MYC2 is a master regulator of the JA response (Lorenzo et al., 2004). However, how JA influences β-C accumulation in *Citrus* remained unknown. In this issue, **Pengtao Yue and colleagues** (Yue et al., 2022) provide evidence that β-C biosynthesis is regulated by a JA-coordinated negative feedback regulatory module involving CsMYC2 (see Figure) in *Citrus sinensis*.

The authors first confirmed that endogenous levels of β-C, JA, and the transcripts encoding carotenoid biosynthetic enzymes showed some degree of correlation in orange peels, as originally shown by Liu et al. (2012). In addition, pretreatment of detached fruits with ethylene or abscisic acid inhibitors did not prevent the promotion of fruit coloration by methyl-jasmonate (MeJA) treatment, suggesting that MeJA-induced fruit coloration was independent of ethylene or ABA. In their quest for the identification of a TF required for JA-induced fruit coloration, the authors observed that among the three detected *MYC*-encoding genes in *Citrus*, only *CsMYC2* transcripts accumulated more in fruit peels after MeJA treatment and during natural fruit coloration. Through a compelling series of in vitro and in vivo experiments, the authors show that CsMYC2 interacted with E-box-containing promoters of carotenoid biosynthesis genes and stimulated their transcription in transactivation assays (see Figure). Importantly, MeJA treatment reinforced the association of CsMYC2 with E-boxes as observed by chromatin immunoprecipitation, followed by quantitative PCR (ChIP-qPCR) experiments. Interestingly, the authors also observed a rapid and transient increase in the accumulation of carotenoid gene transcripts after MeJA treatment, with the peak of expression preceding the peak of JA and β-C accumulation. The authors therefore posited that a negative feedback loop might occur to dampen an excessive JA response.

Among the 37 detected orthologs of JA repressors in Arabidopsis, transcript levels of *Mitogen-Activated Protein Kinase 6* (*CsMAPK6*) and two other putative repressors were increased by MeJA treatment in *C. sinensis*. The authors performed ChIP-qPCR and transactivation experiments suggesting that CsMYC2 interacted with the E-box-containing *CsMAPK6* promoter to activate transcription. They also confirmed that CsMYC2 and CsMAPK6 interacted as observed in Arabidopsis (Sethi et al., 2014). To understand the molecular consequences of the CsMYC2-CsMAPK6 interaction, the authors hypothesized that CsMAPK6 phosphorylates CsMYC2. Using a phos-tag biotin probe, the researchers observed that phosphorylation of CsMYC2 occurred only when *CsMAPK6* was co-expressed. Mass spectrometry analysis of CsMYC2 identified several phosphorylated amino acids. Site-directed mutagenesis of these amino acids to alanine prevented the detection of CsMYC2 by phos-tag biotin assay

Received January 18, 2023. Accepted January 19, 2023. Advance access publication January 21, 2023

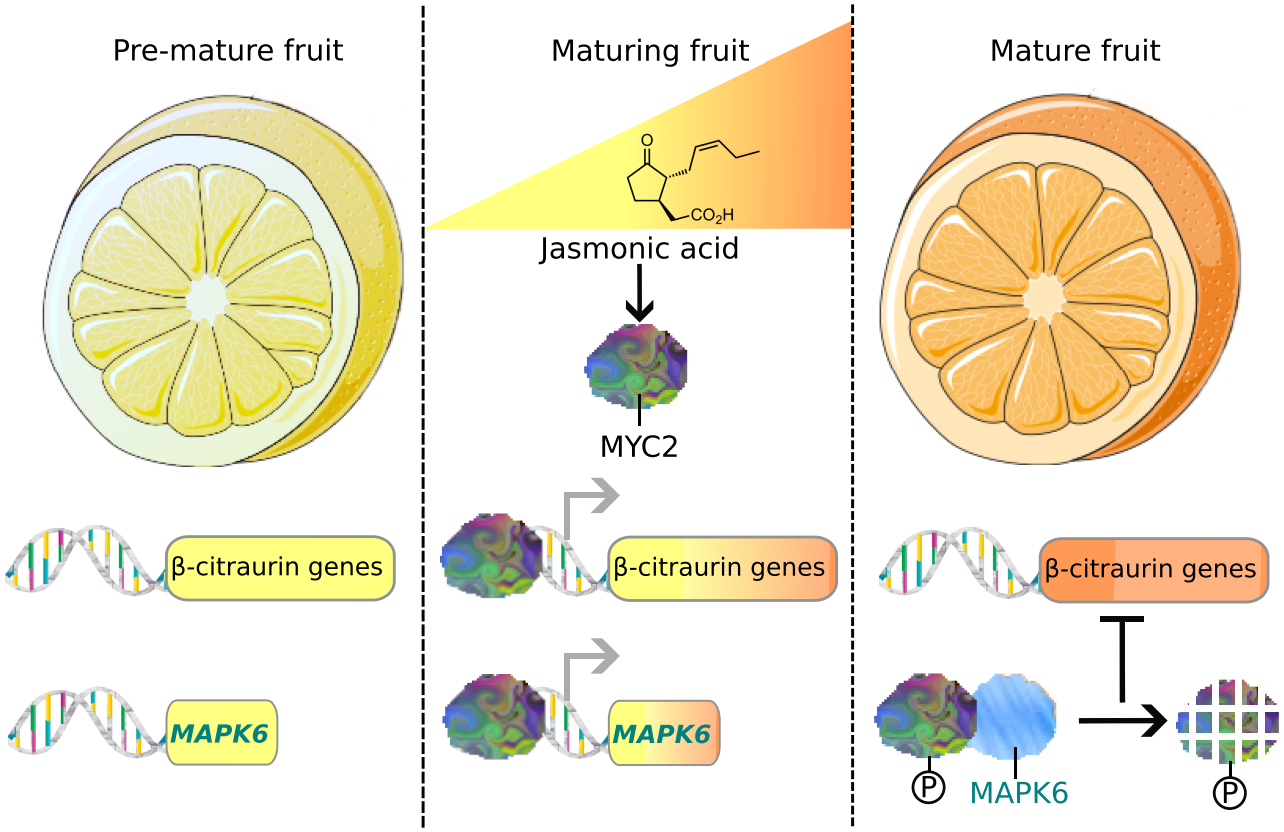

Figure The JA-orchestrated MYC2-MAPK6 negative feedback regulatory module controls orange coloration. During maturation, JA levels increase and activate MYC2 expression. Then, MYC2 binds the E-boxes in carotenoid biosynthetic and *MAPK6* gene promoters to stimulate transcription. At this stage, fruit coloration occurs. Upon accumulation of excessive amounts of JA, MAPK6 binds and phosphorylates MYC2 to induce its degradation, causing an arrest in fruit coloration. Adapted from Yue et al. (2022) with images taken from bioicons.com and commons.wikimedia.org.

when co-expressed with *CsMAPK6*, thus revealing that they are CsMAPK6-dependent phosphorylation sites. The authors then confirmed the effect of phosphorylations on CsMYC2 protein stability by transient expression of phospho-variants in *Citrus* calli. They further showed that co-expression of *CsMYC2* and *CsMAPK6* decreased the transactivation potential of CsMYC2, and co-incubation of CsMYC2 with CsMAPK6 reduced the binding of CsMYC2 with the E-box, suggesting that the interaction alone could reduce CsMYC2 TF activity by steric hindrance. Finally, the authors validated the role of *CsMYC2* and *CsMAPK6* in fruit coloration by transient overexpression or silencing experiments in orange peels. Transient overexpression of *CsMAPK6* or of an antisense *CsMYC2* transcript decreased β-C accumulation and orange coloration.

Collectively, these results led the authors to propose that in parallel to its role in activating carotenoid genes transcription upon increased JA levels, MYC2 also stimulates the transcription of *MAPK6*, which in turn binds and displaces MYC2 from biosynthesis genes. After protein interaction, MAPK6 phosphorylates MYC2 to induce its degradation and avoid an excessive JA response (see Figure).

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