Transcriptional control of strawberry ripening – two to tango

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Although many genomic regions encoding putative transcription factors have been identified in sequencing efforts, the roles of relatively few have been elucidated. Now, working with strawberry, Molina-Hidalgo et al. (2017) have identified a second transcription factor (FaDOF2) involved in regulating eugenol synthase and production of the aroma compound eugenol during strawberry ripening. Not only is this important in understanding the control of ripening in non-climacteric fruit but, potentially, in breeding to reintroduce flavor into modern germplasm.

Eugenol is a phenylpropanoid aroma compound that is an important constituent of the flavor of many spices, including cloves and basil (Koeduka et al., 2006). It is also found in flowers and fruits, including banana, tomato, grape, melon and strawberry (Aubert and Pitrat, 2006; Tieman et al., 2012), all of which are major agricultural species (Box 1). Eugenol synthase catalyzes the last step in the biochemical pathway to eugenol in strawberry (Fragaria × ananassa); the biosynthesis proceeds from coniferyl alcohol through the action of coniferyl alcohol acetyltransferase to form coniferyl acetate, the eugenol synthase substrate (Hoffmann et al., 2011). Three forms of eugenol synthase have been identified in strawberry – FaEGS1a, FaEGS1b and FaEGS2 – with FaEGS2 being the dominant form in ripening receptacles (Aragüez et al., 2013).

Earlier work showed that FaMYB10 (Medina-Puche et al., 2014) works as a major regulator of the expression of genes belonging to the phenylpropanoid pathway, and that it is upstream of another MYB transcription factor, FaEOBII, also regulating its expression. In turn, FaEOBII regulates the expression of FaEGS2 (Medina-Puche, 2015). Molina-Hidalgo et al. (2017) have now identified a second transcription factor, FaDOF2, that regulates the levels of FaEGS2 transcription – silencing showed that it regulates the expression of FaEGS2, eugenol synthase and FaEOBII. FaDOF2 is expressed in red receptacles and flower petals, but not green receptacles; it is localized in the nucleus, binds to a defined DNA sequence found in the promoter of a strawberry eugenol synthase gene (FaEGS2) and interacts with the transcription factor FaEOBII. FaEOBII and FaDOF2 together coordinate the expression of FaEGS2.

Fruit ripening in climacteric fruits is regulated by the plant hormone ethylene, but regulation of ripening in non-climacteric species, such as strawberry, is not well understood. Nevertheless, several plant hormones, including ABA, auxins and gibberellins, have been implicated in the process (reviewed in Fortes et al., 2015; Osorio et al., 2013). While ABA increases during strawberry ripening, auxin and gibberellins decline, suggesting that ABA coordinates strawberry receptacle ripening (Medina-Puche et al., 2015; Medina-Puche et al., 2016). FaDOF2 expression is up-regulated during strawberry ripening and is regulated by ABA.

Multiple changes in color, texture, taste and aroma

Fruit ripening is associated with multiple changes in color, texture, taste and aroma, and many genes exhibit changes in expression during the transition from development to ripening (Giovannoni et al., 2017). These include genes encoding RNAs for cell wall-degrading enzymes, anthocyanin and carotenoid biosynthetic enzymes, chlorophyll-degrading enzymes, and enzymes in the pathways to flavor compounds (reviewed by Giovannoni, 2004, and Tohge and Fernie, 2015). Each fruit species has its own unique flavor, comprising a mixture of sugars, acids and aroma compounds. Although sugars and acids are the base of flavor, the particular mix of aroma compounds constitutes each fruit species’ unique signature. Altering the levels of these compounds in relation to one another can significantly alter the flavor of a fruit, resulting in reduced acceptability by consumers. Therefore, each species has to regulate the production of many biochemicals from multiple pathways to produce its own defining flavor (Wang et al., 2016).

In addition, these aroma compounds are derived from many different primary metabolites, including carotenoids, amino acids, lipids and carbohydrates (reviewed by Schwab et al., 2008). Moreover, each species must have multiple distinct pathways for the formation of aroma compounds, and many of these compounds are only produced in fruit, flowers or ripening fruit. In turn, each of these pathways must be regulated by transcription factors that coordinate the expression of the genes producing many different enzymes in a fruit-ripening-specific manner. Considering that most fruits have hundreds of aroma compounds from many different pathways, the degree of complexity rapidly multiplies. Regulation of these pathways to produce these compounds during the relatively short fruit-ripening process requires transcriptional regulation of many genes and biochemical pathways simultaneously.
Identifying the roles of transcription factors

The multiple complexities of the fruit-ripening process complicate the identification of the role of transcription factors in producing associated volatile compounds. Unlike enzymes involved in primary and secondary metabolism, sequence similarity among transcription factors does not mean that the regulated genes have similar functions (Klie et al., 2014). Indeed DOF-family transcription factors have been shown to regulate a large number of processes: seed germination; the response to light; root and leaf development; responses to salicylic acid; nitrogen assimilation; carbon metabolism; organ growth; hormone signaling; and biosynthesis of glucosinolates, brassinosteroids, jasmonic acid and flavonoids (reviewed by Corrales et al., 2017; Yanagisawa, 2004; Lehti-Shiu et al., 2017). Although many genes encoding transcription factors have been identified through sequencing efforts in multiple plant species, relatively few of these have been assigned a function in controlling gene expression (reviewed by Karlova et al., 2014; Kourmpetli and Drea, 2014; Tohge and Fernie, 2015). Next-generation sequencing will continue to identify transcription factors from more species that are up-regulated during ripening.

Considering the complex regulatory networks regulating processes such as fruit ripening, determining the targets of putative transcription factors and their interactions in ripening and flavor compound accumulation would further our understanding of the ripening process. The approach used by Molina-Hidalgo et al., comparing transcriptomes of green and ripe strawberry receptacles, was effective in defining the role of FaDOF2 in the control of eugenol synthesis and it is a way forward in identifying transcription factors important for other ripening-related processes.

What about other implications? Loss of flavor in fruits and vegetables is a common consumer complaint. Breeding for yield, disease resistance, shelf-life, appearance and firmness for shipping has resulted in loss of flavor quality in modern fruit varieties. Although these traits are essential for modern production processes, it should be possible to reintroduce flavor into modern germplasm. With a greater understanding of the biochemical and regulatory processes in the formation of flavor compounds, breeding for improved flavor should be feasible. Some flavor biochemical pathways have been established, and are now targets for breeding for improved flavor (Tieman et al., 2017). Transcription factors, such as FaDOF2, should only add to the target list.

Key words: DOF transcription factor, eugenol, Fragaria, fruit ripening, phenylpropanoid pathway, strawberry.

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Insight

Auxin transport and conjugation caught together

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Polar auxin transport together with local synthesis and turnover are crucial for establishing auxin gradients, which determine an array of plant developmental pathways. Transcription factor-mediated control of the genes involved in these processes is steadily receiving increased attention, including efforts to find regulators determining the expression of major auxin efflux carriers of the PIN family. Now, Kong et al. (2017) have provided evidence of a direct link between auxin metabolism and transport mediated by PINs that is controlled by the transcription factor WRINKLED1.

In its major form as indole-3-acetic acid (IAA), the plant hormone auxin drives plant growth and development and controls fundamental cellular processes, such as division, expansion and differentiation. Hence, transport of auxin plays a pivotal role in nearly all aspects of plant development, and auxin efflux carriers of the PIN-FORMED (PIN) family have been described as key components exerting this role. Numerous studies have shown that the polar localization of PINs is a critical vectorial feature of auxin flow in Arabidopsis (Zazimalova et al., 2014). Many older studies had shown hormonal and environmental cues to be major regulators of their expression (Vieten et al., 2005; see also review by Krec et al., 2009). However, the identification of molecular components determining PIN expression levels turned out to be very difficult, and the first detailed molecular mechanisms and protein factors acting upstream of these genes have only been uncovered relatively recently. Even less is known about how PINs are co-regulated with other auxin-relevant targets.

Regulation of PINs

Among the first factors shown to regulate PIN expression was the MADS transcription factor XAANTAL2 (XAL2), also known as AGAMOUS-LIKE 14 (AGL14) (Box 1). It was shown that XAL2, which otherwise regulates meristem proliferation and flowering transition, is required for expression of PIN4 and PIN1 (Tapia-López et al., 2008; Garay-Arroyo et al., 2013). Meristem defects in the xal2 mutant resemble those seen in pin4 and/or pin1 knockouts or in their higher order mutant combination, and xal2 mutants also show reduced free IAA levels and polar auxin transport (Friml et al., 2002; Bilou et al., 2005; Garay-Arroyo et al., 2013).

Another transcription factor controlling PIN expression, PPP1 (PIN2 PROMOTER BINDING PROTEIN 1), is a