

RECENT ADVANCES IN THE REGULATION OF CLIMACTERIC FRUIT RIPENING: HORMONE, TRANSCRIPTION FACTOR AND EPIGENETIC MODIFICATIONS

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KEYWORDS

climacteric fruit ripening, phytohormones, TFs, epigenetic modifications

HIGHLIGHTS

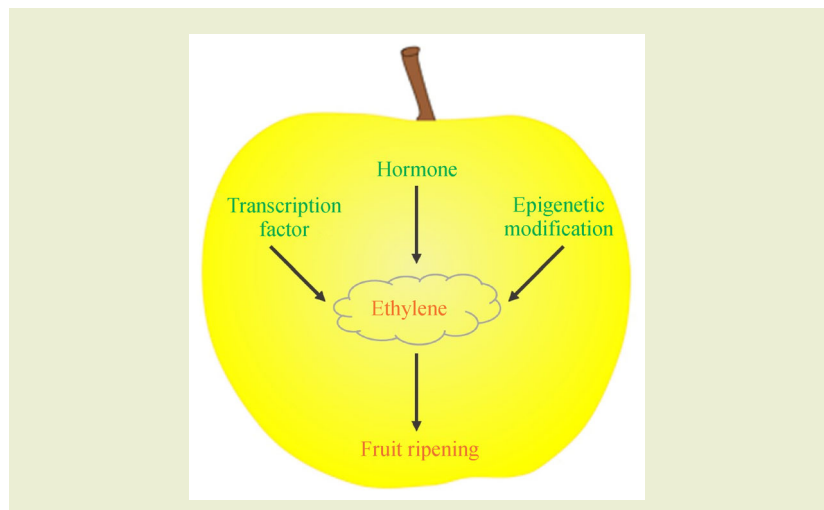
- The dynamic interplay between phytohormones plays an important part in climacteric fruit ripening.
- Transcription factors are critical for the regulation of climacteric fruit ripening.
- Epigenetic modifications act as important regulators of fruit ripening.

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GRAPHICAL ABSTRACT



ABSTRACT

Fruit ripening is a complex developmental process made up of genetically programmed physiological and biochemical activities. It culminates in desirable changes in the structural and textural properties and is governed by a complex regulatory network. Much is known about ethylene, one of the most important metabolites promoting the ripening of climacteric fruits. However, the dynamic interplay between phytohormones also plays an important part. Additional regulatory factors such as transcription factors (TFs) and epigenetic modifications also play vital role in the regulation of climacteric fruit ripening. Here, we review and evaluate the complex regulatory network comprising interactions between hormones and the action of TFs and epigenetic modifications during climacteric fruit ripening.

1 INTRODUCTION

Angiosperms have evolved numerous fruit forms that facilitate the spread of their seeds in different ecological and environmental contexts. There are dry fruits such as nuts, cereal grains and legumes, and fleshy fruit such as apple (*Malus domestica*), peach (*Prunus persica*) and tomato (*Solanum lycopersicum*)^[1]. Nutritious fleshy fruits are more attractive to animals that feed on fruit and have become an indispensable part of the human diet, providing important nutrients such as carbohydrates, dietary fiber, vitamins and special functional active substances. As such, it is a dramatic shift for plants from protecting immature fruits from animal destruction to appealing to animals to eat ripe fruits for seed dispersal^[2,3]. It is crucial to plants to regulate the transition from unripe to ripe for dispersal of mature seed. The ripening of fleshy fruits is an economically important phase in the life cycle of horticultural crops. To this end, the strategy and timing of fruit ripening must be strictly regulated in order to ensure the survival of progeny, and fruit have evolved complex mechanisms to ensure appeal to fruit-eating animals^[4,5].

Fruit ripening is a biochemical and physiological process regulated by genetic processes followed by changes in fruit size, texture, color, aroma and flavor^[6–9]. Fleshy fruits are mostly distinguished by respiration pattern and ethylene production at the onset of the ripening process of non-climacteric fruits such as strawberry (*Fragaria vesca*) and grape (*Vitis vinifera*), and climacteric fruits such as apple and tomato (Fig. 1)^[10–12]. During non-climacteric fruit ripening, ethylene production remains low

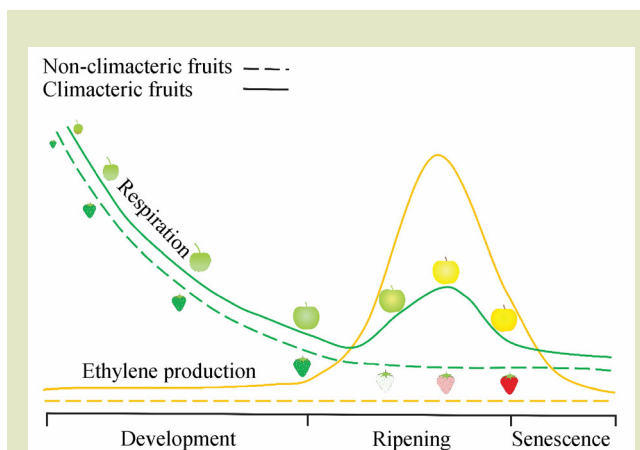


Fig. 1 Changes in respiration rate and ethylene production in climacteric or non-climacteric fruit during development, maturation and senescence^[3]. Dashed lines represent the non-climacteric fruits and solid lines climacteric fruits. Green lines represent the respiration rate and orange lines ethylene production.

and respiration exhibits no dramatic change from beginning to end and the ripening is mainly controlled by gradual accumulation of the phytohormone abscisic acid (ABA)^[13–15]. In contrast, climacteric fruit ripening has a peak in respiration and a burst of ethylene to unleash the ripening process in an autocatalytic response just prior to the initiation of ripening, and is mainly regulated by phytohormone ethylene^[4,10,15,16]. However, an increasing number of studies show that fruit ripening is regulated by the balance of multiple phytohormones rather than by a single phytohormone^[15,17,18]. Also, epigenetics such as DNA methylation and histone modifications are involved in regulating fruit ripening^[2,19–24] involving various transcriptional factors. A better understanding of the regulatory network controlling fruit ripening is not only biologically interesting but also economically important for developing strategies to improve sensorial qualities and fruit nutrition and reduce postharvest losses^[25]. Here, we summarize recent advances in research on climacteric fruit ripening and discuss future perspectives in this field.

2 HORMONE REGULATION

Knowledge of the role of phytohormones other than ethylene during climacteric fruit ripening has long been limited. However, an increasing number of studies point to a role of auxins, abscisic acid, brassinosteroids and jasmonates in climacteric fruit ripening. Tomato, a typical climacteric fruit, is a good model system to investigate the mechanism of fruit ripening^[1] in addition to numerous fleshy climacteric fruit such as apple^[26–28], banana (*Musa acuminata*)^[29,30], mango (*Mangifera indica*)^[31,32], peach^[33,34], pear (*Pyrus ussuriensis*)^[35,36] and plum (*Prunus sibirica*)^[37] which have been used to unravel the mechanisms underlying fruit ripening.

2.1 Ethylene

Ethylene, a gaseous phytohormone, has a central role in climacteric fruit ripening^[8,38]. In the ripening process, ethylene production gradually increases to a peak and then gradually decreases (Fig. 2) and subsequently the fruit moves into the aging stage^[39].

The role of ethylene in fruit is regulated by two pathways: the biosynthesis and signal transduction pathways. The biosynthesis of ethylene in climacteric fruit is divided into two systems in higher plants, designated systems 1 and 2. System 1 is mainly responsible for the biosynthesis of constitutive concentrations of ethylene in vegetative tissues and it is wounding-induced. Ethylene produced in young fruit also belongs to system 1.

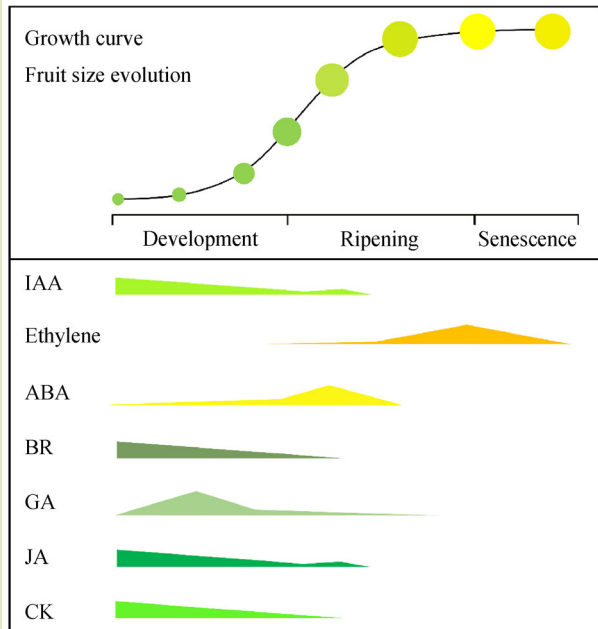


Fig. 2 Concentrations of endogenous phytohormones in climacteric fruit including apple, pear and tomato during development, maturation and senescence^[26,39–47].

Ethylene production in system 1 can be autoinhibited by ethylene. System 2 is mainly responsible for the biosynthesis of ethylene in aging flowers and ripe fruit. System 2 ethylene has auto-catalytic characteristics, that is, ethylene produced *in vivo* can be further promoted by ethylene, and this is unique to climacteric fruit^[48–50]. System 2 is active when climacteric ethylene has to be produced.

The pathway of ethylene biosynthesis is now well understood and the primary steps include the conversion of *S*-adenosyl-methionine to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and further oxidation of ACC to ethylene by ACC oxidase (ACO)^[10,38,51,52]. ACC acts as a direct precursor of ethylene biosynthesis and its concentration is closely related to the ethylene production^[38,51]. Therefore, ACS is the rate-limiting enzyme in ethylene biosynthesis^[27]. Ethylene biosynthesis in plants, especially in fruit, is a complicated process that involves the coordinated action of multiple ACS and ACO genes. In other words, fruit requires different ACS genes to form ethylene at distinct developmental stages. In tomato, nine ACS genes, *SlACS1a*, *SlACS1b* and *SlACS2-SlACS8*, have been reported^[53]. *SlACS1a* and *SlACS6* are specialized to system 1 ethylene biosynthesis in developing fruit, and *SlACS2* works in system 2 ethylene biosynthesis in ripening fruit, while *SlACS4*

operates in the transition period between system 1 and system 2^[48,49]. *MdACS6*, *MdACS3a* and *MdACS1* in apples are three important co-operative ACS genes regulating ethylene biosynthesis during fruit development, maturation and ripening^[27,54,55]. *MdACS6* is expressed in the early stages of fruit development before *MdACS3a* and *MdACS1* are expressed^[54], implying that the ethylene production in the early stages of fruit development is mainly due to catalysis through *MdACS6*. The transcription of *MdACS6* decreases around one month prior to maturation, when *MdACS3a* expression is initiated^[55,56]. This suggests that *MdACS3a* may take the place of *MdACS6* to meet the demand for ethylene in fruit just prior to maturation. Notably, overexpression of *MdACS6* induced *MdACS3a* expression indicates that *MdACS6* can regulate the expression of *MdACS3a*^[54]. At the onset of fruit ripening, *MdACS1* is expressed abundantly and is responsible for ripening-related ethylene biosynthesis (system 2)^[57,58]. Considering the gene structure, *MdACS1* contains an RLSF motif and a long C-terminal tail which is essential for calcium-dependent protein kinase and mitogen-activated protein kinase 6 phosphorylation, respectively but *MdACS6* and *MdACS3a* possess neither of these^[57,58]. This may be one of the reasons why *MdACS1* is expressed specifically during fruit ripening while *MdACS6* and *MdACS3a* are expressed during fruit development. The importance of *SlACS2* and *MdACS1* in fruit ripening was shown in transgenic tomato and apple in which *SlACS2* and *MdACS1* were silenced with RNA interference (RNAi)^[10,59,60]. The RNAi *SlACS2* fruit had an abnormal pattern of ripening, including reduced lycopene accumulation, delayed fruit softening and a significantly reduced climacteric peak. Silencing of *SlACS2*, in which *SlACS4* was also down-regulated, reduced ripening-related ethylene production to 0.1% of that in control fruit^[60]. Dandekar et al. silenced *MdACS1* in apple, and they observed that the transgenic fruit produced 94% less ethylene during respiration and were significantly firmer than controls, displaying increased shelf life^[59].

Many studies show that ACO also has an important role as another rate-limiting enzyme in ethylene biosynthesis pathway. For example, the expression of both *SlACO1* and *SlACO3* is apparently increased at the onset of fruit ripening in tomato^[61]. Silencing *SlACO* in tomato results in substantially reduced or undetectable ethylene production during fruit ripening^[62]. ACO genes have also been identified in several climacteric fruits such as apple, avocado (*Persea americana*), banana, and melon (*Cucumis melo*)^[63]. Silencing *MdACO1* in apple significantly inhibits ethylene production and fruit softening^[59]. Furthermore, the enzyme activity rise of ACO precedes ACS enzyme activity before fruit occurrence of climacteric respiration, indicating that ACO activity is important in controlling ethylene

production^[64]. These findings confirm that the normal functioning of ethylene biosynthesis gene is required for climacteric fruit ripening.

The ethylene signaling transduction pathway begins with ethylene binding to receptors^[65]. Ethylene receptors are closely related to fruit ripening. Three ethylene receptor families have been identified: ETR (ETHYLENE RESISTANT), ERS (ETHYLENE RESPONSE SENSOR) and EIN4 (ETHYLENE INSENSITIVE 4). In apple, nine ethylene receptors^[4] (MdETR1, MdETR1b, MdETR2, MdETR5, MdETR101, MdETR102, MdETR105, MdERS1 and MdERS2) have been identified^[66]. All these receptors are expressed in the fruit except *MdETR101*. The expression of *MdETR2*, *MdETR5*, *MdETR102*, *MdERS1* and *MdERS2* is strongly induced by exogenous ethylene during fruit ripening^[66]. In tomato, six ethylene receptors (*SlETR1*, 2, 4–6 and *NR*) have been isolated^[67–70]. It has been shown experimentally that the receptors act as negative regulators of the ethylene response. For example, downregulation of *SlETR4* and *SlETR6* resulted in earlier fruit ripening^[4].

Acting downstream of the receptors is CTR1 (CONSTITUTIVE TRIPLE RESPONSE 1)^[71], which functions as a negative regulator of ethylene responses^[72]. A *CTR1*-like gene *SICTR1* has been isolated from tomato and shown through complementation of a *ctr1 Arabidopsis* (*Arabidopsis thaliana*) mutant to function in ethylene signaling^[73]. The expression of *SICTR1* is upregulated by ethylene during fruit ripening^[73], and *SICTR1* transcript increased markedly at the onset of ripening^[74], but the function of *SICTR1* in fruit ripening is currently unclear.

In the absence of ethylene the receptors activate the kinase activity of CTR1 which suppresses downstream progression of signaling. In the perception of ethylene the receptors no longer activate CTR1, thus activating downstream positive responses such as EIN2 (ETHYLENE INSENSITIVE 2) and the EIN3/EIL (EIN3 like) family^[75]. EIN3/EIL is the core or primary transcription factor (TF) in the ethylene signal transduction pathway by binding to the PERE (primary ethylene response element) or ECBS (EIL conserved binding sequence) motif of promoters to regulate gene transcription. Kiwifruit (*Actinidia deliciosa*) AdEIL2/3 can bind to *AdACO1* promoter and upregulate *AdACO1* expression and, when overexpressed in *Arabidopsis*, stimulated ethylene production^[76]. In addition, EIN3/EIL proteins have also been identified in apple, banana, peach, pear and other fruits, but are limited to the detection of gene expression levels.

Downstream of EIN3/EIL, ERF (ethylene response factor) is the secondary TF to trigger an ethylene response. ERF is widely

distributed in plants^[77] and is divided into classes I, II and III based on amino acid sequences in which transcription activators generally exist in classes I and III and transcription suppressors generally exist in class II^[78]. Class II usually acts upstream of classes I and III^[79]. ERF can specifically bind to the GCC-box or DRE (dehydration-responsive element) motif of promoters of ethylene-responsive genes^[27,80]. ERF not only regulates downstream genes in response to ethylene signal but also regulates ethylene biosynthetic genes to regulate ethylene production in fruit^[28,54]. For example, in tomato *SlERF2* binds to the GCC box or DRE motif and activates the expression of ethylene biosynthesis genes^[81]. It has been shown that banana *MaERF9* associates with the promoter of *MaACS1* and promotes its activity, whereas *MaERF11* interacts with the promoters of both *MaACS1* and *MaACO1* and depresses their activity in banana^[82]. In apple, silencing of *MdERF2* leads to rapid fruit ripening while overexpression of *MdERF2* leads to delayed fruit ripening compared with controls, indicating that *MdERF2* functions as a negative regulator of apple ripening. The mechanism is that *MdERF2* can bind to the promoter of *MdACS1* and suppress its expression^[27]. In contrast, *MdERF3* can also bind to the promoter of *MdACS1* but enhances its expression, acting as a positive regulator of apple ripening. More interestingly, *MdERF2* functions upstream of *MdERF3* where *MdERF2* binds to the promoter of *MdERF3* and suppresses *MdERF3* transcription^[27]. The existence of ERF with negative regulation might act to balance the fruit ripening, which prevents the fruit from ripening too fast and is helpful in attracting animals to disperse seeds.

2.2 Auxins

Auxins are widely studied growth and development regulators in fruit^[83,84]. Increasing numbers of studies have shown that auxin also acts as a fruit ripening regulator. In general, the most abundant free auxin, indole-3-acetic acid (IAA), is described as the main regulator in fruit^[85]. Endogenous concentrations of IAA are particularly high at fruit set and during initial growth developmental stages, after which IAA concentrations tend to decline to low levels at the onset of ripening in climacteric fruits such as apple^[40], mango^[32] and tomato (Fig. 2)^[85,86], relating the increase in the conjugated form (IAA-Asp). In tomato, overexpressing a *Capsicum chinense* auxin-conjugating enzyme (GH3) leads to a decrease in free IAA and an increased sensitivity to ethylene at an earlier stage of development^[41]. There are 15 members of the *GH3* gene family, yet only *SIGH3-1* and *SIGH3-2* are upregulated at the onset of fruit ripening, indicating that these genes may contribute to fruit ripening^[87]. Therefore, the temporal regulation of ripening may be caused by the ratio between IAA and IAA-Asp rather than the absolute

concentration of IAA itself. However, a significant increase in IAA concentration is accompanied by climacteric ethylene production in the mesocarp tissues of peach^[88]. Further work using a genomic approach shows that the hormone auxin has a role in the regulation of ripening in climacteric peach. Also, many genes involved in auxin biosynthesis (indole-3-glycerol phosphate synthase and tryptophan synthase beta subunit), transport and, in particular, the signaling (receptors, i.e., TIR1, ARF and Aux/IAA) had increased expression in the mesocarp during ripening, thus supporting the idea that auxin functions independently in the ripening of peach^[33].

Signal transduction by auxin is well understood. In the absence of auxin, Aux/IAAs (auxin/indole-acetic acids proteins) interact with ARF (auxin response factor) and suppress their activity, which prevents downstream progression of signaling. When the auxin concentration is high the auxin is recognized by its receptor TIR1 (transport inhibitor response 1) which promotes the interaction between Aux/IAAs (auxin/indole-acetic acids proteins) and TIR1. Then Aux/IAAs are removed by ubiquitin-mediated action so that ARF are released to activate the expression of downstream genes^[89,90]. ARF is the key TF in the auxin signaling pathway. The expression of apple *MdARF5* is enhanced by synthetic auxin naphthaleneacetic acid (NAA) treatment. *MdARF5* is a TF that binds to the promoters of *MdACS3a*, *MdACS1* and *MdACO1*, and promotes their expression and ethylene production^[40].

Exogenous IAA treatment affects ripening in climacteric fruit. For example, a delay in ripening and softening of banana fruit upon treatment with IAA is due to inhibition of cell wall hydrolases polygalacturonase, pectin methyl esterase, pectate lyase and cellulase activities^[91]. In tomato, auxin treatment delays fruit ripening with delaying initiation of climacteric ethylene for about 3 d compared to a control, and also inhibiting ACO activity during the first two weeks after treatment during storage^[92]. In contrast, treatment with auxin or synthetic auxin promotes the ripening of pear (*Pyrus pyrifolia* cv. Whangkeumbae), apple and plum, and triggers the expression of ethylene associated genes as well as ethylene production^[35,40,93]. Auxin can stimulate the biosynthesis of system 2 ethylene by inducing the expression of the key rate-limiting enzyme ACS^[84,94]. Therefore, in such conditions, any effect of auxin on the ripening process would be indirect and mediated by ethylene.

Importantly, the effect of auxin on ethylene production can be in a dose-dependent manner in some species. For example, NAA treatment promoted ethylene production and ripening in peach and plum^[34,93]. However, auxin application reduced ethylene production and delayed ripening in tomato^[92]. Also, treatment

with 100 $\mu\text{mol}\cdot\text{L}^{-1}$ IAA delayed banana ripening^[91] whereas 57 $\mu\text{mol}\cdot\text{L}^{-1}$ IAA had the opposite effect^[95].

2.3 Abscisic acid

Abscisic acid (ABA) has long been considered to be mostly involved in the ripening process of non-climacteric fruit^[96,97] but in recent years, increasing numbers of studies have shown that ABA is also involved in regulating the ripening process of climacteric fruit^[98–100]. For example, 0.1 $\text{mmol}\cdot\text{L}^{-1}$ exogenous ABA application significantly induced ethylene production and fruit ripening by promoting the expression of ethylene biosynthetic genes *SIACS2* and *SIACO1* in tomato^[101]. Mango treated with 1.0 $\text{mmol}\cdot\text{L}^{-1}$ of ABA exhibited a climacteric peak of ethylene production that was 36% higher than in the controls whereas treatment with the ABA biosynthesis inhibitor NDGA (nordihydroguaiaretic acid) prevented ethylene biosynthesis and fruit ripening^[101]. In tomato, treatment with 0.1 $\text{mmol}\cdot\text{L}^{-1}$ NDGA gave a climacteric peak of ethylene production that was 43% lower than in the controls^[101]. During ripening of ABA-treated fruit the ACC concentration, and the ACS and ACO enzyme activities in pulp tissues increased by 55%, 58% and 26%, respectively, compared with the controls^[102].

The endogenous ABA concentration is low in green fruit but increases during climacteric fruit ripening (Fig. 2)^[26,103]. Remarkably, studies have shown that the maximum endogenous ABA precede the burst of ethylene in climacteric fruit such as banana, peach and tomato^[29,42,101]. Also, the ABA biosynthetic gene *NCED1* which encodes 9-cis-epoxycarotenoid dioxygenase 1 was highly expressed only at the breaker (the fruit color changes from green to yellow) stage when the ABA concentration becomes high^[101]. The *SIACS2*, *SIACS4*, and *SIACO1* genes were expressed with some delay after *SINCE1* was expressed^[101]. Many experimental data indicate that ABA treatment induced ethylene biosynthesis, but ACC treatment had no effect on ABA concentration in tomato flesh and seed^[101]. These results indicate that ABA may be the other regulatory factors upstream of ethylene in climacteric fruit ripening.

2.4 Gibberellins

Gibberellins (GAs) are a class of tetracyclic diterpenoid hormones that regulate a large range of developmental processes in higher plants such as seed germination, shoot elongation, flower initiation and fruit development^[43,104–107]. Recent studies on GAs have mainly focused on seed development, flowering, and fruit set and development because of the high concentrations of GAs found in flowers and immature fruits^[96,108]. Only a

limited number of several hundred plant GAs are bioactive in higher plants, such as GA₁, GA₃, GA₄ and GA₇. GA₁ and GA₄, which are highly abundant whereas GA₃ and GA₇ are less abundant^[107].

GAs accumulate in fruit during cell division and expansion during early development of fruit but decrease to a low concentration during fruit ripening (Fig. 2)^[107,108]. For example, concentrations of endogenous GAs decline significantly before the increase in ethylene production from mature-green (MG) to the breaker stage in tomato, yet the transcript levels of *SIACS2*, *SIACS4* and *SIACO1* undergo a significant increase^[107]. Also, overexpression of a fruit-specific *SIGA2ox1*, encoding a catabolic enzyme that converts the active forms of GA (GA₁ and GA₄) to inactive forms (GA₈ and GA₃₄), results in early-maturing phenotypes in tomato^[43]. Injecting the GA biosynthesis inhibitor prohexadione-Ca into MG tomato accelerated fruit ripening^[107]. Additionally, exogenous treatment of GA₃ can reduce ethylene production and depress ripening of various climacteric fruit such as banana, persimmon, mango and tomato^[31,44,95,107,109]. In GA₃-treated tomato, the transcriptional levels of *SIACS2*, *SIACS4* and *SIACO1* were depressed and *SIETR3*, *SIETR4* and *SIEN2* expression showed dramatic decline, indicating that GAs inhibit ethylene biosynthesis and perception during fruit ripening^[107]. These results demonstrate that GA is an inhibitor of fruit ripening. However, the detailed mechanism of signaling genes of GAs in ethylene biosynthetic genes remains unclear.

2.5 Brassinosteroids

Brassinosteroids (BRs) are important growth-promoting steroid hormones that promote cell expansion and division, regulate male fertility, pollen development, fruit ripening and senescence, and modulate plant adaptation to environmental stress throughout plant life cycles^[110]. BRs are actively produced during tomato fruit development^[111] and recent studies have shown that BRs might be another regulator of tomato fruit ripening^[112]. In tomato, the treatment of fruit with the most active BR, brassinolide, accelerated maturation increasing vitamin C and carotenoid concentrations, preventing accumulation of soluble solids, increasing respiratory activity and ethylene production, and decreasing chlorophyll concentrations. However, treatment of tomato with the BR synthetic inhibitor brassinazole inhibited the expression of ethylene synthesis related genes and ethylene production^[113]. In pear (*P. ussuriensis*), treatment of fruit with epibrassinolide (EBR), a brassinosteroid, significantly inhibited ethylene production and maintained fruit firmness compared with untreated control fruit during storage^[114].

The BR signaling pathway in plants is well-studied. Following biosynthesis, BR binds to receptor, BRASSINOSTEROID INSENSITIVE 1 (BRI1). BRI1 interacts with and transphosphorylates with BRI1-associated kinase 1, which allows BRI1 to phosphorylate BR SIGNALING KINASE 1 (BSK1). The phosphorylated BSK1 activates BRI SUPPRESSOR 1 which dephosphorylates and inhibits BRASSINOSTEROID INSENSITIVE 2, leading to unphosphorylated BRASSINAZOLE-RESISTANT 1 (BZR1) and its homolog, BRI1-EMSSUPPRESSOR 1 (BES1), moves to the nucleus^[45]. BZR1 and BES1 are important TFs downstream in the BR signal transduction pathway that bind to the promoters of BR-responsive genes and regulate their expression.

Notably, endogenous BR does affect fruit ripening. Overexpression of the BR biosynthetic gene *DWARF* in tomato leads to an increase in endogenous BR accompanied by an increase in ethylene production in fruit^[115]. Overexpression of *SIBRI1* in tomato promoted ethylene production and fruit ripening with upregulation of the expression of ethylene biosynthetic genes *SIACO1*, *SIACS2* and *SIACS4*, and caused an increase in carotenoids, ascorbic acid, soluble solids and soluble sugars during fruit ripening^[116]. These results suggest that BRs promote fruit ripening by activating the expression of ethylene biosynthetic genes and ethylene production. Evidence that BRs promote fruit ripening has also been obtained in other horticultural crops. A good example is that application of exogenous BRs facilitated the ripening of banana, possibly due to the accelerated expression of *MaACS1* and *MaACO13/14* promoting ethylene production^[30]. Examples of BRs suppressing fruit ripening have also been reported. Ji *et al.* reported that endogenous BRs such as typhasterol (TY), 6-deoxocastasterone (6-deoxoCS) and castasterone (CS) gradually decreased during pear (*P. ussuriensis*) fruit development (Fig. 2), and treatment with 0.2, 3 or 10 $\mu\text{mol}\cdot\text{L}^{-1}$ EBR significantly inhibited ethylene production and well-maintained fruit firmness compared with untreated control fruit^[114]. They further revealed that high concentrations of BRs activate the expression of TF *PuBZR1*, *PuBZR1* suppresses the enzyme activity of *PuACO1* by direct protein interaction in the cytoplasm, and the transcription of *PuACO1* and *PuACS1a* in the nucleus, thereby reducing ethylene production and suppressing fruit ripening. In jujube (*Zizyphus jujuba*), treatment with 5 $\mu\text{mol}\cdot\text{L}^{-1}$ exogenous BRs significantly reduced ethylene production during storage, but 10 $\mu\text{mol}\cdot\text{L}^{-1}$ exogenous BRs promoted ethylene production and fruit ripening^[117]. The positive or negative regulation of BRs on ethylene production may be a consequence of the dose applied. In *Arabidopsis*, low concentrations (10–100 $\text{nmol}\cdot\text{L}^{-1}$) of BRs inhibit ethylene biosynthesis by increasing the activity of BZR1/BES1 to depress the transcription of ACSs but high

concentrations ($> 500 \text{ nmol}\cdot\text{L}^{-1}$) of BRs promote ethylene biosynthesis by enhancing the enzyme stability of ACSs or through the auxin pathway^[118]. These findings suggest that the influence of BRs on ethylene biosynthesis and fruit ripening is different between species and varies in a dose-dependent manner.

2.6 Jasmonates

Most studies on jasmonates (JAs) in plants have focused on plant responses to biotic and abiotic stresses^[119,120] and has been shown that JA is also important in fruit ripening^[28,108,121]. Kondo et al.^[122] reported that JA concentrations were high in early fruit development of apple, decreased along with fruit growth, and then increased again immediately before maturation (Fig. 2). The application of methyl jasmonate (MeJA) to apple results in increased ethylene production and earlier fruit ripening^[28,46,123]. Lalel et al.^[124] reported that MeJA treatment promoted ethylene biosynthesis and ripening in mango fruit. MeJA treatment also significantly stimulated the ripening and ethylene biosynthesis of plum^[37]. MeJA treatment stimulated ethylene production in tomato at all stages of fruit ripening^[125].

JA signal transduction is well documented^[126–128], in which the TF MYC is considered to be the master regulator^[126]. Li et al.^[28] elucidated that the mechanism by which JA induces ethylene production in apple is dependent on MdMYC2. Their study shows that JA-activated MdMYC2 directly binds to the promoters of both *MdACS1* and *MdACO1* and enhances their transcription. Also, MdMYC2 binds to the promoter of *MdERF3* activating *MdACS1* transcription. In addition, MdMYC2 interacts with MdERF2 and prevents MdERF2 from suppressing both *MdERF3* and *MdACS1*^[28].

JAs not only regulate fruit ripening but also affect fruit color development. Application of JA can effectively enhance color development in apple, but it might limit fruit storage by promoting ethylene production^[28,129]. Liu et al. discovered that applying MeJA to apple (cv. Hanfu) three weeks before the commercial harvest enhanced fruit color development without affecting ethylene production or fruit firmness during storage^[130].

2.7 Cytokinins

Cytokinins (CKs) have crucial functions in various phases of plant growth and development^[131] but studies on the effects of CKs on fruit ripening have been limited mostly to climacteric fruit. Varga & Bruinsma^[132] found that ripening of tomato was progressively retarded at increasing doses of endogenous CKs

(Fig. 2). Davey et al. discovered that the fruit of a non-ripening mutant *rin* (*ripening inhibitor*) of tomato contained a higher concentration of endogenous cytokinin than the ripening fruit of a control cultivar (cv. Rutgers)^[47]. In the same year, another study reported that the endogenous CK activity was much higher in MG tomato fruit than in ripe fruit^[133]. These findings suggest that a high concentration or activity of CKs may inhibit fruit ripening. A recent study revealed that kiwifruit fruit treated with synthetic cytokinin N-(2-chloro-4-pyridyl)-N'-phenylurea suppressed ethylene biosynthesis and retained fruit softening, indicating that CKs induce tissue-dependent disturbances of proteins in climacteric ripening^[134].

2.8 Hormone crosstalk

Fruit ripening is a complex process involving subtle changes in physiological and metabolic traits and is coordinated by multiple hormones. However, ethylene and ABA appear to provide the predominant regulation of fruit ripening^[8,135] and ethylene is specifically required for ripening of climacteric fruits^[38]. The other plant hormones primarily act through minor adjustments to ethylene action during fruit ripening. For example, exogenous treatment with ABA promoted ethylene biosynthesis by inducing the transcription of ethylene biosynthetic genes, while the ABA biosynthetic inhibitor fluridone depressed ethylene biosynthesis in tomato fruit^[101,136]. In addition, induction of an auxin-related *GH3* gene by ABA, ethylene and several other phytohormones during tomato ripening indicates that, along with ethylene, auxin can crosstalk with ABA and other hormones^[87]. Compared to ABA the crosstalk between IAA and ethylene during ripening seems to be more complex and interesting. First, in peach (climacteric fruit) it has been shown that, concomitant with ethylene production, an increase in the amount of auxin can also be measured^[33], whereas ethylene promotes IAA reduction through PuERF-activated *PuGH3.1* during ripening of pear (*P. ussuriensis*)^[36]. Second, genes for ethylene biosynthesis (ACSs and ACOs) and the signal pathway (e.g., *ETRs* and *ERFs*) are induced by auxin in apple, peach and tomato^[33,40,137]. However, application of IAA reduced ethylene production and delayed ripening in banana and tomato^[91,92]. Also, components of IAA biosynthesis and signaling can be upregulated by ethylene during ripening^[36,91,137]. These results indicate that fruit ripening is impacted by coordination of multiple hormones.

It is well known that polyamines (PAs) can function as cellular signals in the complex crosstalk between hormonal pathways, including ethylene, ABA and auxin^[138,139]. There are antagonistic effects between ethylene and PAs during postharvest fruit ripening. PAs along with salicylic acid (SA) repress the

expression of the ACS gene and ethylene production in tomato^[140]. Also, PA application induces nitric oxide production in olive fruit^[138,141]. Importantly, nitric oxide has now been shown to interfere with ethylene effects to directly and significantly influence fruit ripening^[6,142]. Nitric oxide has been shown to interact with SA and JA to affect postharvest attributes in some climacteric fruits but the underlying mechanism remains to be elucidated^[143]. Also, JA is another well-studied hormone which promotes ethylene production and ripening of climacteric fruits such as apple^[28,46,123], mango^[124], plum^[37] and tomato^[125], and endogenous JA-Me increases during fruit ripening in apple, mango, pear and tomato^[144]. In addition to these hormones, GAs have been found to repress ethylene biosynthesis in many fruits including mango^[145], peach^[146] and tomato^[107,109]. However, limited information is available on the effect of ethylene on JA or GA biosynthesis, or on signaling pathways during fruit ripening.

Current information indicates that ethylene may be the destination of hormones crosstalk during climacteric fruit ripening. Of course, ethylene signaling in climacteric fruit ripening is tightly coordinated under the influence of multiple phytohormones. Given the complexity of fruit ripening processes, the task of exploring the molecular basis of its regulation by hormones is made more difficult by crosstalk between hormones.

3 TRANSCRIPTION FACTORS

A number of ripening regulator genes control fruit ripening in harmony with ethylene. It is obvious that the presence of an

intricate regulatory network underlies the process. In this section we summarize several TFs that have also been shown to regulate fruit ripening (Fig. 3).

3.1 MADS-box

MADS-box is the most studied class of TFs involved in regulating fruit ripening. The *rin* mutant has enlarged sepals and inhibited fruit ripening which have been attributed to the functions of two MADS-box TFs, SIMADS-MC and SIMADS-RIN^[147]. Therefore, RIN has long been considered to function as a major regulator essential for the induction of fruit ripening. However, a CRISPR/Cas9-mediated *RIN*-knockout mutation of tomato did not have inhibited fruit ripening but rather a moderate red phenotype. Thus, the *rin* mutation was originally thought to be a null mutation^[147] but is now known to be a gain-of-function mutation that generates a protein, RIN-MC, that represses fruit ripening^[148,149]. RIN is still critical for progression of ripening but is not required for the initiation of ripening^[148–150]. The factor operating for ripening initiation in the absence of RIN has not been proven. SIMADS-RIN binds to the *cis*-element of *SIACS2* promoter, indicating that SIMADS-RIN is located upstream of ethylene signal transduction and regulates ethylene biosynthesis^[151]. However, SIMADS-RIN regulates fruit ripening not only by ethylene signaling genes but also by other downstream genes. It has been shown by proteomics and chromatin immunoprecipitation (ChIP) that SIMADS-RIN can target many ripening-related genes: (1) ethylene biosynthesis and perception genes including *SIACS2*, *SIACS4*, *NR* and *E8*; (2) cell-wall-modifying genes including *Polygalacturonase*, *β -Galactosidase 4* and *α -Expansin 1*; (3) carotenoid metabolism genes *PHYTOENESYNTHASE1*;

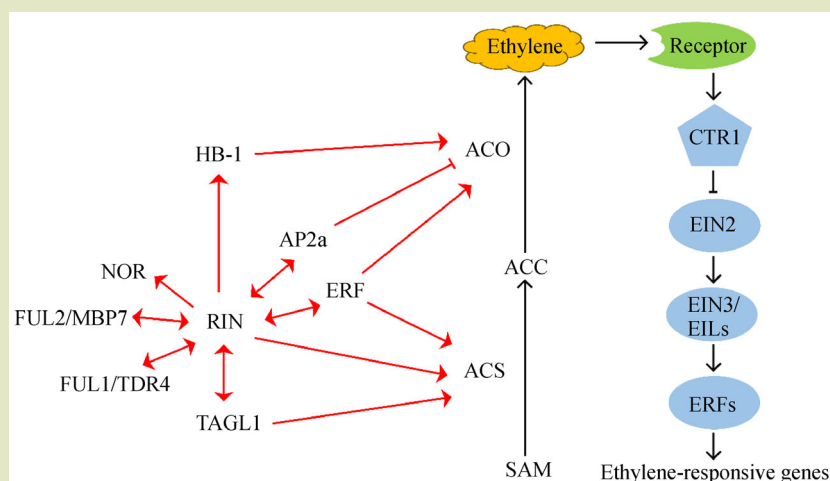


Fig. 3 Transcription factors involved in regulating fruit ripening^[3,62,65,76]. →, positive regulation; ⊥, negative regulation.

(4) aroma volatile metabolism genes *tomato lipoxygenase*, *alcohol dehydrogenase 2* and *hydroperoxidelyase* and (5) glycolysis-related gene *phosphoglycerate kinase*^[152–154]. In addition, RIN can affect the protein ubiquitin in nuclei by binding directly to the promoter of E2 ubiquitin-binding enzyme genes *SIUBC32* and *PSMB2A* and alter their expression during fruit ripening^[24]. Also, there is a close correlation between miRNA expression and RIN as well as ethylene. RIN directly represses *MIR172a* transcription by binding to its promoter regions and affects the accumulation of miR172 to promote ethylene production in tomato^[155].

Other MADS-box TFs involved in fruit ripening include TOMATO AGAMOUS-LIKE 1 (*TAGL1*)^[156,157], *FUL1/TDR4* and *FUL2/MBP7*^[158,159], *MADS1*^[160] and *FYFL*^[161]. These MADS-box proteins generally operate as coregulators by interacting with RIN to control fruit ripening. Vrebalov et al. reported that *TAGL1* is expressed in floral organs, young fruit and ripe tomatoes, and silencing of *TAGL1* resulted in inhibited ripening. *TAGL1* knockdown decreased the transcript level of RIN-targeted *SIACS2*, indicating that *TAGL1* may work with RIN to influence ripening-related ethylene production^[156,157]. Also, overexpression of *SITAGL1* leads to earlier fruit ripening and higher lycopene concentrations^[157,162].

In contrast to the previously identified MADS-box protein *TAGL1*, two homologues of *FRUITFULL* (*FUL1/TDR4* and *FUL2/MPB7*) do not regulate ethylene production but control ripening in an ethylene-independent manner^[158]. Silencing *FUL* genes resulted in orange-ripe fruit and significantly reduced lycopene compared with the wild type fruit^[163]. Silencing tomato *SIFUL1/2* did not significantly affect the expression of ethylene biosynthesis genes *SIACS* or *SIACO* and the ethylene production was similar to the wild type. The expression of ethylene response genes *SIEIL* were not significantly altered in *SIFUL1/2* silenced lines. Thus, ethylene perception appears normal in the *SIFUL1/2*-silenced fruit but the mutant orange-ripe phenotype cannot be rescued by ethylene application^[158]. In addition, Tomato *SIFUL1* and *SIFUL2* seem to have broader functions in ripening since recent data reveal that *FUL1/2*, RIN and *TAGL1* form a protein complex *in vitro*, indicating that a tetramer in the MADS-box proteins could be mainly responsible for the regulation of ripening^[159]. However, most of the *FUL1/2* targets appear not to be RIN because microarray analysis (ChIP-chip) and transcriptome analysis indicate that *FUL* is responsible for regulating the carotenoid pathway, but RIN regulates the lycopene pathway^[159].

Another tomato MADS-box domain protein interacting with RIN is *MADS1* which belongs to the *SEPALLATA* gene subfamily^[160,164]. Notably, *MADS1* acts as a negative regulator

of ethylene biosynthesis and signaling^[160]. The tomato *SIMADS1* transcripts accumulate mainly in mature-green fruit, with decreasing expression during fruit ripening^[160]. *SIMADS1*-silenced fruit showed both elevated ethylene production and also elevated gene expression of ethylene and ripening^[160]. A novel tomato MADS-box TF *SIFYFL*, an ortholog of *Arabidopsis* *FYF* (*FOREVER YOUNG FLOWER*), *AtAGL42*, acts as a repressor of fruit ripening. Overexpression of *SIFYFL* reduced carotenoid accumulation by 30%–40% and ethylene production by 50%^[161].

3.2 AP2/ERF

The ERF (ethylene response factor) proteins belong to the large AP2/ERF protein family and associate with GCC-box (GCCGCC) or DRE (CCGAC) motif of target promoters to regulate gene expression^[27,165,166]. ERF is an important TF in the ethylene signaling pathway and governs the transcriptional level of ethylene-responsive genes^[27,167]. For example, tomato ERF proteins *SIERF2/TERF2* have been shown to upregulate the expression of ethylene biosynthetic genes with promoters that include a GCC box or DRE motif in tomato and ethylene treatment no longer induces ethylene production in antisense *SIERF2/TERF2* transgenic tomato, indicating that *SIERF2/TERF2* is a positive regulator in the feedback loop of ethylene stimulation^[81]. Although some ERF TFs are inhibitors of fruit ripening^[168,169], for example downregulation of *SIAP2a* in tomato resulted in overproduction of ethylene and early ripening^[168,170]. Also, ripening regulators RIN, NOR (No-ripening) and CNR (Colorless non-ripening) function upstream of *SIAP2a* and trigger its expression^[170]. Importantly, mRNA levels of *CNR* were elevated in the pericarp of *SIAP2*-RNAi fruits, indicating that both *SIAP2* and *CNR* are part of the negative feedback loop in the regulation of tomato ripening, a process that needs to be studied further^[170]. Recently, tomato *SIERF6* was identified as a novel ripening inhibitor, which inhibited carotenoid biosynthesis and additional ripening phenotypes by decreasing trans-lycopene and b-carotene accumulation^[169]. Silencing of *SIERF6* enhanced carotenoid concentration and ethylene production during fruit ripening^[169].

3.3 NAC

NAC proteins (comprising NAM, ATAF and CUC members) constitute one of the largest families of plant specific TFs^[171]. Typically, NAC proteins contain a highly conserved N-terminus containing the DNA-binding domain which is responsible for the oligomerization into dimeric proteins and a variable C-terminal domain which is considered to be a transcription regulatory region^[172]. The tomato genome contains 101 NAC

TFs known from The Plant Transcription Factor Database^[173], which have been shown to be involved in diverse processes including development^[174], fruit ripening^[175] and senescence^[176]. NAC TFs associate with the core CGT[G/A] or GCTT NACRS (NAC recognition sequence) or NACBS (NAC binding sequence) of target promoters to regulate gene expression^[177]. NOR, a member of the NAC domain family, was shown to act upstream of ethylene in regulating the ripening of tomato^[121]. The *nor* tomato mutant fails to produce autocatalytic ethylene and ripen in response to exogenous ethylene. Although the ripening-related gene expression in *nor* mutant has been analyzed, its underlying molecular mechanism remains unclear^[178]. Osorio et al. reported that, based on analysis of transcriptome and proteome data, NOR may have more important effects on ethylene and ripening-related genes than RIN, and may even act upstream of RIN in the regulatory network of tomato fruit ripening^[179]. Two other fruit ripening-related NAC TFs *SINAC1*^[180] and *SINAC4*^[175] have been identified in tomato. The ripening time of antisense *SINAC1* transgenic tomato was delayed by 3–4 d compared to the wild type^[181]. Silencing *SINAC1* promoted ethylene biosynthesis mainly by interacting with the regulatory regions of system 2 ethylene biosynthetic genes and upregulating their expression, thus leading to deeper red fruits^[180,181]. In addition, overexpressing *SINAC1* reduced the thickness of the pericarp and produced more ABA, which caused the fruit to soften early^[180], indicating that *SINAC1* affected fruit ripening through ethylene-dependent and ABA-dependent pathways. In contrast, *SINAC4*, which is highly expressed in sepals and at the onset of tomato fruit ripening, functions as a positive regulator of fruit ripening. Silencing *SINAC4* resulted in delayed fruit ripening by about 2–3 d, decreased ethylene synthesis, suppressed chlorophyll degradation, and reduced carotenoids, compared with the wild type^[175]. The transcript levels of *SIACS2*, *SIACS4*, *SIACO1* and *SIACO3* were substantially reduced by silencing *SINAC4* in fruit at the breaker and/or breaker + 4 (4 days after breaker) stages, and the transcript levels of *SIERF1* were reduced by approximately 45% repressed at the MG stage, indicating *SIACS4* is a positive regulator of fruit ripening^[175]. In addition to NOR, *SINAC1* and *SINAC4*, Gao et al. established the role of a new tomato NAC TF NOR-like1 (Solyco07g063420) as a new positive regulator of tomato fruit ripening and defined important NOR-like1 targets^[182]. Silencing *NOR-like1* by CRISPR/Cas9 delayed tomato ripening initiation by 14 d and clearly reduced ethylene production, retarded softening and chlorophyll loss, and reduced lycopene accumulation in fruit. This also confirms that NOR-like1 directly binds to the promoters of genes related to ethylene biosynthesis (*SIACS2* and *SIACS4*), color formation (*SIGgpps2* and *SISGR1*) and cell wall metabolism (*SIPG2a*, *SIPL*, *SICEL2* and *SIEXPI*), and activates their expression^[182].

3.4 Other transcription factors

SIHB-1, encoding a class-I homeodomain leucine zipper protein, is involved in tomato fruit ripening through transcriptional regulation of *SIACO1* expression. Virus-induced silencing of *SIHB-1* greatly reduced *SIACO1* mRNA levels and inhibited fruit ripening^[183]. In peach and tomato a specific orthologous *bZIP* gene has been found to be specifically and highly expressed at the onset of fruit ripening and declined to a much lower concentration when ripening was established^[184]. Overexpressing *bZIP* in tomato resulted in delayed fruit ripening and significantly enhanced metabolism associated with ripening, indicating that such TF might regulate ripening by acting as a pacemaker for some of the ripening metabolic pathways^[184].

The MYB superfamily, one of the largest TF families in plants, is characterized by a conserved DNA-binding domain, the MYB domain^[185]. It has been extensively shown that MYB TFs regulate pigment/anthocyanin production in fruits^[186,187]. Recently, Fan et al. reported that MaMYB3 negatively regulates starch degradation by directly binding to the promoters of starch degradation-related genes *MaGWD1* (glucan water dikinase), *MaSEX4*, *MaBAM7*, *MaBAM8*, *MaAMY2B*, *MaAMY3*, *MaAMY3A*, *MaAMY3C*, *MaMEX1* and *MapGlcT2-1*, and repressing their expression, thereby delaying banana ripening^[188].

4 EPIGENETIC MODIFICATIONS

Epigenetic modifications such as DNA methylation and histone modification regulate gene expression by affecting DNA-binding capacity and transcriptional regulatory activity of TFs or, in contrast, through being regulated by TFs that recruit chromatin remodeling proteins^[189]. Differential epigenetic modifications have been shown to be crucial in plant developmental processes such as vernalization, seed development, flowering and gametogenesis^[190,191]. In recent years research has increasingly demonstrated that epigenetic regulation is important in fruit ripening.

DNA methylation is a conserved epigenetic mark which is generally associated with inactive transcription and is important for genome integrity, gene imprinting, development and environmental responses in mammals and plants^[192,193]. Earlier research demonstrates that genome-wide or gene-specific DNA methylation markedly decreases during fruit ripening^[20,194,195] and the DNA methylation directly hinders the binding of TFs to their downstream *cis*-elements^[196]. For example, *Cnr* is a ripening-inhibited mutant of tomato which is caused by hypermethylation of an upstream region of the *CNR*. The *Cnr* phenotype results from the methylation of several normally

unmethylated cytosines in the SBP-box (SQUAMOSA promoter binding protein-like) promoter^[20].

Furthermore, it has been shown that inducing DNA cytosine demethylation of the whole genome leads to premature ripening, and differential methylation sites of ripening-related genes occur near RIN-binding sites^[194]. Several studies agree that the level of DNA methylation can be a key factor affecting gene transcription levels during ripening of fruit^[20,194,195]. More recently it has been shown that the DNA methylation of *MdACS3a* promoter gradually declines as fruit approaches ripening in apple^[40].

DNA methylation levels are dynamically controlled by DNA methylation and demethylation reactions. Active DNA demethylation in plants is triggered by a positive family of 5-methylcytosine DNA glycosylases/lyases (DNA demethylases). Recent reports suggest that tomato undergoes active DNA demethylation during ripening^[20,194,195]. Treatment of tomato fruit with 5-azacytidine (a DNA methylation inhibitor) promoted ripening whereas silencing of *SIDML2* encoding a DNA demethylase resulted in inhibited fruit ripening^[194,197]. Tomato contains four putative DNA demethylases (*SIDML1-4*) according to sequence homology with the *Arabidopsis* DNA demethylase genes. *SIDML2*, the most abundant DNA demethylase in fruits, is critical in tomato fruit ripening^[197,198]. In the fruit of CRISPR/Cas9-mediated *SIDML2*-knockout mutation, DNA methylation level was increased in thousands of genes which included both numbers of ripening-induced genes such as RIN and genes involved in ethylene and pigment synthesis and cell wall hydrolysis, and also many ripening-repressed genes^[198]. These results indicate that active DNA demethylation is responsible for both the activation of ripening-induced genes and the inhibition of ripening-repressed genes during ripening.

RNA methylation (m⁶A), is a type of epigenetic modification and has been shown to regulate fruit ripening in tomato. It is proposed that DNA methylation affects m⁶A methylation by targeting RNA demethylase gene *SLALKBH2*, which in turn influences DNA methylation via DNA demethylase gene *SIDML2* by a feedback loop to regulate fruit ripening^[199]. These results identify the interplay between DNA and RNA methylation and reveal a novel layer of gene regulation in fruit ripening.

Histone methylation, which mostly occurs at specific lysine and arginine residues located on the N-terminal end of the core histones, is critical in the regulation of chromatin configuration and gene expression. Generally, the methylation of K4, K36 and K79 on histone H3 is associated with activating chromatin and positively regulating gene expression, whereas methylation of K9

and K27 on histone H3 is related to repression of gene expression. Histone lysine residues can be monomethylated, dimethylated or trimethylated, and are regulated by histone lysine methyltransferases and demethylases^[200]. Recently it has been shown that histone H3 K27 trimethylation (H3K27me3) has a conserved function in regulating the pivotal ripening-related genes and their homologs in ethylene-dependent fruit ripening^[201], indicating that H3K27me3 may regulate ripening as a new epigenetic mark. From this point of view, tomato Like Heterochromatin Protein 1b was demonstrated to regulate ripening-related genes and repress fruit color development during ripening with epigenetic mark H3K27me3^[202]. Twenty-five histone demethylases were identified in the tomato genome, of which SIJM6, a member of the plant-specific KDM5/JAR2DI sub-family of JmjC domain-containing proteins, is an H3K27 demethylase with apparent demethylation activity for trimethyl at H3K27^[203]. It has been reported that SIJM6 removes H3K27me3 of a number of ripening-related genes and up-regulates the expression of these genes to promote fruit ripening in tomato. Notably, SIJM6 promotes fruit ripening also via directly activating the expression of *SIDML2*^[203], suggesting that histone demethylation may affect DNA demethylation via an unknown mechanism.

Emerging evidence has shown that histone acetylation influences several aspects of development in different plant species^[204]. Also, the involvement of histone acetylation in regulating fruit ripening and senescence has been indicated^[2]. Chromatin remodeling and transcription activation are associated with the level of histone acetylation which is controlled by histone acetyltransferases and histone deacetylases (HDACs)^[205]. In general, histone acetylation and deacetylation are related to the transcription activation and repression, respectively. Fourteen HDACs have been identified in tomato, three of which belong to the histone deacetylase 2 (HD2) subfamily, two of which are members of the Silent Information Regulator Protein 2 subfamily^[206], and nine of which are part of the reduced potassium dependency protein 3 (RPD3)/HDA1 subfamily^[207]. In tomato, silencing of RPD3/HDA1-type histone deacetylase genes *SIHDA1* and *SIHDA3* results in promoted ethylene biosynthesis and fruit ripening by upregulating the transcript levels of ethylene biosynthetic genes (*SIACO1*, *SIACO3*, *SIACS2* and *SIACS4*) and ethylene response factor *SIERF1*^[23,208]. However, silencing of the HD2-type histone deacetylase gene *SIHDT3* represses ethylene production and fruit ripening and prolongs the shelf life of the fruit with down-regulation of the expression of *SIACO1*, *SIACO3*, *SIACS2*, *SIACS4* and *SIERF1* from breaker to breaker + 7 (7 days after breaker stage) stages^[209]. *SIPSY1* (phytoene synthase 1) was down-regulated and *SICYC-B* (chromoplast-specific lycopene- β -cyclase),

SILCY-B (chloroplast-specific lycopene- β -cyclase-B) and *SILCY-E* (chloroplast-specific lycopene- β -cyclase-E) were upregulated in *SIHDT3* silenced fruit. Total carotenoids decreased by 30% in *SIHDT3* silenced fruit compared with wild type fruits^[209]. Emerging evidence indicates that HDACs are recruited by various TFs in repression of gene expression^[205]. For example, JAZ proteins recruit HDA6 to repress *EIN3/EIL1*-dependent transcription and inhibit jasmonate signaling^[210]. TF WOX5 recruit HDA19 at the *CDF4* promoter region to repress the differentiation factor *CDF4* in regulation of stem cell maintenance^[211]. Also, MaERF11 recruit histone deacetylase MaHDA1 to repress the expression of ripening-related genes such as *MaACO1* during banana ripening^[212]. These findings indicate that recruitment of HDAC-related proteins is a common mechanism in the repression of gene expression. Thus, future studies will focus on the identification of more histone modification factors to unravel the regulator of fruit ripening.

5 CONCLUSIONS AND PERSPECTIVES

Knowledge about the phytohormones regulating climacteric fruit ripening is becoming substantial. Numerous key genes associated with these hormones have been identified and many of them may be used to improve postharvest quality and food security through genetic engineering. The release of the whole genome sequence of numerous fleshy fruit species including apple^[213], banana^[214], melon^[215], papaya (*Carica papaya*)^[216], pear^[217] and tomato^[218] has now provides powerful tools to

study the underlying mechanisms governing climacteric fruit ripening. Although many studies have documented the influence of phytohormones on ethylene production and fruit ripening, most have focused only on the changes in gene expression and have not investigated how these hormone signaling genes, especially TFs, regulate ethylene biosynthesis and signaling genes.

Fruit ripening has been demonstrated to be controlled by a number of TFs in conjunction with ethylene but little is known about their direct ethylene-responsive target genes. With the development of *in vivo* ChIP combined with high-throughput sequencing, the identification of these TFs targets will no doubt soon be revealed. RIN has long been considered an indispensable factor for ripening initiation; recent studies have revealed that RIN is critical for progression of ripening but is not required for ripening initiation. However, the factors actually responsible for ripening initiation remain unknown. It will be a difficult, but worthwhile, challenge to identify the precise regulatory factors that are required for ripening initiation.

Although recent studies have shown that epigenetic modifications are important in fruit ripening^[20,40], knowledge of mechanisms regarding how they regulate ripening-related genes, especially TFs, and how they crosstalk with phytohormone biosynthesis and signal transduction to regulate fruit ripening remains limited. More work is required to elucidate these mechanisms and this is becoming a major focus of research on fruit ripening.

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Compliance with ethics guidelines

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