Chapter 16
Mechanism of Fruit Ripening

M. Bouzayen, A. Latché, P. Nath, and J.C. Pech

16.1 Introduction: Fruit Ripening as a Developmentally Regulated Process

The making of a fruit is a developmental process unique to plants. It requires a complex network of interacting genes and signaling pathways. In fleshy fruit, it involves three distinct stages, namely, fruit set, fruit development, and fruit ripening. Of these, ripening has received most attention from geneticists and breeders, as this important process activates a whole set of biochemical pathways that make the fruit attractive, desirable, and edible for consumers. In recent years, the scientific goal has been to reveal the mechanisms by which nutritional and sensory qualities are developed during fruit development and ripening using advanced genomics and post-genomics tools. These genome-wide technologies have been combined to physiological approaches to decipher the networks of interactions between the different pathways leading to the buildup of fruit quality traits. From a scientific point of view, fruit ripening is seen as a process in which the biochemistry and physiology of the organ are developmentally altered to influence appearance, texture, flavor, and aroma (Giovanonni 2001, 2004). For the consumers and distributors, the process of ripening corresponds to those modifications that allow fruit to become edible and attractive for consumption. Since the majority of the quality attributes are elaborated during the ripening process, it has always been considered

M. Bouzayen
Génomique et Biotechnologie des Fruits, INRA, Chemin de Borde Rouge, F-31326, Castanet-Tolosan, France
e-mail: bouzayen@ensat.fr

A. Latché and J.C. Pech
Génomique et Biotechnologie des Fruits, Université de Toulouse, INP-ENSA Toulouse, Avenue de l'Agrobiopole, BP 32607, F-31326 Castanet-Tolosan, France

P. Nath
Plant Gene Expression Laboratory, National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India

essential to better understand the mechanisms underlying this ultimate fruit developmental stage.

The fruit ripening process has been viewed over the last decades as being successively of physiological, biochemical, and molecular nature. Fruit ripening is accompanied by a number of biochemical events, including changes in color, sugar, acidity, texture, and aroma volatiles that are crucial for the sensory quality (Fig. 16.1). At the late stages of ripening, some senescence-related physiological changes occur that lead to membrane deterioration and cell death. In that regard, fruit ripening can thus be considered as the first step of a programmed cell death process. All biochemical and physiological changes that take place during fruit ripening are driven by the coordinated expression of fruit ripening-related genes. These genes encode enzymes that participate directly in biochemical and physiological changes. They also encode regulatory proteins that participate in the signaling pathways, and in the transcriptional machinery that regulate gene expression and set in motion the ripening developmental program (Fig. 16.1).

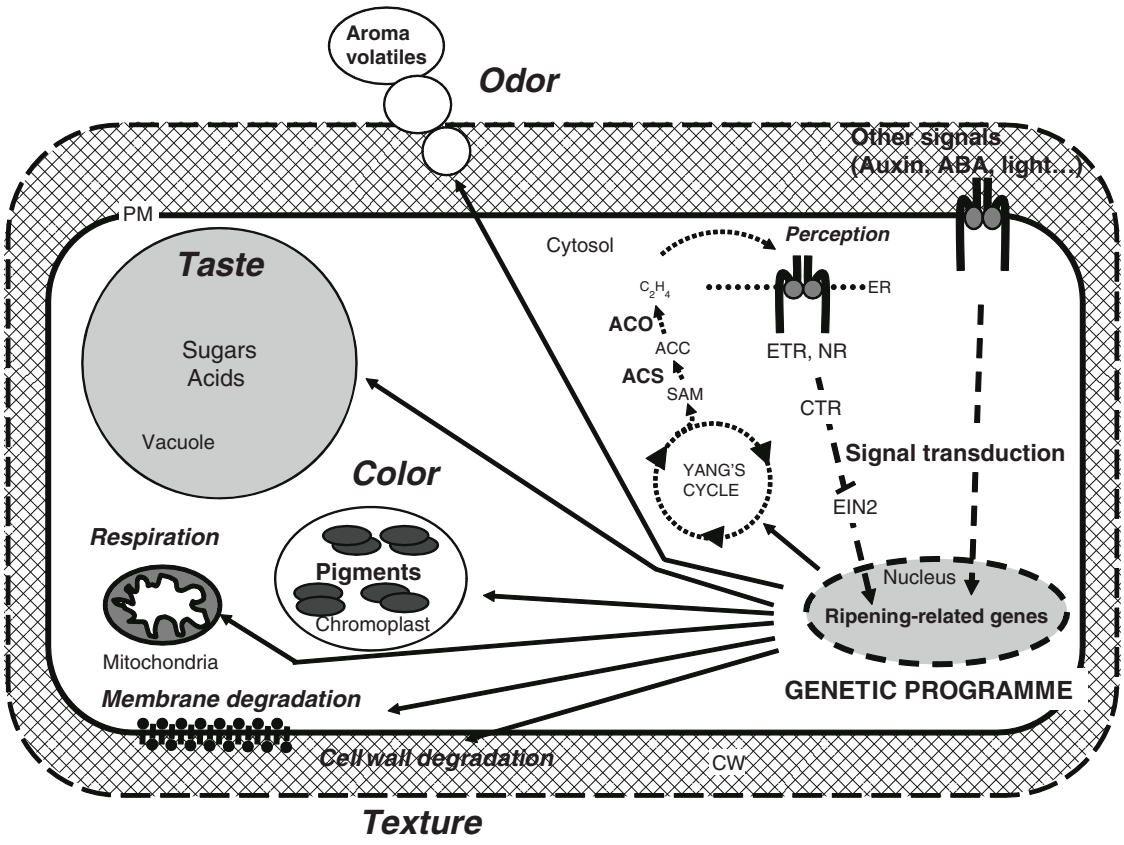

Fig. 16.1 Schematic representation depicting the molecular mechanisms controlling the ripening of climacteric fruit. The fruit ripening process is a genetically regulated developmental process involving the activation of a high number of primary and secondary metabolic pathways that all contribute to the overall sensory and nutritional quality of the fruit. This process involves the expression of ripening-related genes that encode enzymes (proteins) involved in the various ripening pathways (e.g., softening, color development). The whole process is under the control of hormonal and environmental signals, amongst which ethylene plays a major role

16.2 Climacteric and Non-Climacteric Fruit Ripening

Fruit can be divided into two groups according to the regulatory mechanisms underlying the ripening process. Climacteric fruit, such as tomato, apple, pear, and melon (Table 16.1), are characterized by a ripening-associated increase in respiration and in ethylene production. By contrast, non-climacteric fruits, such as orange, grape, and pineapple (Table 16.1), are characterized by the lack of ethylene-associated respiratory peak. At the onset of ripening, climacteric fruit present a peak in respiration, and a concomitant burst of ethylene production. The relationship existing between the climacteric respiration and fruit ripening has been questioned following the discovery that ripening on the vine of a number of fruit may occur in the absence of any increase in respiration (Salveit 1993; Shellie and Salveit 1993). More recently, it has been reported that the presence or absence of a respiratory climacteric on the vine depends upon prevailing environmental conditions (Bower et al. 2002). These observations indicate that the respiratory climacteric is probably not an absolute trigger of the ripening process, but secondary and consequential to the process of ripening. An ethylene burst that precedes respiratory climacteric has been shown during the ripening of banana (Pathak et al. 2003).

Table 16.1 A list of representative climacteric and non-climacteric fruit. A more extensive list is provided by Watkins (2002)

Climacteric fruits	Non-climacteric fruits
Apple (*Malus domestica* Borkh.)	Asian pear (*Pyrus serotina* Rehder)
Apricot (*Prunus armeniaca* L.)	Cactus pear (*Opuntia amyclaea* Tenore)
Avocado (*Persea americana* Mill.)	Carambola (*Averrhoa carambola* L.)
Banana (*Musa sapientum* L.)	Cashew (*Anacardium occidentale* L.)
Cherimoya (*Annona cherimola* Mill.)	Cherry (*Prunus avium* L.)
Corossol (*Annona muricata* L.)	Cucumber (*Cucumis sativus* L.)
Durian (*Durio zibethinus* Murr.)	Grape (*Vitis vinifera* L.)
Feijoa (*Feijoa sellowiana* Berg.)	Grapefruit (*Citrus grandis* Osbech)
Fig (*Ficus carica* L.)	Lime (*Citrus aurantifolia* Swingle)
Guava (*Psidium guajava* L.)	Limon (*Citrus limonia* Burm.)
Kiwifruit (*Actinidia sinensis* Planch.)	Litchee (*Litchi sinensis* Sonn.)
Mango (*Mangifera indica* L.)	Mandarin (*Citrus reticulata* Blanco)
Melon Cantaloup and Honeydew (*Cucumis melo* L.)	Mangoustan (*Garcinia mangostana* L.)
Papaya (*Carica papaya* L.)	Olive (*Olea europaea* L.)
Passion fruit (*Passiflora edulis* Sims.)	Orange (*Citrus sinensis* Osbeck)
Peach (*Prunus persica* Batsch)	Pepper (*Capsicum annuum* L.)
Pear (*Pyrus communis* L.)	Pineapple (*Ananas comosus* Merr.)
Persimmon (*Diospyros kaki* Thunb.)	Pomegranate (*Punica granatum* L.)
Physalis (*Physalis peruviana* L.)	Rambutan (*Nephelium lappaceum* L.)
Plum (*Prunus domestica* L.)	Raspberry (*Rubus idaeus* L.)
Sapota (*Manilkara achras* Fosb.)	Strawberry (*Fragaria* sp.)
Tomato (*Solanum lycopersicum L.*)	Tamarillo (*Cyphomandra betacea* Sendtu)
	Watermelon (*Citrullus lanatus* Mansf.)

16.2.1 Ethylene Production, and Its Role in Climacteric and Non-Climacteric Fruit

Two distinct ethylene biosynthesis systems have been described. System 1 corresponds to low ethylene production in the pre-climacteric period of climacteric fruit, and is present throughout the development of non-climacteric fruit. System 2 refers to an auto-stimulated massive ethylene production called "autocatalytic synthesis", and is specific to climacteric fruit. Therefore, the major ethylene-related differences between climacteric and non-climacteric fruit is the presence or absence of autocatalytic ethylene production (McMurchie et al. 1972; Alexander and Grierson 2002). The ethylene biosynthetic pathway is now well established (Fig. 16.1; Yang and Hoffmann 1984). This ripening hormone is synthesized from methionine via *S*-adenosyl-L-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). Two major enzymes are involved in the biosynthetic pathway, namely, ACC synthase (ACS), which converts SAM into ACC, and ACC oxidase (ACO), which converts ACC into ethylene. The corresponding genes have been identified and characterized (Sato and Theologis 1989; Hamilton et al. 1990, 1991). Both ACO and ACS are encoded by a multigene family of five and nine members, respectively in tomato, with expressions differentially regulated during fruit development and ripening (Barry et al. 1996, 2000). While *LeACO1* and *LeACO4* genes are up-regulated at the onset of ripening, and continue being active throughout ripening, *LeACO3* displays only transient activation at the breaker stage of fruit ripening (Fig. 16.2). It was shown that *Le ACS6* and *LeACS1A* are expressed at the pre-climacteric stage (system 1), while at the transition to ripening, *LeACS4* and *LeACS1A* are the most active genes (Fig. 16.2). Subsequently, *LeACS4* continues to express highly during climacteric phase, whereas the expression of *LeACS1A* declines. The rise in ripening-associated ethylene production results in the induction of *LeACS2*, and the inhibition of *Le ACS6* and *LeACS1A* expression. This fine tuning of the *ACS* genes is thought to be critical for the switch from pre-climacteric system 1 to climacteric system 2. Noteworthy is that system 1 is characterized by inhibitory feedback of ethylene in its own biosynthetic pathway, whereas the transition to system 2 is characterized by autocatalytic production. The requirement for ethylene to trigger the ripening of climacteric fruit has been clearly demonstrated by down-regulating *ACO* and *ACS* genes in transgenic plants using an antisense strategy. The ethylene-suppressed lines showed strongly delayed ripening in tomato (Oeller et al. 1991; Picton et al. 1993), and in other fruits, e.g., melon (Ayub et al. 1996) and apple (Dandekar et al. 2004). However, ethylene-independent ripening pathways exist in climacteric fruit, as illustrated in melon fruit, where part of softening, sugar accumulation, and coloration of the flesh occur in ethylene-suppressed fruit (Flores et al. 2001). These results have led to the conclusion that climacteric (ethylene-dependent) and non-climacteric (ethylene-independent) regulation coexists in climacteric fruit (Pech et al. 2008a).

Although the ripening of non-climacteric fruit is not associated with any significant change in ethylene production, some ethylene-dependent processes do exist in

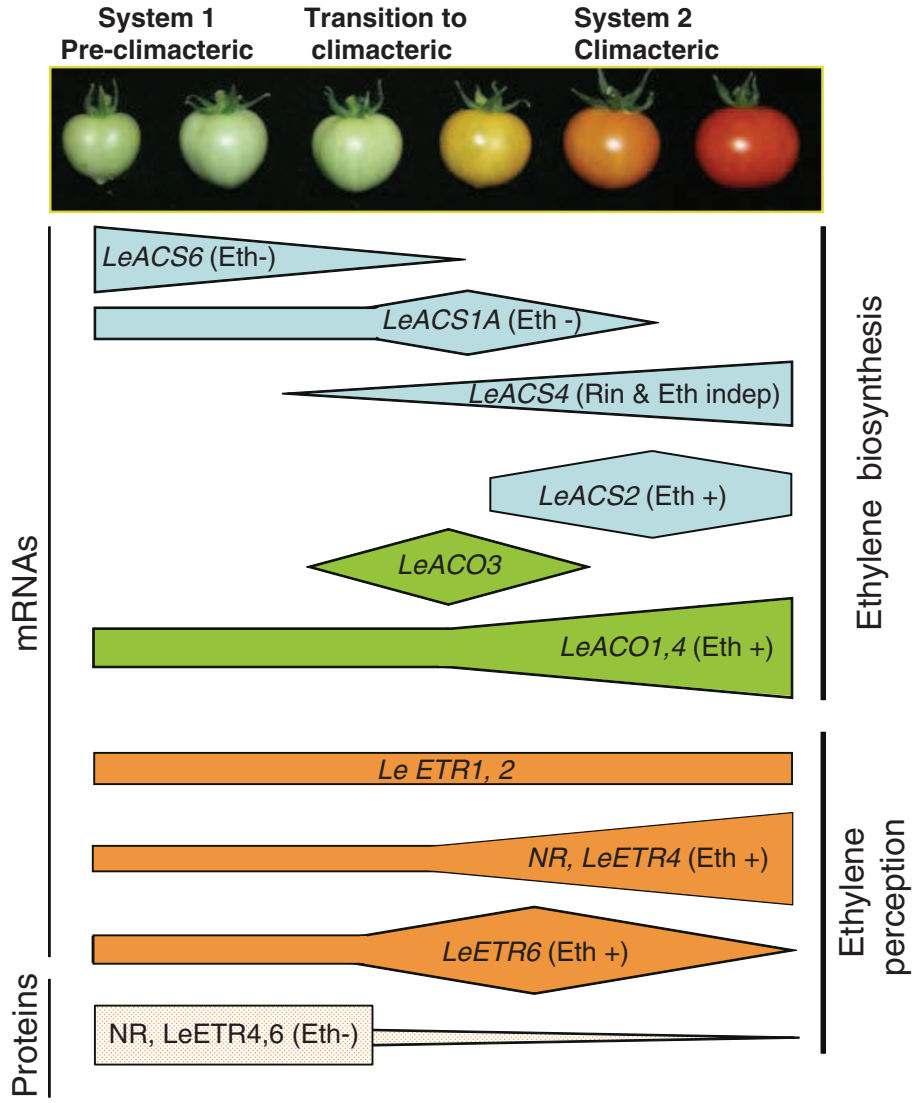

Fig. 16.2 Schematic representation describing the expression of ethylene biosynthesis and ethylene perception genes during the transition to climacteric in tomato. System 1 refers to pre-climacteric ethylene production, and System 2 to climacteric autocatalytic ethylene production. LeACS, *Lycopersicon esculentum* ACC synthase; LeACO, *Lycopersicon esculentum* ACC oxidase; LeETR and NR, ethylene receptors. Eth+ and Eth– refer to the stimulation and repression, respectively of gene or protein expression (adapted from Barry et al. 1996, 2000; Kevany et al. 2007)

this type of fruit. In grape berries, the ethylene synthesis pathway is activated at the inception of the ripening, the so-called veraison stage. Treatments with exogenous ethylene stimulate the long-term expression of genes related to anthocyanin synthesis, and ethylene signals appear to be involved in the regulation of vascular flux, acid content, and in some steps of aroma volatile production (Mailhac and Chervin 2006). In citrus, another class of typically non-climacteric fruit, the existence of an autocatalytic system of ethylene production similar to that of climacteric fruit has been suggested (Katz et al. 2004), and it is well known that these types of fruit have the ability to respond to exogenous ethylene in terms of chlorophyll degradation. Moreover, in all non-climacteric fruits, exogenous ethylene accelerates senescence

via the deterioration of cell membranes. While the role of ethylene in climacteric fruit ripening is beginning to be well understood, the main signaling pathways involved in non-climacteric ripening remain very poorly understood.

16.2.2 Ethylene Perception and Signal Transduction

Breakthrough advances in the field of ethylene perception have been made possible by the use of the model plant Arabidopsis, and the implementation of molecular genetics strategies. Following the identification of the ethylene-insensitive mutants, named ETR1 (Bleecker et al. 1988), the gene encoding the ethylene receptor was isolated by positional cloning (Chang et al. 1993). The ethylene receptor was the first plant hormone receptor to be isolated and characterized, and this paved the way toward the isolation of the other components of the ethylene transduction pathway (Klee and Clark 2004). Based on these discoveries, the use of Arabidopsis has been critical in helping to isolate the ethylene receptor from other plant species, and to understand the role of the receptors in the ripening process. The ethylene receptors are encoded by a small multigene family for structurally distinct but functionally redundant proteins working either as hetero- or homo-multimers. In tomato, six ethylene receptor genes have been isolated and found to be expressed in all plant tissues, three of these showing a net increase during ripening, while two express constitutively (Fig. 16.2). Interestingly, it was demonstrated that the tomato *Never ripe* (*Nr*) mutation, which results in impaired ripening, occurs in one of the ethylene receptor genes. Recent studies demonstrated that the ethylene receptors are rapidly degraded during fruit ripening, while the transcription rate remains high, and that the receptor level determines the timing of ripening (Kevany et al. 2007). Moreover, the suppression of the ethylene receptor LeETR4 led to an early ripening of tomato fruits (Kevany et al. 2008).

In more applied terms, the search for ethylene antagonists led to the discovery of 1-methylcyclopropene (MCP), a powerful antagonist of ethylene action (Sisler et al. 1999). This compound is now widely used both by academic researchers as a tool for understanding ethylene-regulated developmental processes (Blankenship and Dole 2003), and by the producers and shippers of fresh fruit and flowers on a commercial scale for extending the shelf life of these products. MCP probably represents the most remarkable innovation in the past two decades in the field of post-harvest horticulture (http://www.hort.cornell.edu/department/faculty/watkins/ethylene/).

The *CTR1* gene (*Constitutive Triple Response*), first isolated from Arabidopsis, encodes another major component of ethylene signaling lying downstream of the receptor acting as a negative regulator of the ethylene transduction pathway (Kieber et al. 1993). The tomato *CTR1* gene (*Sl-CTR1*) was first isolated from fruit tissue (Leclercq et al. 2002), and in spite of being a negative regulator of ethylene responses, its transcripts are up-regulated during fruit ripening, commensurate with the rise in ethylene production. Subsequently, it was shown that the CTR

family was composed of four genes in tomato, each displaying a specific pattern of expression during ripening and in response to ethylene, with *Sl-CTR1* being the most actively expressed during fruit ripening (Adams-Phillips et al. 2004). Strikingly, reverse genetic strategies have to date failed to show any impact of altered *CTR1* expression on the fruit ripening process, indicating a potential functional redundancy among the *CTR* genes.

16.2.3 Control of Ethylene Response in Fruit

Because of the tremendous change in the expression level of a large number of genes during fruit ripening, and in order to gain better insight into the control mechanisms underlying this process, differential screening approaches were attempted to isolate and characterize ethylene-regulated genes (Lincoln et al. 1987). Genes encoding cell wall-degrading, ethylene production, and pigment biosynthesis enzymes were among the first ethylene-responsive genes to be isolated from tomato fruit. Later, a set of early ethylene-regulated genes were isolated from mature green tomatoes that are responsive to exogenous ethylene, but not yet producing elevated levels of ripening-associated ethylene (Zegzouti et al. 1999). Expression studies revealed that the ethylene-responsive genes can be up-regulated, down-regulated, or transiently induced following short periods of hormone treatment, supporting the idea that ethylene can act as negative or positive regulator of gene expression (Gupta et al. 2006; Kesari et al. 2007). Noteworthy is that many of the early ethylene-responsive genes encode putative regulatory proteins involved in transduction pathways and transcriptional or post-transcriptional regulation, indicating that the ethylene control of the ripening process operates in a complex multilevel way. More recently, the work by Giovannoni's group (Alba et al. 2005) demonstrated the importance of ethylene control during tomato fruit development. In the tomato *Nr* mutant, impaired in ethylene sensing and fruit ripening, up to one third of ripening-associated genes showed altered expression compared to wild type (Alba et al. 2005). Moreover, in a non-climacteric fruit like strawberry, microarray analyses comparing akene and receptacle tissues show high levels of ethylene response factor (*ERF*) and ethylene regulated (*ER*) gene expression in akene tissue, suggesting a role for ethylene in the maturation of the akene (Aharoni and O'Connell 2002). Together, these data demonstrate the important role of ethylene in fruit ripening in both climacteric and non climacteric fruit. However, the mechanistic insight into how ethylene acts to bring about the activation of all the ripening-associated metabolic pathways remains unclear. Ethylene is known to have numerous effects on a wide range of developmental processes, including germination, flower and leaf senescence, fruit ripening, leaf abscission, root nodulation, programmed cell death, and responsiveness to abiotic stress and pathogen attack (Johnson and Ecker 1998; Bleecker and Kende 2000; Pirrello et al. 2006). This diversity of plant responses to ethylene raises the question on how this phytohormone selects the desired target genes with respect to their tissue and

developmental specificity. This question becomes even more relevant when considering that the ethylene transduction pathway is linear in its upstream part from the receptor to *ein3*, the first transcription regulator. It is therefore tempting to speculate that most of the diversity of ethylene responses may arise largely from fine tuning of the expression and/or activity of ERFs, transcriptional regulator proteins lying downstream of EIN3 (Fig. 16.3). Indeed, ERFs belong to one of the largest families of transcription factors in plants (Riechmann et al. 2000), thus offering different branching possibilities to channel the hormone signaling to a variety of responses. The diversity and complexity of ethylene responses can also arise from the cross-talk between ethylene and other hormones (Rosado et al. 2006; Stepanova et al. 2007).

ERF genes encode a type of *trans*-acting factors unique to plants that specifically bind the GCC box, a conserved motif of the *cis*-acting element found in the promoter of ethylene-responsive genes (Ohme-Takagi and Shinshi 1995; Solano et al. 1998). ERFs are known to be the last actors of the ethylene signaling pathway,

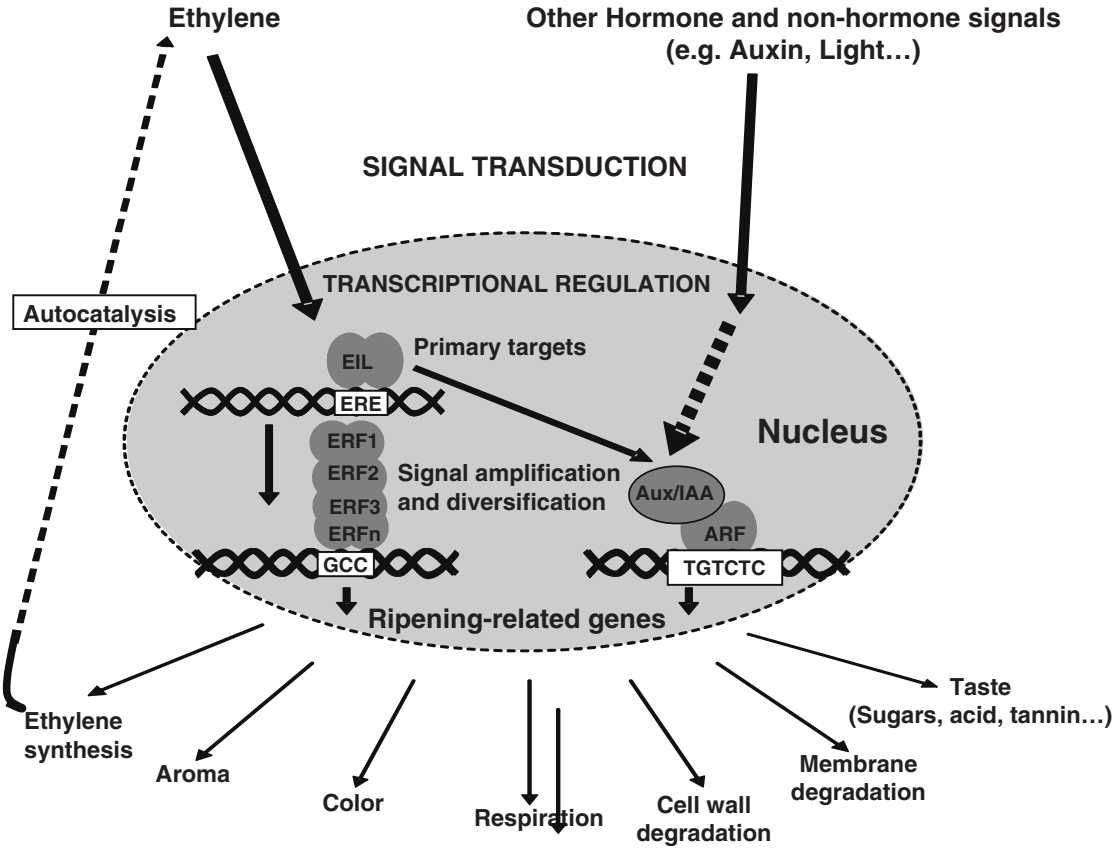

Fig. 16.3 The hormone-dependent transcriptional regulation associated with fruit ripening. A main focus is made on ethylene and auxin, aiming at exemplifying the importance of cross-talk between hormone signaling. Ethylene transduction cascade leads to the activation of *EIN3-Like* (*EIL*) genes, which activates primary target genes (ethylene-response factors, ERFs). ERFs in turn activate the expression of secondary ripening-related genes. Other signals, such as auxin, are also involved in this process. Some auxin response factors (ARFs) and Aux/IAA transcription factors are also ethylene-responsive, and therefore are likely to participate in the expression of ripening-related genes (Jones et al. 2002)

and the ERF family is part of the AP2/ERF superfamily of transcription factors, which also contains the AP2 and RAV families (Riechmann et al. 2000). Since *ERFs* belong to a large multigene family, it is expected that members of this family have varied functionality, and diverse binding activities. Using combined reverse genetics and transcriptomics approaches, intensive studies are in progress to uncover the specific role of each ERF in the ripening process, and to establish the set of target genes regulated by each member of this transcription factor family. In the long term, the objective of these studies is to set up tools enabling targeted control of the ripening process, thus allowing engineering fruit ripening in specific ways, such as slowing down the loss of firmness, while enhancing desired metabolic pathways.

16.3 Hormone Cross-Talk and Fruit Ripening

As mentioned above, fruit ontogeny and ripening are genetically regulated processes involving a complex multi-hormonal control (Fig. 16.3). While the roles of ethylene in triggering and regulating the ripening of climacteric fruit have been clearly demonstrated, little is known on the roles of other hormones. Phytohormones exert their effect on plant development via a chain of transduction pathways that ultimately activates specific transcription factors, which in turn regulate the expression of a set of target genes. In order to uncover the role of hormones that act in concert with ethylene to regulate tomato fruit development, a screen for transcription factors showing differential expression from fruit set through ripening led to the isolation of a number of genes encoding auxin transcriptional regulators of the ARF and Aux/IAA type (Jones et al. 2002). Among the isolated auxin-response factors, some showed fruit-specific and ethylene-regulated expression that clearly correlated with their pattern of ethylene responsiveness, suggesting a cross-talk between ethylene and auxin throughout fruit development (Jones et al. 2002; Wang et al. 2005). Combined reverse genetic and transcriptomic approaches have been carried out to uncover the functional significance of these genes. Molecular and physiological characterization of transgenic tomato plants under- and over-expressing these transcription factors confirmed their crucial role in both early and late stages of fruit development. Important quality traits, such as sugar content, firmness, and parthenocarpy, are strongly affected in the transgenic lines (Jones et al. 2002; Wang et al. 2005). These genes offer new potential targets for improving fruit quality, either by marker-assisted selection or by biotechnological means.

16.4 Biochemical Changes and Sensory Traits Associated with Fruit Ripening

One of the major factors associated with the post-harvest deterioration of fruit is the rate of softening. Excessive softening results in shorter shelf life during storage, transportation and distribution, and increased wastage. A number of genes potentially

involved in cell wall degradation, rearrangement and structure have been isolated, and most of these have been studied in the tomato model. However, unexpectedly, it has been shown that the suppression of candidate genes, such as those encoding polygalacturonase, pectin-methyl-esterase, and β-glucanase, did not have a major impact on the evolution of fruit firmness (Giovannoni et al. 1989; Tieman et al. 1992; Brummell et al. 1999a). Up to 40% reduction of tomato fruit softening has been achieved by down-regulating the *TBG4* β-galactosidase gene (Smith et al. 2002), but in antisense *TBG4* fruit, *TBG3* gene expression was also reduced, indicating a possible cooperation of the two genes. Expansins are cell wall proteins that loosen cell walls by reversibly disrupting hydrogen bonds between cellulose microfibrils and matrix polysaccharides. The *LeExp1* (*tomato expansin 1*) gene encodes a protein that is specifically expressed in ripening fruit. Down-regulation resulted in strong reduction of softening throughout ripening, probably by alteration of the microfibril-/matrix glycan interface that facilitates access of cell wall hydrolases to the matrix glycan substrates (Brummell et al. 1999b). Another class of cell wall-degrading enzymes, pectate lyases, appears to have a more important role in ripening than previously expected. In strawberry, a non-climacteric fruit, suppression of the pectate lyase mRNA resulted in significantly firmer fruits (Jiménez-Bermúdez et al. 2002), with the highest reduction in softening being shown to occur during the transition from the white to the red stage. Within the gene families of cell wall-degrading genes of climacteric fruit, some members are regulated by ethylene, while others are not, confirming the coexistence of ethylene-dependent and -independent processes (Flores et al. 2001; Nishiyama et al. 2007). In general, it appears that fruit softening involves many genes that encode a variety of cell wall-degrading enzymes and non-enzymatic proteins. Each protein, and each protein isoform, may play a specific role in softening and textural changes.

Pigments are essential for the attractiveness of fruits, accumulating most often in the skin during the ripening process, although many climacteric fruits accumulate pigments also in their pulp tissue. The most important pigments of fruit are carotenoids and anthocyanins. Beside their role in pigmentation, they are important for human health as a source of vitamin A and antioxidant compounds. Carotenoids comprise carotenes, such as lycopene and β-carotene, and xanthophylls, such as lutein. They are derived from terpenoids, and are synthesized in fruit at a high rate during the transition from chloroplast to chromoplast. Many genes involved in the biosynthesis of carotenoids have been cloned (Cunningham and Gantt 1998; Hirschberg 2001), and extensive information is available on the regulation of carotenoid formation during fruit ripening (Bramley 2002). Anthocyanins belong to the flavonoid subclass of phenolic compounds. The flavonoid biosynthetic pathway has been elucidated in plants, and many enzymes and corresponding genes have been isolated and characterized (Winkel-Shirley 2001). In grape, where anthocyanins are crucial for the quality of wine, it has been demonstrated that ethylene (or the ethylene generator ethephon) stimulates berry coloration, demonstrating that this hormone is involved in the regulation of anthocyanin biosynthesis genes (El-Kereamy et al. 2003). A number of factors and signals influence the accumulation of anthocyanins and the expression of related genes,

including photochrome and light, hormones (gibberellins, methyl jasmonate), and various stresses such as wounding and low temperature (Mol et al. 1996). Environmental conditions and orchard management, including irrigation, pruning, and fertilization, are also known to strongly impact on fruit coloration.

Aroma volatiles contribute strongly to the overall sensory quality of fruit and vegetables. Extensive studies have been focused on the identification of volatile compounds, and to the elucidation of some of the biosynthetic routes either by bioconversion or by tracing of precursors (Sanz et al. 1997; D'Auria et al. 2002; Dudareva et al. 2004). In recent years, research efforts have been directed toward the isolation of the corresponding genes in fruits and vegetables (Aharoni et al. 2000; Yahyaoui et al. 2002; Beekwilder et al. 2004; El-Sharkawy et al. 2005; Pech et al. 2008b). Aroma is generally a complex mixture of a wide range of compounds. Each product has a distinctive aroma, which is function of the proportion of the key volatiles, and the presence or absence of unique components. The most important classes of aromas are monoterpenes, sesquiterpenes, and compounds derived from lipids, sugars, and amino acids. Ethylene is known to control the rate of ripening, the duration of storage life, and most of the ripening events in climacteric fruit. Therefore, breeders have "incidentally" reduced ethylene synthesis or action by generating genotypes with extended shelf life. Because many genes of aroma biosynthesis are ethylene-regulated (El-Sharkawy et al. 2005; Manriquez et al. 2006), this has often resulted in a severe loss of flavor in long-keeping genotypes that have commonly been generated by breeding with non-ripening mutants (McGlasson et al. 1987; Aubert and Bourger 2004). One major challenge for the future is to uncouple the down-regulation of ethylene from inhibition of aroma volatile production.

16.5 Molecular Markers and QTL Mapping of Fruit Ripening Traits

The advent of genetic approaches based on quantitative trait loci (QTLs) opens new prospects toward genetic improvement of fruit. Indeed, most fruit quality traits are under multigenic control, and the QTL approach allows the localization on genetic maps of loci responsible for at least part of the phenotypic variation, and enables the quantification of their individual effects. Because of the low molecular polymorphism observed in cultivated tomato, which is usually used as model species in fruit research, the majority of these studies (Tanksley and McCouch 1997; Causse et al. 2002) rely on interspecific progeny. Surprisingly, in spite of their characteristics inferior to those of cultivated species, wild species can possess alleles useful for improving fruit traits. A good example is given by a QTL improving fruit color, detected in a *Solanum habrochaites* (*Lycopersicon hirsutum*), a green-fruited species. The molecular markers localized in the vicinity of this QTL are now being used in marker-assisted selection to create parent lines with increased potential, or in contrast, to avoid certain unfavorable traits (Fulton et al. 2002). A fruit weight QTL, common to several studies, has been precisely localized and then cloned by

chromosome walking (Frary et al. 2000). Another QTL controlling sugar concentration in fruit has also been cloned (Fridman et al. 2000), and the gene responsible for this QTL has been shown to encode a cell wall invertase (Fridman et al. 2004).

As emphasized above, the climacteric character represents an important determinant of the ripening rate and storability. Because genetically compatible climacteric and non-climacteric types of melon are available, it has been possible to study the inheritance of the climacteric character. A segregating population resulting from a cross between a typical climacteric-type Charentais melon (*Cucumis melo* var. *cantalupensis* cv. *Védrantais*) and a non-climacteric melon, Songwhan Charmi PI 161375 (*Cucumis melo* var. *chinensis*), has been generated and used to study the segregation of the formation of the abscission layer (*Al*) of the peduncle and ethylene production (Périn et al. 2002). It was found that the climacteric character was controlled by two duplicated independent loci (*Al-3* and *Al-4*), and the intensity of ethylene production was controlled by at least four QTLs localized in other genomic regions. None of the QTLs matched with known genes of the ethylene biosynthetic or transduction pathways. Recently, it was reported that some introgression lines generated from two non-climacteric melons, Piel de Sapo (var. *inodorus*) and Songwhan Charmi PI 161375 (var. *chinensis*) possessed a climacteric character (Obando et al. 2007). The QTLs associated with ethylene production and respiration rate in this work have not been mapped at the same position as the *Al* loci described by Périn et al. (2002). Collectively, these data suggest that different and complex genetic regulation exists for the climacteric character.

Improvement of fruit quality arose in some cases randomly, like in the apple, where a chance seedling, Golden Delicious, was discovered with good agronomic characters. It has been crossed with old apple varieties having good sensory attributes to generate new apple cultivars that combine good agronomic and good sensory characters (Vaysse et al. 2000). Similarly, the poor-keeping qualities of Delicious have been improved by crossing with long-keeping apples (Rall's Janet), giving rise to the Fuji group of apples (Vaysse et al. 2000). Likewise, in Charentais-type melons, long or mid-shelf life commercial genotypes are available. Some of these have been generated using a non-ripening melon named "Vauclusien". However, the long shelf life character is often associated with poor sensory qualities (Aubert and Bourger 2004). Low ethylene production is generally correlated with long storage life. The delayed ripening of these genotypes was found to result in alteration of ethylene biosynthetic or response genes. The amount of ethylene in ripening Fuji apples parallels the transcript levels of the ripening-specific *ACS* gene, *MdACS1* (Harada et al. 1985). An allele of this gene (*MdACS1-2*) contains an insertion of a retro-transposon-like sequence in the 5′-flanking region, and is transcribed at a lower level than the wild-type allele *MdACS1-1*. Cultivars that are homozygous for the *MdACS1-2* allele have low ethylene production and long storage life (Sunako et al. 1999). Two *ERF* genes (*MdERF1* and *MdERF2*) have been isolated from ripening apple fruit. The *MdERF1* gene has been shown to express predominantly in ripening fruit, and *MdERF2* exclusively in ripening fruit (Wang et al. 2007). Expression of both genes was repressed by treatment with 1-MCP. Apple cultivars with low ethylene production had a tendency to show lower

expression of these two *MdERF* genes than those with high ethylene production. By screening different cantaloupe melons, Zheng and Wolff (2000) reported a correlation between ethylene production and post-harvest decay. In addition, using *ACO* cDNA probes, they were able to demonstrate that low ethylene production was associated with the presence of an RFLP *ACO* allele *Ao*, whereas high ethylene production was associated with the *Bo* allele in homozygous conditions (Zheng et al. 2002).

Amongst climacteric fruits, there are genetic differences in the capacity to induce the ripening process. The most striking case is given by fruit cultivars that require exposure to post-harvest low temperatures for ripening. Some winter pear varieties, such as D'Anjou, Beurre Bosc, and Passe Crassane, require chilling temperatures for the induction of autocatalytic ethylene production (Blankenship and Richardson 1985; Morin et al. 1985; Knee 1987). Furthermore, it has been reported that the cold-requirement character can be transmitted by breeding, as exemplified by crossing of Passe-Crassane pears and a cold-independent variety, Old Home, to give a mixed population of cold-dependent and cold-independent hybrids (El-Sharkawy et al. 2004). Cold requirement appears to be linked to the possibility of inducing ethylene biosynthesis genes. In Passe Crassane pears, a 3-month chilling treatment at 0°C strongly stimulated ACC oxidase activity, and to a lesser extent, ACC synthase activity (Lelièvre et al. 1997). It has been shown that the presence of some *ACS* alleles was correlated with the chilling requirements for ripening, and with the induction of autocatalytic ethylene production (El-Sharkawy et al. 2004).

16.6 Natural Mutants Affected in the Ripening Phenotype

Among the reasons why tomato has emerged as a model species for studying fleshy fruit development is the presence of well-characterized, spontaneous mutants or wild-allele variants that have been recovered from production fields or breeding programs. A number of genes corresponding to various mutations have been isolated by positional cloning (Giovannoni 2007). The first ripening-impaired mutant to be characterized at the molecular level is *Never-ripe* (*Nr*), which bears a dominant mutation that affects the ethylene response, and results in fruit producing reduced amounts of ethylene and retaining very low ethylene responsiveness (Lanahan et al. 1994). It was shown that the *NR* gene encodes an ethylene receptor from the ERS family devoid of receiver domain (Wilkinson et al. 1995). The *Green-ripe* (*Gr*) mutant corresponds also to a dominant ripening mutation lying in a gene encoding a new component of ethylene signaling (Barry and Giovannoni 2006), corresponding to the *Reversion To Ethylene Sensitivity1* (*RTE1*) shown to interact and regulate the ETR1 ethylene receptor in Arabidopsis (Resnick et al. 2006; Zhou et al. 2007).

One of the tomato mutations most commonly used by the breeders affects the transcriptional control of fruit ripening. The *ripening-inhibitor (rin)* mutation is a recessive mutation that blocks the ripening process, and prevents ethylene

production and responsiveness. In the last decade, the *rin* locus has been widely used for generating long shelf life commercial varieties. The *rin* mutation encodes a MADS box-type transcription factor that is present in both climacteric and non-climacteric fruit (Vrebalov et al. 2002), suggesting that it probably acts upstream of the climacteric switch. The *Colorless non-ripening* (*Cnr*) mutant is a dominant mutant corresponding to an epigenetic mutation that alters the methylation of the promoter of a SPB box transcription factor (Manning et al. 2006). Although it has been proposed that both *rin* and *cnr* act upstream of ethylene production (Giovannoni 2007), the location of these two transcription factors in the ripening regulatory network is not clear.

A number of other mutants affect the fruit composition in terms of secondary metabolites. Because of the ease of visual screening, most of the mutants affected in fruit composition are altered in pigment accumulation. The color change from green to red associated with ripening in tomato results from both chlorophyll degradation and carotenoid pigment accumulation. The numerous tomato mutants affected in pigmentation represent a valuable genetic resource, which has been exploited to facilitate the identification of the genes involved in carotenoid biosynthetic pathways, and understanding the complex mechanisms regulating pigment accumulation (Bramley 2002). The role of light has been reported in the regulation of fruit pigmentation (Giovannoni 2001). The *yellow-flesh* (*r*) mutation that results in the absence of carotenoid accumulation corresponds to a deletion within the ethylene-regulated *phytoene synthase-1* gene (Fray and Grierson 1993). The *delta* mutant displays an orange color resulting from the accumulation of δ-carotene at the expenses of lycopene (Tomes 1969), due to a dominant mutation within the *CrtL-e* gene encoding a lycopene ε-cyclase (Ronen et al. 1999). The *Beta* (*B*) partially dominant mutation also results in orange color, due to the accumulation of β-carotene instead of lycopene. The gene responsible for the *B* mutation encodes a fruit- and flower-specific lycopene, β-cyclase, capable of converting lycopene into β-carotene. Its expression is strongly increased in the *B* mutant (Ronen et al. 2000). Deep-red fruit of *old-gold* and *old-gold-crimson* mutants are null mutations of an allele of the B gene (Ronen et al. 2000). *Tangerine* is a recessive mutation conferring orange color by accumulation of pro-lycopene instead of normal lycopene. It corresponds to an impairment of the expression of a carotenoid isomerase gene that is suspected to enable carotenoid biosynthesis in the dark, and in non-photosynthetic tissues (Isaacson et al. 2002). The *hp1* and *hp2* mutants exhibiting elevated content of flavonoid and carotenoid are mutated in *Damaged DNA Binding Protein1* (Liu et al. 2004), and *Detiolated1* (Mustilli et al. 1999) genes, respectively. The corresponding genes in Arabidopsis encode nuclear-localized light signal transduction proteins.

16.7 Conclusions and Future Directions

While tremendous progress has been made in understanding the mechanisms of fruit ripening, a number of questions remain unanswered. In climacteric fruit, amongst the major issues that remain to be addressed are the role of hormones

other than ethylene, and the way in which they interact with ethylene signaling to control different aspects of fruit ripening. The mechanism by which ethylene selects specific ripening-regulated genes is another important topic that needs to be investigated. In non-climacteric fruit, the detailed mechanisms that regulate the ripening process remain largely unknown, although molecular data are accumulating. So far, the regulation of gene expression during fruit ripening has been viewed mostly at the transcriptional level. Recent studies on the ethylene receptor (Kevany et al. 2007) illustrate that post-transcriptional regulation plays an essential role, and deserves more attention. Regulation of gene expression by epigenetic variations is now recognized as an important determinant of plant development. Epigenetic variations do not affect the primary DNA sequence, but consist of DNA methylation or histone modifications that affect gene expression generally at the level of chromatin organization. In fruit, the *Cnr* mutation is the only well-characterized, natural and stably inherited epigenetic mutation (Seymour et al. 2007). Research efforts are now being directed toward understanding the epigenetic regulation of the fruit ripening process. It is predictable that the answers to these questions will require cooperation between fruit physiologists, molecular biologists, and geneticists.

The recent development of high-throughput technology for analyzing genome structure and functions is starting to have an impact on fruit research. A number of national and multinational programs are attempting to combine genomics, proteomics, metabolomics, and reverse genetic approaches to unravel the molecular mechanisms of fruit development (Wang et al. 2009). The implementation of these genome-wide (Alba et al. 2004) and metabolomic technologies (Overy et al. 2005), together with bioinformatics tools, is expected to provide new understanding of the fruit developmental program, and reveal the networks of interactions between different pathways leading to the accumulation of fruit quality traits. The most important programs are being implemented on the tomato model species. A multinational consortium has been established recently, which has made available centralized facilities for tomato ESTs and derived DNA chips (Mueller et al. 2005). This is enabling the elucidation of global changes in gene expression during fruit development and ripening, and researchers to mine and analyze the expression profiling data in order to cluster the complete set of genes involved in specific metabolic and regulatory mechanisms. By comparing differences between natural variants, ripening mutants, or introgression lines, genes will be identified that are essential for specific aspects of fruit ripening, with their corresponding impact on fruit metabolism (Fei et al. 2004; Overy et al. 2005). In addition, reverse genetics approaches for high-throughput functional identification of target genes are being developed, amongst which the emerging TILLING (targeting induced local lesions in genomes) technology is most promising. The completion of the tomato genome sequencing project, and the availability of the tomato genome sequence in the near future will represent a major breakthrough likely to change our understanding in the area of the fundamentals of fruit growth and development, and open new avenues to address the varied topics in fruit research.

Acknowledgments The authors greatly acknowledge the financial support of the IFCPAR-CEFIPRA Indo-French programme (Grant 3303-02).

References

Adams-Phillips L, Barry C, Kannan P, Leclercq J, Bouzayen M, Giovannoni J (2004) Evidence that CTR1-mediated ethylene signal transduction in tomato is encoded by a multigene family whose members display distinct regulatory features. Plant Mol Biol 54:387–404

Aharoni A, O'Connell AP (2002) Microarray gene expression analysis during strawberry achenes and receptacle maturation. J Exp Bot 53:2073–2087

Aharoni A, Keizer LCP, Bouwmeester HJ, Sun Z, Alvarez-Huerta M, Verhoeven HA, Blass J, van Houwelingen AMML, de Vos RCH, Van der Voet H, Jansen RC, Guis M, Mol J, Davis RW, Schena M, van Tunen AJ, O'Connell A (2000) Identification of the *SAAT* gene involved in strawberry flavor biogenesis by use of DNA microarrays. Plant Cell 12:647–661

Alba R, Fei Z, Payton P, Liu Y, Moore SL, Debbie P, Cohn J, D'Ascenzo M, Gordon JF, Rose JKC, Martin G, Tanksley SD, Bouzayen M, Jahn MM, Giovannoni J (2004) ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development. Plant J 39:697–714

Alba R, Payton P, Fei Z, McQuinn R, Debbie P, Martin GB, Tanksley SD, Giovannoni JJ (2005) Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. Plant Cell 17:2954–2965

Alexander L, Grierson D (2002) Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J Exp Bot 53:2039–2055

Aubert C, Bourger N (2004) Investigation of volatiles in Charentais cantaloupe melons (*Cucumis melo* var. *cantalupensis*). Characterization of aroma constituents in some cultivars. J Agric Food Chem 52:4522–4528

Ayub R, Guis M, Ben Amor M, Gillot L, Roustan JP, Latché A, Bouzayen M, Pech JC (1996) Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. Nature Biotechnol 14:862–866

Barry CS, Giovannoni JJ (2006) Ripening inhibition in the tomato *Green-ripe* mutant results from ectopic expression of a novel protein which disrupts ethylene signal transduction. Proc Natl Acad Sci USA 103:7923–7928

Barry CS, Blume B, Bouzayen M, Cooper W, Hamilton AJ, Grierson D (1996) Differential expression of the 1-aminocyclopropane-1-carboxylate oxidase gene family of tomato. Plant J 9:525–535

Barry CS, Llop-Tous MI, Grierson D (2000) The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiol 123:979–986

Beekwilder J, Alvarez-Huerta M, Neef E, Verstappen FW, Bouwmeester HJ, Aharoni A (2004) Functional characterization of enzymes forming volatile esters from strawberry and banana. Plant Physiol 135:1865–1878

Blankenship SM, Dole JM (2003) 1-methylcyclopropene: a review. Postharvest Biol Technol 28:1–25

Blankenship SM, Richardson DR (1985) Development of ethylene biosynthesis and ethylene-induced ripening in D'Anjou pears during the cold requirement for ripening. J Am Soc Hort Sci 107:807–812

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18

Bleecker AB, Estelle MA, Somerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science 241:1086–1089

Bower J, Holford P, Latché A, Pech JC (2002) Culture conditions and detachment of the fruit influence the effect of ethylene on the climacteric respiration of melon. Postharvest Biol Technol 26:135–146

Bramley PM (2002) Regulation of carotenoid formation during tomato fruit ripening and development. J Exp Bot 53:2107–2113

Brummell DA, Hall BD, Bennett AB (1999a) Antisense suppression of tomato endo-1,4-β-glucanase *Cel2* mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. Plant Mol Biol 40:615–622

Brummell DA, Harpster MH, Civello PM, Palys JM, Bennett AB, Dunsmuir P (1999b) Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant Cell 11:2203–2216

Causse M, Sabina-Colombani V, Lecomte L, Duffé P, Rouselle P, Buret M (2002) QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. J Exp Bot 53:2089–2098

Chang C, Kwok SF, Bleecker AB, Meyerowtz EM (1993) *Arabidopsis* ethylene-response gene ETR: similarity of product to two component regulators. Science 262:539–545

Cunningham FX, Gantt E (1998) Genes and enzymes of carotenoids biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 49:557–583

Dandekar AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kader AA, Stow JR, Colgan RJ, James DJ (2004) Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Res 13:373–384

D'Auria JC, Chen F, Pichersky E (2002) Characterization of an acyltransferase capable of synthesizing benzylbenzoate and other volatile esters in flowers and damaged leaves of *Clarkia breweri*. Plant Physiol 130:466–476

Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiol 135:1893–1902

El-Kereamy A, Chervin C, Roustan JP, Cheynier V, Souquet JM, Moutounet M, Raynal J, Ford CM, Latché A, Pech JC, Bouzayen M (2003) Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. Physiol Plant 119:175–182

El-Sharkawy I, Jones B, Gentzbittel L, Lelièvre JM, Pech JC, Latché A (2004) Differential regulation of ACC synthase genes in cold-dependent and -independent ripening in pear fruit. Plant Cell Environ 27:1197–1210

El-Sharkawy I, Manríquez D, Flores FB, Regad F, Bouzayen M, Latché A, Pech JC (2005) Functional characterization of a melon alcohol acyl-transferase gene family involved in the biosynthesis of ester volatiles. Identification of the crucial role of a threonine residue for enzyme activity. Plant Mol Biol 59:343–360

Fei Z, Tang X, Alba RM, White JA, Ronning CM, Martin GB, Tanksley SD, Giovannoni JJ (2004) Comprehensive EST analysis of tomato and comparative genomics of fruit ripening. Plant J 40:47–59

Flores F, Ben Amor M, Jones B, Pech JC, Bouzayen M, Latché A, Romojaro F (2001) The use of ethylene-suppressed lines to assess differential sensitivity to ethylene of the various ripening pathways in Cantaloupe melons. Physiol Plant 113:128–133

Frary A, Nesbitt TC, Frary A, Grandillo E, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB (2000) *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88

Fray R, Grierson D (1993) Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. Plant Mol Biol 22:589–602

Fridman E, Pleban T, Zamir D (2000) A recombination hotspot delimits a wild species QTL for tomato sugar content to 484-bp within an invertase gene. Proc Natl Acad Sci USA 97:4718–4723

Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science 305:1786–1789

Fulton TM, Bucheli P, Voirol E, López J, Pétiard V, Tanksley SD (2002) Quantitative trait loci (QTL) affecting sugars, organic acids and other biochemical properties possibly contributing to flavor, identified in four advanced backcross populations of tomato. Euphytica 127:163–177
Giovannoni JJ (2001) Molecular biology of fruit maturation and ripening. Annu Rev Plant Physiol Plant Mol Biol 52:725–749
Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. Plant Cell Suppl 16: S170–S180
Giovannoni JJ (2007) Fruit ripening mutants yield insights into ripening control. Curr Opin Plant Biol 10:283–289
Giovannoni JJ, DellaPenna D, Bennett A, Fischer R (1989) Expression of a chimeric polygalacturonase gene in transgenic *rin* (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. Plant Cell 1:53–63
Gupta SM, Srivastava S, Sane AP, Nath P (2006) Differential expression of genes during banana fruit development, ripening and 1-MCP treatment: presence of distinct fruit specific, ethylene induced and ethylene repressed expression. Postharvest Biol Technol 42:16–22
Hamilton AJ, Lycett GW, Grierson D (1990) Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature 346:284–287
Hamilton AJ, Bouzayen M, Grierson D (1991) Identification of a tomato gene for the ethylene-forming enzyme by expression in yeast. Proc Natl Acad Sci USA 88:7434–7437
Harada T, Sunako T, Wakasa Y, Soejima J, Satoh T, Niizeki M (1985) An allele of the 1-aminocyclopropane-1-carboxylate synthase gene accounts for the low level of ethylene production in climacteric fruits of some apple cultivars. Theor Appl Genet 101:742–746
Hirschberg J (2001) Carotenoid biosynthesis in flowering plants. Curr Opin Plant Biol 4:210–218
Isaacson T, Ohad I, Beyer P, Hirschberg J (2002) Cloning of tangerine from tomato reveals a carotenoid isomerise essential for the production of β-carotene and xanthophylls in plants. Plant Cell 14:333–342
Jiménez-Bermúdez S, Redondo-Nevado J, Munoz-Blanco J, Caballero JL, López-Aranda JM, Valpuesta V, Pliego-Alfaro F, Quesada MA, Mercado JA (2002) Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant Physiol 128:751–759
Johnson P, Ecker JR (1998) The ethylene gas signaling pathway in plants: a molecular perspective. Annu Rev Genet 32:227–254
Jones B, Frasse P, Olmos E, Zegzouti H, Li ZG, Latché A, Pech JC, Bouzayen M (2002) Down-regulation of an ARF-like gene in the tomato results in a pleiotropic phenotype including dark-green and blotchy ripening fruit. Plant J 32:603–614
Katz E, Martinez-Lagunes P, Riov J, Weiss D, Goldsmidt EE (2004) Molecular and physiological evidence suggests the existence of a system II-like pathway of ethylene production in non-climacteric Citrus fruit. Planta 219:243–252
Kesari R, Trivedi PK, Nath P (2007) Ethylene-induced ripening in banana evokes expression of defense and stress related genes in fruit tissue. Postharvest Biol Technol 6:136–143
Kevany BM, Tieman DM, Taylor MG, Dal Cin V, Klee HJ (2007) Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J 51:458–467
Kevany BM, Taylor MG, Klee HJ (2008) Fruit-specific suppression of the ethylene receptor *LeETR4* results in early-ripening tomato fruit. Plant Biotechnol J 6:295–300
Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) *CTR1*, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. Cell 72:427–441
Klee HJ, Clark DG (2004) Ethylene signal transduction in fruits and flowers. In: Davies PJ (ed) Plant hormones: biosynthesis, signal transduction, action. Kluwer, Dordrecht, pp 369–390
Knee M (1987) Development of ethylene biosynthesis in pear fruit at -1°C. J Exp Bot 38:1724–1733
Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ (1994) The *Never ripe* mutation blocks ethylene perception in tomato. Plant Cell 6:521–530

Leclercq J, Adams-Phillips L, Zegzouti H, Jones B, Latché A, Giovannoni J, Pech JC, Bouzayen M (2002) *Lectr1*, a tomato ctr1-like gene, demonstrates ethylene signaling ability in Arabidopsis and novel expression patterns in tomato. Plant Physiol 130:1132–1142

Lelièvre JM, Latché A, Jones B, Bouzayen M, Pech JC (1997) Ethylene and fruit ripening. Physiol Plant 101:727–739

Lincoln JE, Corde S, Read E, Fischer RL (1987) Regulation of gene expression by ethylene during *Lycopersicon esculentum* (tomato) fruit development. Proc Natl Acad Sci USA 84:2793–2797

Liu Y, Roof S, Ye Z, Barry C, van Tuinen A, Vrebalov J, Bowler G, Giovannoni J (2004) Manipulation of light signal transduction as means of modifying fruit nutritional quality in tomato. Proc Natl Acad Sci USA 26:9897–9902

Mailhac N, Chervin C (2006) Ethylene and grape berry ripening. Stewart Postharvest Rev 2:7

Manning K, Tor M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nature Genet 38:948–952

Manríquez D, El-Sharkawy I, Flores FB, Regad F, Bouzayen M, Latché A, Pech JC (2006) Fruit-specific gene expression and biochemical characteristics of two highly divergent alcohol dehydrogenases of melon. Plant Mol Biol 61:675–685

McGlasson WB, Last JH, Shaw KJ, Meldrum SK (1987) Influence of the non-ripening mutants *rin* and *nor* on the aroma of tomato fruit. HortScience 22:632–634

McMurchie EJ, McGlasson WB, Eaks IL (1972) Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237:235–236

Mol J, Jenkins G, Schäfer E, Weiss D (1996) Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Crit Rev Plant Sci 15:525–557

Morin F, Rigault C, Hartmann C (1985) Conséquences d'un séjour au froid sur le métabolisme de l'éthylène au cours de la maturation de la poire Passe Crassane après récolte. Physiol Vég 23:353–359

Mueller LA, Solow TH, Taylor N, Skwarecki B, Buels R, Binns J, Lin C, Wright MH, Ahrens R, Wang Y, Herbst EV, Keyder ER, Menda N, Zamir D, Tanksley SD (2005) The SOL Genomics Network: a comparative resource for Solanaceae biology and beyond. Plant Physiol 138:1310–1317

Mustilli AC, Fenzi F, Ciliento R, Alafano F, Bowler G (1999) Phenotype of the tomato *high pigment-2* mutant is caused by a mutation in the tomato homolog of *DEETIOLATED1*. Plant Cell 11:145–157

Nishiyama K, Guis M, Rose JKC, Kubo Y, Bennett KA, Wangjin L, Kato K, Ushijima K, Nakano R, Inaba A, Bouzayen M, Latché A, Pech JC, Bennett A (2007) Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. J Exp Bot 58:1281–1290

Obando J, Miranda CM, Jowkar M, Moreno E, Sour MK, Martinez JA, Arus P, Garcia-Mas J, Monforte AJ, Fernandez-Trujillo JP (2007) Creating climacteric melon fruit from non-climacteric parentals: postharvest implications. In: Ramina A, Chang C, Giovannoni J, Klee H, Woltering PE (eds) Advances in plant ethylene research. Springer, Berlin Heidelberg New York, pp 197–205

Oeller DC, Min-Wong L, Taylor LP, Pike DA, Theologis A (1991) Reversible inhibition of tomato fruit senescence by antisense RNA. Science 254:437–439

Ohme-Takagi M, Shinshi H (1995) Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 7:173–182

Overy SA, Walker HJ, Malone S, Howard TP, Baxter CJ, Sweetlove LJ, Hill SA, Quick WP (2005) Application of metabolite profiling to the identification of traits in a population of tomato introgression lines. J Exp Bot 56:287–296

Pathak N, Asif MH, Dhawan P, Srivastava MK, Nath P (2003) Expression and activities of ethylene biosynthesis enzymes during ripening in banana fruits and effect of 1 MCP treatment. Plant Growth Regul 40:11–19

Pech JC, Bouzayen M, Latché A (2008a) Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit. Plant Sci 175:114–120

Pech JC, Latché A, van der Rest B (2008b) Genes involved in the biosynthesis of aroma volatiles in fruit and vegetables and biotechnological applications. In: Brückner B, Wyllie SG (eds) Fruit and vegetable flavour: recent advances and future prospects. Woodhead, Cambridge, pp 254–271

Périn C, Gomez-Jimenez MC, Hagen L, Dogimont C, Pech JC, Latché A, Pitrat M, Lelièvre JM (2002) Molecular and genetic characterisation of a non-climacteric phenotype in melon reveals two loci conferring altered ethylene response in fruit. Plant Physiol 129:300–209

Picton SJ, Barton SL, Bouzayen M, Hamilton AJ, Grierson D (1993) Altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene-forming enzyme transgene. Plant J 3:469–481

Pirrello J, Jaimes-Miranda F, Sanchez-Ballesta MT, Tournier B, Khalil-Ahmad Q, Regad F, Latché A, Pech JC, Bouzayen M (2006) *Sl-ERF2*, a tomato ethylene response factor involved in ethylene response and seed germination. Plant Cell Physiol 47:1195–1205

Resnick JS, Wen CK. Shockey JA, Chang C (2006) *REVERSION-TO-ETHYLENE SENSITIVITY1*, a conserved gene that regulates ethylene receptor function in Arabidopsis. Proc Natl Acad Sci USA 103:7917–7922

Riechmann JL, Heard J, Martin G, Reuber L, Jiang CZ, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu GL (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. Science 290:2105–2110

Ronen G, Cohen M, Zamir D, Hirschberg J (1999) Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant *Delta*. Plant J 17:341–351

Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to β-carotene formation in plant chloroplasts discovered by map-based cloning of beta and old-gold color mutations of tomato. Proc Natl Acad Sci USA 97:11102–11107

Rosado A, Schapire AL, Bressan RA, Harfouche AL, Hasegawa PM, Valpuesta V, Botella MA (2006) The Arabidopsis tetratricopeptide repeat-containing protein TTL1 is required for osmotic stress responses and abscisic acid sensitivity. Plant Physiol 142:1113–1126

Salveit ME Jr (1993) Internal carbon dioxide and ethylene levels in ripening tomato fruit attached to or detached from the plant. Physiol Plant 89:204–210

Sanz C, Olias JM, Perez AG (1997) Aroma biochemistry of fruits and vegetables. In: Tomas-Barberan FA (ed) Phytochemistry of fruit and vegetables. Oxford Science, Oxford, pp 125–155

Sato T, Theologis A (1989) Cloning of the mRNA encoding 1-aminocyclopropane-1-carboxylate synthase, the key enzyme for ethylene biosynthesis in plants. Proc Natl Acad Sci USA 86:6621–6625

Seymour G, Poole M, Manning K, King G (2007) Genetics and epigenetics of fruit development and ripening. Curr Opin Plant Biol 10:1–6

Shellie KC, Salveit ME Jr (1993) The lack of a respiratory rise in muskmelon fruit ripening on the plant challenges the definition of climacteric behaviour. J Exp Bot 44:1403–1406

Sisler EC, Serek M, Dupille E, Goren R (1999) Inhibition of ethylene responses by 1-methylcyclopropene and 3-methylcyclopropene. Plant Growth Regul 27:105–111

Smith DL, Abbott JA, Gross KC (2002) Down-regulation of tomato β-galactosidase 4 results in decreased fruit softening. Plant Physiol 129:1755–1762

Solano R, Stepanova A, Chao QM, Ecker JR (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 12:3703–3714

Stepanova AN, Yun J, Likhacheva AV, Alonso JM (2007) Multilevel interactions between ethylene and auxin in *Arabidopsis* roots. Plant Cell 19:2169–2185

Sunako T, Sakuraba W, Senda M, Akada S, Ishikawa R, Niizeki M, Harada T (1999) An allele of the ripening-specific 1-aminocyclopropane-1-carboxylate synthase gene (ACS1) in apple fruit with a long storage life. Plant Physiol 119:1297–1303

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tieman DM, Harriman RW, Ramamohan G, Handa AK (1992) An antisense pectin methylesterase gene alters pectin chemistry and soluble solids in tomato fruit. Plant Cell 4:667–669

Tomes ML (1969) Delta-carotene in the tomato. Genetics 62:769–780

Vaysse P, Scandella D, Masseron A, Mathieu V, Trillot M, Marion M (2000) Recognizing apple and pear varieties. Centre Technique Interprofessionnel des Fruits Légumes, Paris

Vrebalov J, Ruezinsky D, Padmanabahn V, White R, Medrano D, Drake R, Schuch W, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato *ripening-inhibitor* (*rin*) locus. Science 296:343–346

Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, Chaabouni S, Latché A, Pech JC, Bouzayen M (2005) Le*IAA9*, a tomato *Aux*/*IAA* gene involved in fruit development and leaf morphogenesis. Plant Cell 17:2676–2692

Wang H, Schauer N, Usadel B, Frasse P, Zouine M, Hernould M, Latché A, Pech JC, Fernie AR, Bouzayen M (2009) Regulatory features underlying pollination-dependent and -independent tomato fruit set revealed by transcript and primary metabolite profiling. Plant Cell 21:1428–1452

Wang A, Tan D, Takahashi A, Zhong Li T, Harada T (2007) MdERFs, two ethylene-response factors involved in apple fruit ripening. J Exp Bot 58:3743–3748

Watkins CB (2002) Ethylene synthesis: mode of action, consequences, and control. In: Knee M (ed) Fruit quality and its biological basis. Sheffield Academic Press, Sheffield, pp 180–224

Wilkinson JQ, Lanahan MB, Yen H-C, Giovannoni JJ, Klee HJ (1995) An ethylene inducible component of signal transduction encoded by Never-ripe. Science 270:1807–1809

Winkel-Shirley B (2001) Flavonoids biosynthesis. A colourful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol 126:485–493

Yahyaoui EF, Wongs-Aree C, Latché A, Hackett R, Grierson D, Pech JC (2002) Molecular and biochemical characteristics of a gene encoding an alcohol acyl-transferase involved in the generation of aroma volatile esters during melon ripening. Eur J Biochem 269:2359–2366

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35:155–189

Zegzouti H, Jones B, Frasse P, Marty C, Maitre B, Latché A, Pech JC, Bouzayen M (1999) Ethylene-regulated gene expression in tomato fruit: characterization of novel ethylene-responsive and ripening-related genes isolated by differential display. Plant J 18:589–600

Zheng XY, Wolff DW (2000) Ethylene production, shelf-life and evidence of RFLP polymorphisms linked to ethylene genes in melon (*Cucumis melo* L.). Theor Appl Genet 101:613–624

Zheng XY, Wolff D, Crosby WKM (2002) Genetics of ethylene biosynthesis and restriction fragment length polymorphism (RFLPs) of ACC oxidase and synthase genes in melon (*Cucumis melo* L.) Theor Appl Genet 105:397–403

Zhou X, Liu Q, Xie F, Wen C (2007) RTE1 is a Golgi-associated and ETR1-dependent negative regulator of ethylene responses. Plant Physiol 145:75–86