THE MOLECULAR BIOLOGY AND BIOCHEMISTRY OF FRUIT RIPENING

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Preface

Evolution has fashioned multiple means of protecting seed and dispersing them upon maturation. None is as fascinating nor as consequential to humankind as the ripe and delectable fleshy fruit. Ripe fruits comprise a significant and expanding proportion of human and animal diets, which the medical community contends should only be increased. In addition to being visual delights with seductive tastes and aromas, ripe fruits deliver a diverse array of antioxidants and nutrients to those who consume them, in addition to healthy doses of carbohydrates and fiber. The chemistry of fruits comprises attributes that producers, processors, and distributors alike seek to understand, optimize, and deliver to increasingly health-conscious consumers expecting high quality and diversity of choices. Plant scientists have endeavored to unravel the mysteries of fleshy fruit biology and the underlying molecular and biochemical processes that contribute to fruit ripening and the resulting desirable attributes of fruits and fruit products.

This book offers a useful overview of fruit ontology and evolution emphasizing the exponential growth in advances and discoveries in ripening-related chemistry and associated regulatory processes accumulated in the last decade. The reader will appreciate the broad and deep impact of comprehensive genomics and metabolomics in addition to the computational tools necessary to decipher the resulting data on the progress of the field. As a consequence of these all-encompassing approaches, fruit biology has advanced from the investigation of single genes and enzymatic reactions to the development of nuanced molecular regulatory models overseeing complex biochemical pathways leading to numerous metabolic outputs. Looking at the physiological and molecular symphony of events impacting textural changes of the ripening fruit, the array of novel phenolic metabolites, or the network of genes and signaling processes regulating ethylene hormone response, it becomes strikingly clear that recent technical advances have moved ripening biology forward at an astounding rate. This book captures the advances of the field and couches them in an evolutionary context and a fundamental knowledge of fruit biology, making it an excellent primer for those interested in the field and a comprehensive reference for those familiar with it. *The Molecular Biology and Biochemistry of Fruit Ripening* is essential reading for any student of plant science and those especially interested in fruit biology and its relationship to human diet and nutrition.

1 Biochemistry of Fruit Ripening

Sonia Osorio and Alisdair R. Fernie

Introduction

This chapter is intended to provide an overview of the key metabolic and regulatory pathways involved in fruit ripening, and the reader is referred to more detailed discussions of specific topics in subsequent chapters.

The quality of fruit is determined by a wide range of desirable characteristics such as nutritional value, flavor, processing qualities, and shelf life. Fruit is an important source of supplementary diet, providing minerals, vitamins, fibers, and antioxidants. In particular, they are generally rich sources of potassium, folate, vitamins C, E, and K as well as other phytonutrients such as carotenoids (beta-carotene being a provitamin A) and polyphenols such as flavonols (Saltmarsh et al., 2003). A similar, but perhaps more disparate, group of nutrients is associated with vegetables. Thus nutritionists tend to include fruits and vegetables together as a single "food group," and it is in this manner that their potential nutritional benefits are normally investigated and reported. Over the past few decades, the increased consumption of fruits and vegetables has been linked to a reduction in a range of chronic diseases (Buttriss, 2012). This has led the WHO to issue a recommendation for the consumption of at least 400 g of fruits and vegetables per day. This in turn has prompted many countries to issue their own recommendations regarding the consumption of fruits and vegetables. In Britain this has given rise to the five-a-day recommendation. A portion in the United Kingdom is deemed to be around 80 g; so five-a-day corresponds to about 400 g per day. Other countries have opted for different recommendations (Buttriss, 2012), but all recognize the need for increased consumption.

The rationale for the five-a-day and other recommendations to increase fruit and vegetable consumption comes from the potential link between high intake of fruits and vegetables and low incidence of a range of diseases. There have been many studies carried out over the last few decades. The early studies tended to have a predominance of case-control approaches while recently more cohort studies, which are considered to be more robust, have been carried out. This has given rise to many critical and systematic reviews, examining this cumulative

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evidence base, over the years which have sometimes drawn disparate conclusions regarding the strength of the links between consumption and disease prevention (Buttriss, 2012). One of the most recent (Boeing et al., 2012) has concluded that there is convincing evidence for a link with hypertension, chronic heart disease, and stroke and probable evidence for a link with cancer in general. However, there might also be probable evidence for an association between specific metabolites and certain cancer states such as between carotenoids and cancers of the mouth and pharynx and beta-carotene and esophageal cancer and lycopene and prostate cancer (WRCF and American Institute for Cancer Research, 2007). There is also a possible link that increased fruit and vegetable consumption may prevent body weight gain. This reduces the propensity to obesity and as such could act as an indirect reduction in type 2 diabetes, although there is no direct link (Boeing et al., 2012). Boeing et al. (2012) also concluded there is possible evidence that increased consumption of fruits and vegetables may be linked to a reduced risk of eye disease, dementia, and osteoporosis. In almost all of these studies, fruits and vegetables are classed together as a single "nutrient group." It is thus not possible in most cases to assign relative importance to either fruits or vegetables. Similarly, there is very little differentiation between the very wide range of botanical species included under the banner of fruits and vegetables and it is entirely possible that beneficial effects, as related to individual disease states, may derive from metabolites found specifically in individual species.

Several studies have sought to attribute the potential beneficial effects of fruits and vegetables to specific metabolites or groups of metabolites. One such which has received a significant amount of interest is the antioxidants. Fruit is particularly rich in ascorbate or vitamin C which represents one of the major water-soluble antioxidants in our diet and also in carotenoids such as beta-carotene (provitamin A) and lycopene which are fat-soluble antioxidants (Chapter 4). However, intervention studies using vitamin C or indeed any of the other major antioxidants, such as beta-carotene, often fail to elicit similar protective effects, especially in respect of cancer (Stanner et al., 2004). Polyphenols are another group of potential antioxidants that have attracted much attention in the past. The stilbene—resveratrol—which is found in grapes, for example, has been associated with potential beneficial effects in a number of diseases (Baur and Sinclair, 2006). Similarly, the anthocyanins (Chapter 5), which are common pigments in many fruits, have again been implicated with therapeutic properties (Zafra-Stone et al., 2007). It is possible that these individual molecules may be having quite specific nutrient–gene expression effects. It is difficult to study these effects *in vivo*, as bioavailability and metabolism both in the gut and postabsorption can be confounding factors.

Although there are recommendations across many countries regarding the consumption of fruits and vegetables, in general, the actual intake falls below these recommendations (Buttriss, 2012). However, trends in consumption are on the increase driven potentially by increasing nutritional awareness on the part of the consumer and an increasing diversity of available produce. Fruit is available either fresh or processed in a number of ways the most obvious being in the form of juices or more recently smoothies. The list of fruits and vegetables traded throughout the world is both long and diverse. The FAO lists over 100 "lines" of which 60 are individual fruits or vegetables or related groups of these commodities. The remaining "lines" are juices and processed or prepared material. However, the top five traded products are all fruits and these are banana, tomato, apple, grape, and orange. In 1982–1984 these five between

Table 1.1 Global production, consumption, and net export of the five major (million tons) fruit commodities in 2002–2004. Data from European Commission Directorate-General for Agriculture and Rural Development (2007).

Commodity	Production	Consumption	Net Export
Banana	71	58	12.9
Tomato	119	103	2.1
Apple	59	56	3
Grape	64	59	1.7
Orange	63	53	2.5

them accounted for around half of global trade in fruits and vegetables; by 2002–2004, this had fallen to around 40% (European Commission Directorate-General for Agriculture and Rural Development, 2007). This probably reflects a growing trend toward diversification in the fruit market, especially in respect of tropical fruit. These figures represent traded commodities and in no way reflect global production of these commodities. In fact only about 5–10% of global production is actually traded. The EU commissioned a report in 2007 to examine trends in global production, consumption, and export of fruits and vegetables between 1980–1982 and 2002–2004. This demonstrated that fruits and vegetables represented one of the fastest growing areas of growth within the agricultural markets with total global production increasing by around 94% during this period. Global fruit production in 2004 was estimated at 0.5 billion tonnes. The growth in fruit production, at 2.2% per annum, was about half that for vegetables during this period. The report breaks these figures down into data for the most commonly traded commodities and the results for production, consumption, and net export in 2002–2004 are summarized in Table 1.1. Not all of the five major fruit commodities increased equally during this period. Banana and tomato production both doubled; apple and orange production both went up by about 50% while grape stagnated or even declined slightly during this period.

Global consumption of fruits and vegetables rose by 52% between 1992–2004 and 2002–2004 (European Commission Directorate-General for Agriculture and Rural Development, 2007). This means that global fruit and vegetable consumption rose by around 4.5% per annum during this period. This exceeded the population growth during the same period and as such suggested an increased consumption per capita of the population. Again the results for the consumption amongst the five major traded crops were variable with increases of banana, tomato being higher at 3.9% per annum and 4.5% per annum, respectively, while grapes (1.6% per annum) and oranges (1.9% per annum) were lower.

The net export figures reported above do not include trade between individual EU countries; however, even taking this into account, it is clear that only a small proportion of fruit production enters international trade. A major problem with trade in fresh fruit is the perishable nature of most of the commodities. This requires rapid transport or sophisticated means of reducing or modifying the fruits' metabolism. This can be readily achieved for some fruits, such as apple, by refrigeration; however, several fruits, such as mango, are subject to chilling injury that limits this approach. Other methods that are employed are the application of controlled or modified atmospheres (Jayas and Jeyamkondan, 2002). Generally an increase in carbon

dioxide accompanied by a reduction in oxygen, will serve to reduce ethylene synthesis and respiration rate. The application of chemicals such as 1-MCP, an ethylene analog, can also significantly reduce ripening rates (Blankenship and Dole, 2003). Genetically modifying the fruit, for instance to reduce ethylene production, can also lead to an increase in shelf life (Picton et al., 1993).

Fruit ripening is highly coordinated, genetically programmed, and an irreversible developmental process involving specific biochemical and physiological attributes that lead to the development of a soft and edible fruit with desirable quality attributes (Giovannoni, 2001). The main changes associated with ripening include color (loss of green color and increase in nonphotosynthetic pigments that vary depending on species and cultivar), firmness (softening by cell-wall-degrading activities), taste (increase in sugar and decline in organic acids), and odor (production of volatile compounds providing the characteristic aroma). While the majority of this chapter will concentrate on central carbon metabolism, it is also intended to document progress in the understanding of metabolic regulation of the secondary metabolites of importance to fruit quality. These include vitamins, volatiles, flavonoids, pigments, and the major hormones. The interrelationship of these compound types is presented in Figure 1.1. Understanding the mechanistic basis of the events that underlie the ripening process will be critical for developing more effective methods for its control.

Central Carbon Metabolism

Sucrose, glucose, and fructose are the most abundant carbohydrates and are widely distributed food components derived from plants. The sweetness of fruits is the central characteristic determining fruit quality and it is determined by the total sugar content and by their ratios among those sugars. Accumulation of sucrose, glucose, and fructose in fruits such as melons, watermelons (Brown and Summers, 1985), strawberries (Fait et al., 2008) and peach (Lo Bianco and Rieger, 2002) is evident during ripening; however, in domesticated tomato (*Solanum lycopersicum*) only a high accumulation of the two hexoses is observed, whereas some wild tomato species (i.e., *Solanum chmielewskii*) accumulate mostly sucrose (Yelle et al., 1991). The variance in relative levels of sucrose and hexoses is most likely due to the relative activities of the enzymes responsible for the degradation of sucrose, invertase, and sucrose synthase.

The importance of the supply to, and the subsequent mobilization of sucrose in, plant heterotrophic organs has been the subject of intensive research effort over many years (Miller and Chourey, 1992; Zrenner et al., 1996; Wobus and Weber, 1999; Heyer et al., 2004; Roitsch and Gonzalez, 2004; Biemelt and Sonnewald, 2006; Sergeeva et al., 2006; Lytovchenko et al., 2007). While the mechanisms of sucrose loading into the phloem have been intensively studied over a similar time period (Riesmeier et al., 1993; Burkle et al., 1998; Meyer et al., 2004; Sauer et al., 2004), those by which it is unloaded into the sink organ (the developing organs attract nutrients) have only been clarified relatively recently and only for a subset of plants studied (Bret-Harte and Silk, 1994; Viola et al., 2001; Kuhn et al., 2003; Carpaneto et al., 2005). Recently, in the tomato fruit, the path of sucrose unloading in early developmental stages has been characterized as apoplastic. The study used tomato introgression lines containing

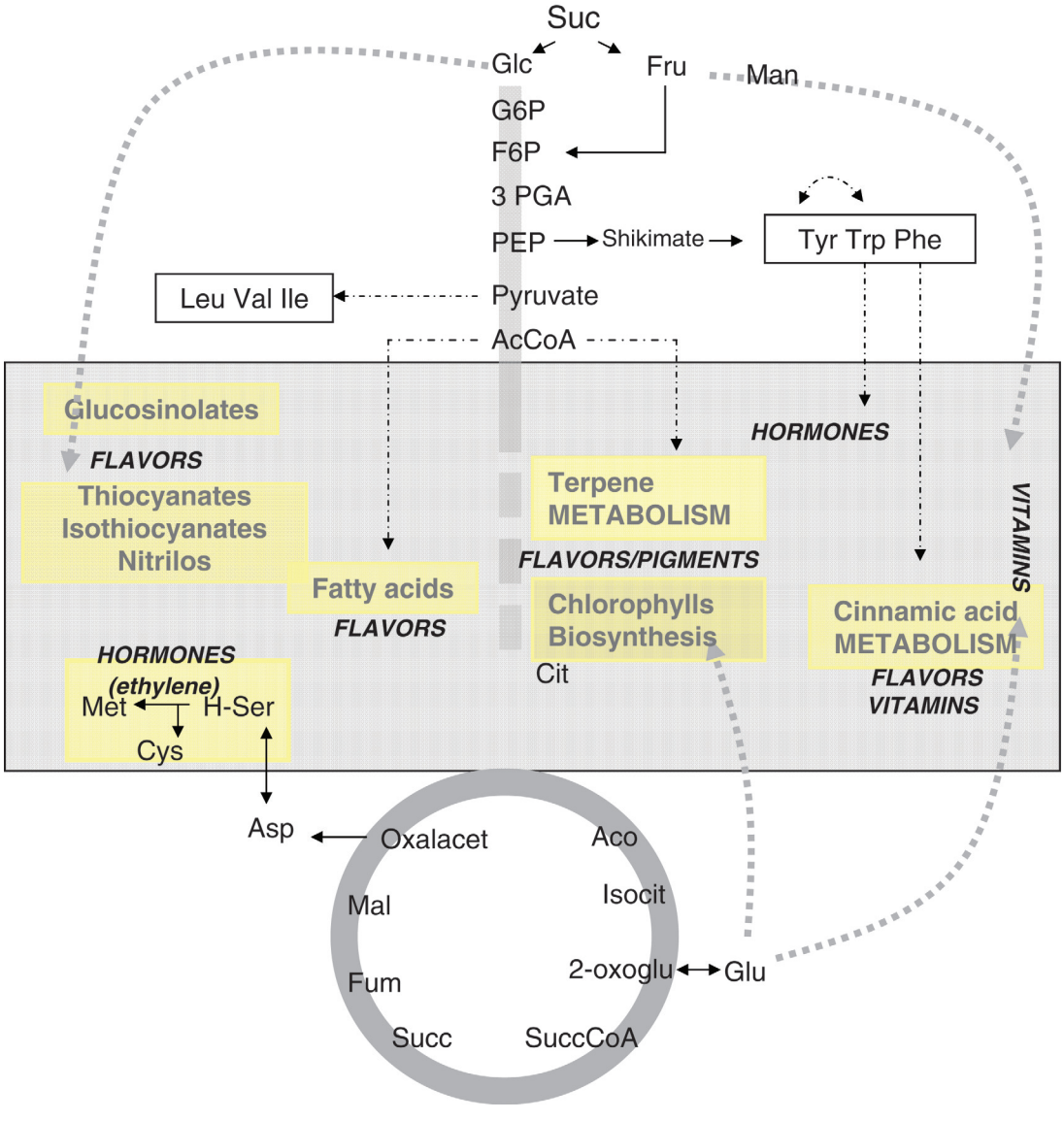

Figure 1.1 Interrelationships of primary and secondary metabolism pathways leading to the biosynthesis of aroma volatiles, hormones, pigments and vitamins (adapted from Carrari and Fernie (2006)).

an exotic allele of LIN5, a cell wall invertase that is exclusively expressed in flower (mainly ovary but also petal and stamen) and in young fruit (Godt and Roitsch, 1997; Fridman and Zamir, 2003), and it has been demonstrated that alterations in the efficiency of this enzyme result in significantly increased partitioning of photosynthate to the fruit and hence an enhanced agronomic yield (Fridman et al., 2004; Baxter et al., 2005; Schauer et al., 2006). Utilizing the reverse genetic approach, Zanor et al. (2009a) reported that *LIN5* antisense plants had decreased

glucose and fructose in the fruit proving *in planta* the importance of LIN5 in the control of the total soluble solids content. The transformants were characterized by an altered flower and fruit morphology, displaying increased numbers of petals and sepals per flower, an increased rate of fruit abortion, and a reduction in fruit size. Evaluation of the mature fruit revealed that the transformants had a reduction of seed number per plant as well as altered levels of phytohormones. Interestingly, a role for apoplastic invertase in the control of sink size has been postulated previously in other species; the apoplastic invertase-deficient *miniature1* mutant of maize exhibits a dramatically decreased seed size as well as altered levels of phytohormones (Miller and Chourey, 1992; Sonnewald et al., 1997; LeClere et al., 2008). This raises interesting questions regarding the regulation of carbon partitioning in fruits. Recently, a metabolic and transcriptional study using introgression lines resulting from a cross between *S. lycopersicum* and *S. chmielewskii* have revealed that the dramatic increase in amino acid content in the fruit is the result of an upregulated transport of amino acids via the phloem, although the mechanism is still unknown (Do et al., 2010).

Starch is another carbohydrate that undergoes modifications during ripening. The tomato introgression lines containing the exotic allele of LIN5 (IL 9-2-5) accumulated significantly more starch in both, pericarp and columella tissues (Baxter et al., 2005). This is in agreement with the finding that starch accumulation plays an important role in determining the soluble solids content or Brix index of mature fruit (Schaffer and Petreikov, 1997). Recently, in tomato fruits, reduction of the activities of either mitochondrial malate dehydrogenase (mMDH) or fumarase via targeted antisense approaches have demonstrated the physiological importance of malate metabolism in the activation state of ADP-glucose pyrophosphorylase (AGPase) that is correlated with the accumulation of transitory starch and also with the accumulation of soluble solids at harvest (Centeno et al., 2011).

Organic acid manipulation is highly valuable from a metabolic engineering perspective because the organic acid to sugar ratio defines quality parameters at harvest time in fruits. However, their study has received much less attention than that of the sugars to date. Malate is the predominant acid in many fruits, both climacteric, including tomato (Kortstee et al., 2007), apple (Beruter, 2004), and nonclimacteric, including pineapple (Saradhuldhat and Paull, 2007), cherry (Usenik et al., 2008), strawberry (Moing et al., 2001), and grape (Kliewer et al., 1967). Interestingly, levels of both citrate and malate were also highly correlated to many important regulators of ripening in an independent study that was focused on early fruit development (Mounet et al., 2009). Patterns of malate accumulation differ between plant species and even cultivars (Kliewer et al., 1967). In fruits, patterns of malate accumulation and degradation cannot be explained by the classification of species as climacteric or nonclimacteric, nor can they be attributed to changes in overall respiration rates. Some climacteric fruits such as plum and tomato appear to utilize malate during the respiratory burst (Goodenough et al., 1985; Kortstee et al., 2007), while others such as banana and mango continue to accumulate malate throughout ripening, even at the climacteric stage (Selvaraj and Kumar, 1989a; Agravante et al., 1991). Nonclimacteric fruits also display widely varying malate accumulation and degradation events (Moing et al., 2001; Saradhuldhat and Paull, 2007); some fruits, including mango, kiwifruit, and strawberry display no net loss of malate throughout ripening (Selvaraj and Kumar, 1989a; Walton and De Jong, 1990; Moing et al., 2001). For this reason, the metabolism of malate

has been a strong focus of research on grapes and tomato fruits, in which the acid plays a more metabolically active role (Goodenough et al., 1985). In grapefruit, malate is increasing in earlier stages and then is decreasing during ripening (Ruffner and Hawker, 1977). In earlier stages, malate is accumulated mostly through the metabolism of sugars (Hale, 1962) and during ripening, malate is a vital source of carbon for different pathways: TCA cycle and respiration, gluconeogenesis, amino acid interconversion, ethanol fermentation, and production of secondary compounds such as anthocyanins and flavonols (Ruffner, 1982; Famiani et al., 2000). Work with tomato fruit suggests that in early development, the majority of malate oxidation occurs through the TCA cycle.

The structure of the TCA cycle is well known in plants; however, until recently its regulation was poorly characterized. In our laboratory, several studies have been pursued to determine the role of mitochondrial TCA cycle in plants. Biochemical analysis of the *Aco1* mutant revealed that it exhibited a decreased flux through the TCA cycle, decreased levels of TCA cycle intermediates, enhanced carbon assimilation, and dramatically increased fruit weight (Carrari et al., 2003). Nunes-Nesi et al. (2005) produced tomato plants with reduced mMDH activity. Plants showed an increment in fruit dry weight likely due to the enhanced photosynthetic activity and carbon assimilation in the leaves, which also led to increased accumulation of starch and sugars, as well as some organic acids (succinate, ascorbate, and dehydroascorbate). Reduction of fumarase activity has been investigated in tomato plants (Nunes-Nesi et al., 2007), which led to lower fruit yield and total dry weight. Those plants showed opposite characteristics to plants that were impaired for mMDH activity. Additionally, biochemical analyses of antisense tomato mitochondrial NAD-dependent isocitrate dehydrogenase plants revealed clear reduction in flux through the TCA cycle, decreased levels of TCA cycle intermediates, and relatively few changes in photosynthetic parameters; however, fruit size and yield were reduced (Sienkiewicz-Porzucek et al., 2010). All those studies have been performed on the illuminated leaf; recently, it has characterized tomato plants independently exhibiting a fruit-specific decreased expression of genes encoding consecutive enzymes of the TCA cycle, fumarase, and mMDH (Centeno et al., 2011). Detailed biochemical characterization revealed that the changes in starch concentration, and consequently soluble solids content, were likely due to a redox regulation of AGPase. Those plants showed also a little effect on the total fruit yield as well as unanticipated changes in postharvest shelf life and susceptibility to bacterial infection. Despite the fact that much research work is needed to understand the exact mechanism for the increment in the fruit dry matter, manipulation of central organic acids is clearly a promising approach to enhance fruit yield (Nunes-Nesi et al., 2011).

Ethylene in Ripening

Based on the respiratory pattern and ethylene biosynthesis during ripening, fruits have been classified either as “climacteric” or “nonclimacteric.” Climacteric fruits such as tomato show an increase in respiration rate and ethylene formation. Nonclimacteric fruits do not increase respiration, although they produce a little ethylene during ripening and do not respond to external ethylene treatment (Giovannoni, 2001). This difference is one of the main reasons

that the majority of biochemical research has concentrated on this hormone. The role of ethylene in ripening of climacteric fruits has been known for more than 50 years (see Chapter 3). Since then, considerable effort has been focused on the studies of ethylene biosynthesis (S-adenosylmethionine, SAM; SAM synthetase; 1-aminocyclopropane carboxylic acid; ACC synthase; and ACC oxidase), ethylene perception (ethylene receptors, ETRs); signal transduction (ethylene response factor, ERFs); and ethylene-regulated genes such as cell-wall-disassembling genes (endopolygalacturonase; pectin methyl esterase, PME; and pectate lyase).

The *Arabidopsis* model system has served as starting point in the knowledge of the steps involved in ethylene perception and signal transduction; however, more efforts in understanding the ethylene response during fruit ripening have focused on the characterization of tomato homologs (Giovannoni, 2007). In this vein, six ethylene receptors have been isolated in tomato (*ETHYLENE RECEPTOR1*, *LeETR1; ETHYLENE RECEPTOR2*, *LeETR2; ETHYLENE RECEPTOR5*, *LeETR5; NEVER-RIPE, NR; ETHYLENE RECEPTOR4*, *LeETR4;* and *ETHYLENE RECEPTOR6*, *LeETR6*) compared to five members in *Arabidopsis* (*ETHYLENE RECEPTOR1*, *ETR1; ETHYLENE RECEPTOR2*, *ETR2; ETHYLENE RESPONSE SENSOR1, ERS1; ETHYLENE RESPONSE SENSOR2, ERS2; and ETHYLENE INSENSITIVE4, EIN4*) (Bleecker, 1999; Chang and Stadler, 2001). Five of the six tomato receptors have shown to bind ethylene (Klee and Tieman, 2002; Klee, 2002) but expression studies have been shown different profiles. Transcript levels of *LeETR1*, *LeETR2*, and *LeETR5* change little upon treatment of ethylene in fruit, where *NR*, *LeETR4*, and *LeETR6* are strongly induced during ripening (Kevany et al., 2007). Interestingly, analysis of transgenic plants with reduced *LeETR4* and *LeETR6*, caused an early ripening phenotype (Kevany et al., 2007; Kevany et al., 2008). On the other hand, *NR* mutation resulted in not fully ripened fruit (Wilkinson et al., 1995; Yen et al., 1995). Nevertheless, analysis of transgenic plants with reduction in *NR* levels suggested that this gene was not necessary for ripening to proceed (Hackett et al., 2000), suggesting that the other fruit-specific member of the receptor family has compensatory upregulation (Tieman et al., 2000). Overexpression of the *NR* receptor in tomato resulted in reduced sensitivity in seedlings and mature plants (Ciardi et al., 2000). This is in agreement with models where ethylene receptors act as negative regulators of ethylene signaling (Klee and Tieman, 2002; Klee, 2002). Consistent with this model, an exposure of immature fruits to ethylene caused a reduction in the amount of ethylene receptor protein and earlier ripening (Kevany et al., 2007). Recently, further ethylene-inducible (*CONSTITUTIVE TRIPLE RESPONSES MAP kinase kinase, CTR*) family of four genes have been identified in tomato (*LeCTR1, LeCTR2, LeCTR3, and LeCTR4*). Like *NR, LeETR4,* and *LeETR6, LeCRT1* is also upregulated during ripening (Adams-Phillips et al., 2004). Recently, studies of two-hybrid yeast interaction assay of tomato ethylene receptor and LeCTR proteins have demonstrated that those proteins are capable of interacting with NR (Zhong et al., 2008), reinforcing the idea that ethylene receptors transmit the signal to the downstream CTRs.

Recently, genomics approaches have provided insight into primary ripening control upstream of ethylene (Chapter 8). Tomato pleiotropic ripening mutations, *ripening inhibitor* (*rin*), *non-ripening* (*nor*), and *Colorless nonripening* (C*nr)* have added great insights in this regard. The *rin*, *nor,* and *Cnr* mutations are affected in all aspects of the tomato fruit ripening process that are unable to respond to ripening-associated ethylene genes (Vrebalov et al., 2002; Manning

et al., 2006). Furthermore, in fruits from those mutants, the ripening-associated ethylene genes are induced by exogenous ethylene indicating that all three genes operate upstream of ethylene biosynthesis and are involved in process controlled exclusively by ethylene. The three mutant loci encode putative transcription factors. The *rin* encoded a partially deleted MADS-box protein of the SEPALLATA clade (Hileman et al., 2006), where *Cnr* is an epigenetic change that alters the promoter methylation of SQUAMOSA promoter binding (SPB) proteins. Manning et al. (2006) and J. Vrebalov and J. Giovannoni (unpublished results) suggest that the *nor* loci encodes a transcription factor, although not a member of MADS-box family. The observed ethylene-independent aspect of ripening suggests that RIN, NOR, and CNR proteins are candidates for conserved molecular mechanisms of fruit in both the climacteric and nonclimacteric categories.

Biochemical evidence suggests that ethylene production may be influenced or regulated by interactions between its biosynthesis and other metabolic pathways. One such example is provided by the fact that SAM is the substrate for both the polyamine pathway and the nucleic acid methylation; the competition for substrate was demonstrated by the finding that the overexpression of a SAM hydrolase has been associated with inhibited ethylene production during ripening (Good et al., 1994). On the other hand, the methionine cycle directly links ethylene biosynthesis to the central pathways of primary metabolism.

Polyamines

The most common plant polyamines are the diamine putrescine and the higher polyamines spermidine and spermine and it is known to be implicated in different biological processes, including cell division, cell elongation, embryogenesis, root formation, floral development, fruit development and ripening, pollen tube growth and senescence, and in response to biotic and abiotic stress (Kaur-Sawhney et al., 2003). In plants, putrescine is synthesized from arginine, a reaction catalyzed by arginine decarboxylase, or from ornithine by ornithine decarboxylase. Spermidine is synthesized from putrescine and SAM. SAM as a key intermediate for ethylene (Good et al., 1994; Fluhr and Mattoo, 1996; Giovannoni, 2004) has the potential to commit the flux of SAM either into polyamine biosynthesis, ethylene biosynthesis, or both. The overexpression of a SAM hydrolase has been associated with inhibited ethylene production during ripening (Good et al., 1994) which led to suggestions that changes in the levels of polyamines and ethylene may influence specific physiological processes in the plant (Kaur-Sawhney et al., 2003).

Mattoo et al. (2007) produced tomato fruits with increased SAM decarboxylase, in an attempt to over-accumulate spermidine and spermine whose levels decline during normal ripening process in tomato (Mehta et al., 2002). In the metabolite levels, those fruits showed prominent changes which influence multiple cellular pathways in diverse subcellular compartments such as mitochondria, cytoplasm, chloroplasts, and chromoplasts during fruit ripening. Red fruits showed upregulation of phosphoenolpyruvate carboxylase (PEPC) and cytosolic isocitrate dehydrogenase (ICDHc) as well as increase in the levels of glutamate, glutamine, asparagine, and organic acids; those of aspartate, valine, glucose, and sucrose showed a decrease compared to the wild type. The authors suggested that spermidine and spermine are perceived as signals of carbon metabolism in order to optimize C and N budgets following similar N regulatory