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MOLECULAR BIOLOGY OF FRUIT MATURATION AND RIPENING

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■ **Abstract** The development and maturation of fruits has received considerable scientific scrutiny because of both the uniqueness of such processes to the biology of plants and the importance of fruit as a significant component of the human diet. Molecular and genetic analysis of fruit development, and especially ripening of fleshy fruits, has resulted in significant gains in knowledge over recent years. Great strides have been made in the areas of ethylene biosynthesis and response, cell wall metabolism, and environmental factors, such as light, that impact ripening. Discoveries made in *Arabidopsis* in terms of general mechanisms for signal transduction, in addition to specific mechanisms of carpel development, have assisted discovery in more traditional models such as tomato. This review attempts to coalesce recent findings in the areas of fruit development and ripening.

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INTRODUCTION

In their constant effort to yield subsequent generations of viable and competitive progeny, plant species have evolved numerous mechanisms for seed dispersal. Fruit are an integral part of this endeavor and can be narrowly defined as mature carpels. This definition accurately describes the fruits of tomato, melons, and stone fruits, to name just a few. A more accurate and inclusive definition encompasses extracarpellary tissues that are included at the mature fruiting stage. Examples of such additional tissues in more complex fruits include the receptacle in strawberry and the bracts of pineapple. Fruits can be additionally separated into dehiscent, or dry, fruits and non-dehiscent, or fleshy, fruits. Examples of dehiscent fruits include the pods of legumes and the siliques of many of the Brassicaceae, including *Arabidopsis thaliana*. Analysis of floral development–related MADS-box genes in *Arabidopsis* has been particularly relevant toward initiating the dissection of the molecular basis of fruit development and make up a portion of the discussion here (for recent review, see 38).

The ripening process renders fruit attractive and palatable to a variety of seed-dispersing organisms and typifies non-dehiscent (fleshy) fruits. Because of the dual role of non-dehiscent fruits as both a unique aspect of plant development and the source of a large portion of the human diet, the molecular basis of development and ripening of fleshy fruits has received considerable scientific attention in recent years and constitutes the majority of this review. Previous reviews of the molecular regulation of fruit ripening have focused primarily on tomato, cell wall metabolism in particular, and the effects of the gaseous hormone ethylene (19, 46, 48, 52, 81, 161). The ripe phenotype is the summation of biochemical and physiological changes that occur at the terminal stage of fruit development and render the organ edible and desirable to seed-dispersing animals. Ripening also imparts value to fruit as agricultural commodities. These changes, although variable among species, generally include modification of cell wall ultrastructure and texture, conversion of starch to sugars, increased susceptibility to post-harvest pathogens, alterations in pigment biosynthesis and accumulation, and heightened levels of flavor and aromatic volatiles [for reviews on fruit physiology and biochemistry, see (116, 128)]. One of the key regulatory questions relative to the ripening process is, "How is the collection of otherwise unrelated pathways and processes coordinated to act efficiently and synchronously during this stage of fruit development?" Additionally, from a practical viewpoint, several ripening attributes translate to decreased shelf-life and high-input harvest, shipping, and storage practices, particularly as a result of changes in firmness and the overall decrease in resistance to microbial infection of ripe fruit. The 1990s have been a time of significant advances in our understanding of the molecular regulation of individual ripening parameters in which significant insights into their coordination have been revealed. The resulting knowledge has contributed to a more complete view of molecular ripening control and has

produced the first molecular tools for addressing problems in fruit production and quality.

CLIMACTERIC AND NON-CLIMACTERIC RIPENING

Although most fruit display modifications in color, texture, flavor, and pathogen susceptibility during maturation, two major classifications of ripening fruit, climacteric and non-climacteric, have been utilized to distinguish fruit on the basis of respiration and ethylene biosynthesis rates. Climacteric fruit, such as tomato, cucurbits, avocado, banana, peaches, plums, and apples, are distinguished from non-climacteric fruits, such as strawberry, grape, and citrus, by their increased respiration and ethylene biosynthesis rates during ripening (81). Although non-climacteric fruits, such as citrus, may respond to ethylene (an example being ethylene-induced mRNA and pigment accumulation in the flavedo of orange; 5), ethylene is not required for fruit ripening from species in this classification. In contrast, ethylene is necessary for the coordination and completion of ripening in climacteric fruit via analysis of inhibitors of ethylene biosynthesis and perception (148, 157), in transgenic plants blocked in ethylene biosynthesis (69, 105, 113), and through examination of the *Never-ripe* (*Nr*) ethylene receptor mutant of tomato (77, 149, 155, 159). It is also important to note that, though not nearly as well characterized in this regard, plant hormones, in addition to ethylene, are likely to influence climacteric fruit ripening (28, 92).

COMMON GENETIC REGULATORY MECHANISMS

A clarification of the common genetic regulatory elements that are shared among climacteric and non-climacteric species is central to a full understanding of fruit ripening. Such primary regulators of fruit maturation might be shared by, or at least related to, those that regulate maturation of dehiscent fruit. Although such common regulatory elements remain elusive, *Arabidopsis* silique development genes, such as those from the MADS-box family of transcription factors (38), may represent starting points in a search for common control mechanisms. Indeed, although antisense repression had no obvious effect on fruit ripening (114), ectopic expression of the tomato *AGAMOUS* gene (*TAG1*) results in fleshy expansion, ripening-like cell wall metabolism, and carotenoid accumulation in the sepals of transgenic tomatoes (60). Though not conclusive, these results are consistent with a hypothesis in which TAG1 represents a redundant ripening control function. Alternatively, TAG1 may not regulate in vivo ripening, but it may be related to, and thus mimic, a similar regulatory gene when over-expressed in sepals. In addition to a further pursuit of candidate genes or gene families, investigators have identified a number of climacteric ripening mutants that fail to ripen in response to ethylene and represent an additional track toward identification of common ripening regulators (52).

PRACTICAL AND SCIENTIFIC IMPORTANCE

Fruit development and ripening are processes unique to plant species and, from this perspective, represent an opportunity for novel insights regarding plant developmental regulatory mechanisms. The development and maturation of fruit tissues represent a final phase of floral development typically proceeding and signaled by successful pollination (106). Although parthenocarpic (seedless) fruit development occurs, such phenomena typically result from either (*A*) genetic alterations (including gene mutations or changes in genome ploidy) or environmental and/or hormonal alterations that ultimately mimic and trigger the fruit developmental cascade (true parthenocarpy) or (*B*) premature embryo abortion that results in fruit with minimal residual seed tissue (95). Indeed, parthenocarpy is of considerable agricultural importance as a means of both consumer satisfaction and variety protection. Although much is known of the hormonal and physiological signals that trigger fruit development (39), maturation and ripening are aspects of late floral development for which the molecular regulatory signals remain largely unknown.

From the standpoint of agriculture, ripening confers both positive and negative attributes to the resulting commodity. Although ripening imparts desirable flavor, color, and texture, considerable expense and crop loss result from negative ripening characteristics. For example, ripening-related increase in fruit pathogen susceptibility is a major contributor to fruit loss both before and after harvest. This genetically regulated change in fruit physiology currently necessitates the use of pesticides, post-harvest fumigants, and controlled atmosphere storage and shipping mechanisms in attempts to minimize loss. In addition to being wasteful of energy and potentially harmful to the environment, such practices represent major expenses in fruit production.

Finally, it is important to reiterate that ripening imparts numerous quality and nutritional characteristics upon a significant component of the human diet, fruit. Ripening impacts various critical aspects of mature fruit, including fiber content and composition, lipid metabolism, and the levels of vitamins and various antioxidants (123). The ability to understand key control points in global ripening regulation or within specific ripening processes, such as carotenoid, flavonoid, vitamin, and flavor volatiles, will allow for manipulation of nutrition and quality characteristics associated with ripening. The most convincing argument for the promotion of safe plant–genetic engineering will be the development of products with direct consumer impact and appeal, such as quality and nutritionally enhanced fruits.

MODEL SYSTEMS FOR FRUIT DEVELOPMENT AND RIPENING

As the fruit of numerous plant species have been studied in terms of development, maturation, ripening, and associated quality and yield characteristics, several have emerged as model systems from which the majority of available information

regarding the molecular regulation of development and ripening has been derived. Specifically, these include tomato, *Arabidopsis*, and important but to a significantly lesser extent, strawberry. Each of these model systems represents unique fruit development and maturation programs, and each has attributes reflective of a useful model system. All three, for example, can be utilized for direct assessment of gene function via stable integration of transgenes (27, 41, 93).

In large part due to its importance as a crop species, tomato has long served as the primary model for climacteric fruit ripening. This practical importance combined with diploid inheritance, ease of seed and clonal propagation, efficient sexual hybridization, a short generation period (~45–100 days, depending on variety and season), and year-round growth potential in greenhouses has made tomato the plant of choice for ripening research. From the standpoint of genetic and molecular investigations, tomato has the additional advantage of a relatively small genome (0.9 pg/haploid genome; 9) for which over 1000 molecular markers have been identified, with an average genetic spacing of less than 2 cM (138). The resulting genetic map has been especially useful in the identification and localization of quantititive trait loci (QTLs) that influence numerous fruit development, ripening, and quality loci (22, 34, 51, 74). High-molecular weight insert genomic libraries are available in both yeast artificial chromosome (17, 91, 101) and bacterial artificial chromosome (23, 43, 58) vector systems to facilitate positional cloning, and a limited number of characterized heterologous T-DNA insertion lines have been created (14, 70, 96, 107). A recently added tool to the repertoire of tomato and other plant science researchers is the National Science Foundation–sponsored development of a tomato expressesd sequence tag (EST) database. Over 20 cDNA libraries from various tissues have been created, followed by partial (single-pass 5′) sequencing of 2000–10,000 clones from each. The database will be at or near completion at the publication of this review and can be accessed prior to and following completion via the following URL, http://www.tigr.org/tdb/lgi/index.html. The finished database will include approximately 30,000 sequences derived from fruit at various stages of development, and a recent query indicated approximately 1000 non-redundant ESTs that are found exclusively in the subset of fruit libraries.

In addition to the molecular tools noted above, years of breeding and mutagenesis have resulted in a valuable germplasm resource, representing genes that influence multiple aspects of fruit development and ripening. QTL analysis has resulted in the identification of loci that regulate shape (74), size (51), and ripening time (34), while a variety of single gene mutants have been described that influence comprehensive ripening effects or subsets of ripening attributes, such as pigment accumulation (Table 1; 52, 54). In addition, Eshed & Zamir (36) created a series of introgressions of a wild tomato species (*Lycopersicon pennellii*) into cultivated tomato (*L. esculentum*), resulting in 50 introgression lines that span the tomato genome and yield variation in numerous phenotypes, including fruit development and ripening. The potential for further examination and discovery using this genetic resource remains considerable.

TABLE 1 Tomato germplasm altered in ripening. The dashed line separates mutants for which the corresponding gene has been cloned (1st tier) from those that have not (2nd tier). The third tier indicates transgenic lines altered in ethylene signaling

Genotype	Activity	Function	Reference
rin, ripening-inhibitor	Transcription factor	Comprehensive ripening	147*
nor, non-ripening	Transcription factor	Comprehensive ripening	147*
Nr, Never-ripe	C2H4 receptor	Ethylene signaling	155
hp-2, high-pigment-2	DET1 homolog	Light signaling	100
cr, crimson	Lycopene cyclase	Carotenoid metabolism	123
B, Beta	Lycopene cyclase	Carotenoid metabolism	123
r, Phytoene Synthase	Phytoene synthase	Carotenoid metabolism	45
hp-1, high-pigment-1	NA	Light signaling	152, 160
alc, alcobaca	NA	Comprehensive ripening	72
Nr-2, Never-ripe-2	NA	Comprehensive ripening	65
Gr, Green-ripe	NA	Comprehensive ripening	64
Cnr, Clear non-ripening	NA	Comprehensive ripening	143
Gf	NA	Comprehensive ripening	3
t, tangerine	NA	Carotenoid metabolism	119
at, apricot	NA	Carotenoid metabolism	61
ACO	ACC oxidase	C2H4 biosynthesis	113
ACS	ACC synthase	C2H4 biosynthesis	105
ACD	ACC deaminase	C2H4 biosynthesis	69
LeETR4	Ethylene receptor	Ethylene signaling	146
TCTR1	Putative MAPKKK	Ethylene signaling	**

*Vrebalov, Ruezinsky, Padmanabhan, and Giovannoni, unpublished.

**Adams, Kannan, Barry, and Giovannoni, unpublished.

Arabidopsis remains unsurpassed as a model for dehiscent fruit development in particular and plant biology in general. At 0.15 pg/haploid genome, the *Arabidopsis* genome is small, gene-dense, and almost completely sequenced. Combined, these attributes make positional cloning strategies fairly straightforward (87). Numerous mutants have resulted from large-scale mutagenesis programs, with insertional mutagenesis efforts resulting in particularly powerful tools for ascertaining gene function (reviewed in 11, 71, 90, 108). With respect to genetic control of fruit development, recent functional analyses of *AGAMOUS*-like (*AGL*) genes has resulted in identification of several MADS-box genes that regulate fruit (silique) development and maturation (38).

Finally, although several non-climacteric species, including citrus (67) and grape (30, 139, 140), have received considerable attention as systems for molecular analysis of fruit maturation, strawberry has emerged as the most widely studied and tractable non-climacteric model system. Several differential screens have resulted in a number of novel ripening-related genes (89, 103, 154), and a strawberry fruit microarray has been developed for use in identifying genes associated with quality characters (2).

MOLECULAR ANALYSIS OF FRUIT DEVELOPMENT

Arabidopsis MADS-Box Genes

To date, molecular factors influencing fruit development have been best described via mutant and subsequent gene cloning in *Arabidopsis* and *Antirrhinum*. Classic floral homeotic genes, such as the *AGAMOUS* and *SQUAMOSA* MADS-box genes, represent molecular determinants necessary for the formation of floral organs, including carpels. However, these genes are not fruit specific in effects and thus are not the focus of this review (for recent reviews of floral development, see 97, 141). Nevertheless, recent analysis of *Arabidopsis* MADS genes (of which there are at least 45) (8) reveals several that have clear fruit-specific activities. MADS genes are defined by the presence of a highly conserved amino-terminal DNA-binding motif, denoted as the MADS-box, followed by less well conserved I, K, and C domains. The I and K domains may be involved in the formation of homo- and heterodimers, with additional MADS proteins, whereas the C domain is the most variable and likely to confer functional specificity (120).

Inactivation of the *FRUITFUL* MADS gene (*AGL8*) resulted in siliques that failed to fully expand, although that produced no discernable effect on seed development (55). Mutant siliques also fail to dehisce as a result of abnormal formation of valve-replum boundaries (38). In this latter regard, *FRUITFUL* does not seem to directly influence silique maturation per se. Rather, it does so indirectly because it mediates silique expansion and development processes that result in appropriate definition of valve-replum boundaries and normal formation of the dehiscence zone.

Two functionally redundant MADS-box genes (*AGL1* and *AGL5*) required for normal silique dehiscence–zone formation were also recently reported. The *AGL1* and *AGL5* MADS-box genes are highly homologous and demonstrate similar gene expression patterns. Inactivation of either gene yields no discernable phenotype. This fact, together with sequence and expression similarities, suggests the possibility of functional redundancy. To test this hypothesis, *AGL1/AGL5* double mutant lines were generated and were found to yield siliques that failed to dehisce but were otherwise normal (82, 83). *AGL1* and *AGL5* were renamed *SHATTERPROOF1* and *SHATTERPROOF2* (*SHP1*, *2*), respectively, and are negatively regulated by *FRUITFUL* (37, 82). These results suggest that a cascade of MADS-box gene activities coordinate aspects of fruit development in *Arabidopsis* and possibly other species. As mentioned above, MADS-box genes have been correlated with the

induced ripening of tomato sepals and have also been associated with development and ripening of additional fruit-bearing species, including apple (135, 136, 158), strawberry (125), and cucurbits (40). Although specific functions of MADS-box genes in the development of these fleshy fruits remain unknown, their expression in various stages of fruit development is consistent with possible roles in fruit development and expansion, as well as later stages of development that may include ripening and senescence.

Tomato Fruit Mass QTLs

Quantitative trait loci (QTLs) are responsible for the majority of important crop characteristics, including regulation of fruit development and ripening. Thus, the ability to isolate QTLs, though important, has been thwarted by their very nature as multiple locus traits. This recalcitrance to isolation results from the fact that genes revealed only by allelic variation (as is typical of QTLs), in the absence of additional biochemical or molecular clues, are typically targeted for isolation through positional cloning or insertion mutagenesis strategies. Both approaches are dependent on fully accurate target locus segregation analysis that can be confused by additional segregating loci.

Tomato fruit mass genes have been the proving ground for a strategy to isolate QTLs based on effective conversion of the target gene to a single gene trait. In summary, germplasm is developed through advanced backcross breeding to fix the genotype of all non-target QTLs while selecting nearly isogenic lines (NILs) for the target locus. The resulting NILs can be used simultaneously for both gene isolation and accurate assessment of the contribution of specific alleles at an individual locus to the trait in question. Advanced backcross breeding was initially used to genetically isolate a QTL that plays a major role in fruit mass variation between cultivated tomato and the considerably smaller fruited wild species *L. pennellii* (6). This locus was designated *fruit weight 2.2* (*fw2.2*). Once all other major fruit weight loci were fixed for genotype, a large segregating population could be accurately scored to permit high-resolution genetic mapping (7) as a prelude to eventual positional cloning (44). Gene isolation was eventually confirmed via transfer of the dominant (*L. pennellii*) allele to the recessive (*L. esculentum*) genotype via *Agrobacterium*-mediated T-DNA transfer (Figure 1). This accomplishment represents the first targeted isolation of a QTL known only through phenotype.

The *fw2.2* sequence is only indicative of the route through which this gene influences fruit mass. Analysis of the predicted amino acid sequence indicates a similarity to a human oncogene RAS protein, thus suggestive of a possible role in developmental regulation. Additional clues stem from the facts that (*a*) variation in fruit mass can be at least partially attributable to a corresponding variation in pre-anthesis carpel cell number between NILs harboring the *L. esculentum* versus *L. pennellii* alleles of *fw2.2*, (*b*) a corresponding difference in cell size was not observed between the fruit of NILs, and (*c*) *fw2.2* is expressed in pre-anthesis floral organs at low levels, with highest expression in carpel tissues. These results suggest

that *fw2.2* may regulate fruit mass through modulation of pre-anthesis carpel cell number. Furthermore, no obviously significant changes in coding sequence are observed between the *L. esculentum* and *L. pennellii* alleles of *fw2.2*, though mRNA accumulation was higher in pre-anthesis carpels of the *L. pennellii* NIL. This observation suggests that the dominance observed for the *L. pennellii* allele results from elevated expression (presumably due to promoter sequence variation). This observation supports a model in which the *fw2.2* gene product acts as a negative regulator of cell division during early carpel development (44), and selection for weaker alleles at the *fw2.2* locus may have occurred during domestication. Following a search of the EST and genome sequence databases, researchers identified *fw2.2* homologs in *Arabidopsis*. Whether any of these related genes influence fruit mass or additional aspects of fruit development, and how such genes may interact with MADS-box fruit development genes, should prove to be interesting lines of future investigation.

RIPENING OF FLESHY FRUITS

As mentioned above, climacteric fruits are distinguished from non-climacteric fruits by their increased respiration and ethylene biosynthesis rates during ripening (81). Using the tomato system, investigators have long known that ethylene is necessary for manifestation of ripening in climacteric fruit (148, 157). The critical role of ethylene in coordinating climacteric ripening at the molecular level was first observed via analysis of ethylene-inducible, ripening-related–gene expression in tomato (85, 94). Numerous fruit development–related genes were isolated using differential gene expression patterns and biochemical function in the late 1980s and early 1990s (reviewed in 53), with more recent screens focused on gene isolation strategies that are likely to detect less abundant mRNAs (162). The in vivo functions of fruit development– and ripening-related–genes, including HMG-CoA reductase, polygalacturonase (PG), pectin methylesterase, ACC synthase, ACC oxidase, phytoene synthase, and the *NR* ethylene receptor, have been tested via antisense gene repression and/or mutant complementation in tomato (52). This is demonstrated by the following examples: PG is necessary for ripening-related pectin depolymerization and pathogen susceptibility, yet it has little effect on fruit softening (49, 73, 131). Inhibition of phytoene synthase results in reduced carotenoid biosynthesis and reduction in fruit and flower pigmentation (45). Reduced ethylene evolution results in ripening inhibition of ACC synthase and ACC oxidase antisense lines (57, 105), whereas introduction of a dominant mutant allele of the *NR* ethylene receptor results in tomato plants that are inhibited in virtually every measurable ethylene response, including fruit ripening (155, 159).

Ethylene Signal Transduction

Analysis of *Arabidopsis* ethylene response mutants has yielded the clearest model for hormone signal transduction in plants (Figure 2) (35, 42, 62, 66, 134). Demonstration that the tomato *Nr* mutant represents a lesion in an ethylene receptor

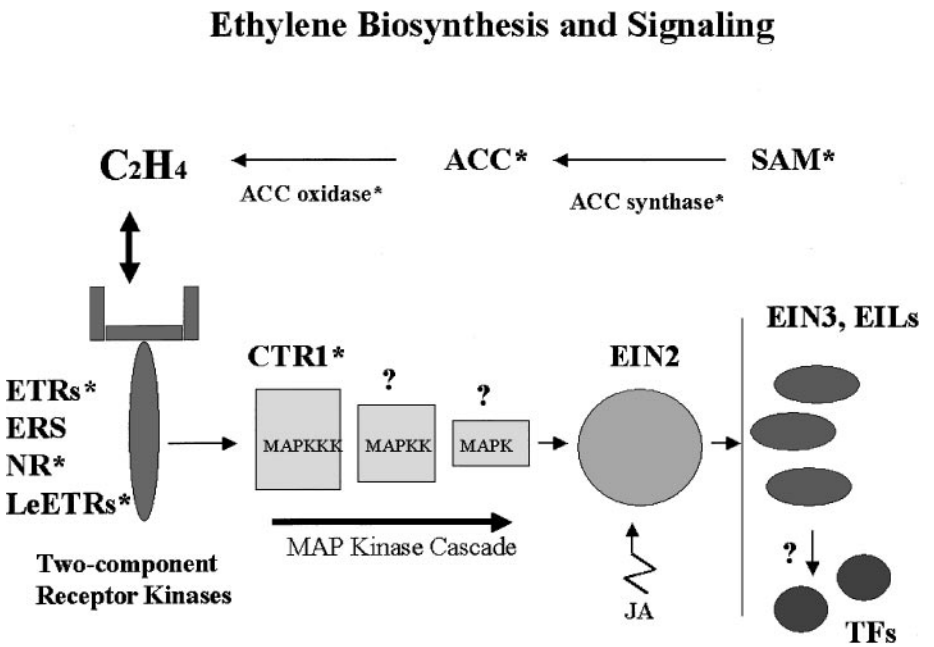

Figure 2 Model for ethylene synthesis and signal transduction. A composite model derived from the work of Yang (157) on ethylene biosynthesis and as reviewed in Stepanova & Ecker's research on ethylene signal transduction (134). Steps and intermediates designated with an asterisk have been targeted for transgene modification in ripening fruit. EIN3, EILs, and EREBPs are localized in the nucleus.

gene (155), combined with the isolation of additional fruit species homologues [tomato (80, 109, 163), cucurbits (126, 156), peach (13)], has permitted comparative analysis of ethylene receptor expression in several species, as well as functional analysis during fleshy fruit ripening in tomato. As would be predicted by the *Arabidopsis* model, mutation in the putative ethylene-binding domain of the tomato *NR* gene results in global ethylene insensitivity, including inhibition of ripening (77, 155). Genetic mapping of putative tomato ethylene receptor loci employing the *Arabidopsis ETR1* ethylene receptor as a probe suggested the presence of several tomato receptors in addition to *NR* (159). Corresponding loci have since been isolated and characterized for expression by several groups (80, 109, 163). *NR* and *LeETR4* demonstrated elevated expression during ripening and were thus targeted for antisense repression. In summary, repression of *NR* had no obvious effects on ethylene signaling other than elevated expression of *LeETR4*, suggesting a feedback mechanism resulting in compensation for missing *NR* with increased *LeETR4*. Repression of *LeETR4* did not elicit any alteration of *NR* expression but did result in leaf epinasty, premature floral senescence, and accelerated ripening suggestive of a negative regulatory role in ethylene signaling. Transgene

mediated expression of *NR* in *LeETR4* repression lines resulted in complementation of the enhanced ethylene response phenotype, confirming functional redundancy (146).

Initially, this result seems odd as the inactivation of single *Arabidopsis* ethylene receptor genes has no obvious effect on ethylene signal transduction. An ethylene constitutive response phenotype analogous to tomato *LeETR4* repression was not observed until multiple *Arabidopsis* ethylene receptor loci were rendered inactive (59). One possible explanation for this result is that *LeETR4* may make a greater contribution to net receptor levels in tomato versus individual receptor genes in *Arabidopsis*. Inactivation of the remaining tomato ethylene receptors should confirm or deny this possibility and will provide insights into how evolution has tailored ethylene perception to suit the developmental programs deployed by these two species.

Analyses of gene knockouts and repression in *Arabidopsis* and tomato, respectively, do indicate clear functional redundancy in the ethylene receptor gene families of both species. Wilkinson et al (153) demonstrated that ethylene receptor function is also highly conserved across species boundaries. Specifically, expression of a mutated *Arabidopsis ETR1* transgene yielded a receptor gene product that was altered in its ability to bind ethylene (127) and resulted in ethylene insensitivity in *Arabidopsis* plants that harbored the normal complement of ethylene receptor genes. This result is consistent with a model in which ethylene phenotypes result from ethylene inactivation of receptors, thus allowing dominant mutant (active) receptors to continue repression of responses attributed to the recognition of ethylene (56). Wilkinson et al also expressed the mutated *Arabidopsis ETR1* transgene in petunia and tomato, resulting in similar repression of ethylene phenotypes (153). (Figure 3) Both species demonstrated general ethylene insensitivity in response to transgene expression, though most notably in the agriculturally significant attributes of fruit ripening and floral senescence. This result demonstrates functional conservation across species and suggests that the mutant *Arabidopsis* receptor gene will have wide-range potential for modification of ethylene responses (such as climacteric fruit ripening) across diverse taxa.

Developmental Regulation

Further analysis of transgenic and mutant tomato lines that are inhibited in ethylene biosynthesis or perception demonstrates that climacteric ripening represents a combination of ethylene regulation and developmental control. Indeed, the gene encoding the rate limiting activity in ethylene biosynthesis, *ACC synthase*, is initially induced during ripening by an unknown developmental signaling system (12, 142).

Expression analysis of a number of additional ripening-related genes indicates that developmental or non-ethylene–mediated regulation of a subset of ripening-related genes is evident in climacteric fruits. Examples in tomato include members of the *ACO* and *ACS* gene families (12, 16, 84, 102, 142), the *NR* ethylene receptor (80, 109, 155), and E8 (32). Additional evidence for non-ethylene–mediated

ripening control comes from analysis of gene expression in a number of ripening impaired mutants, such as *rin* (*ripening-inhibitor*) and *nor* (*non-ripening*), that fail to ripen in response to exogenous ethylene yet display signs of ethylene sensitivity and signaling, including induction of some ethylene-regulated genes (Figure 4; 159). Other researchers and we have interpreted these results to indicate that additional regulatory constraints are placed on climacteric fruit maturation in addition to general ethylene biosynthesis and signaling. Such regulatory mechanisms could include fruit-specific regulation of certain subsets of ethylene-regulated genes or regulatory mechanisms that operate separately from and in addition to ethylene (Figure 5). Genes corresponding to both the *rin* and *nor* mutations have been recently cloned; although unrelated at the level of DNA or protein sequence, both have features suggestive of roles in regulation of gene transcription (Vrebalov, Ruezinsky, Padmanabhan, White, Noensie, & Giovannoni, unpublished data). Availability of these ripening regulatory genes should allow analysis of steps in the ripening regulatory hierarchy that precede ethylene. They should also permit

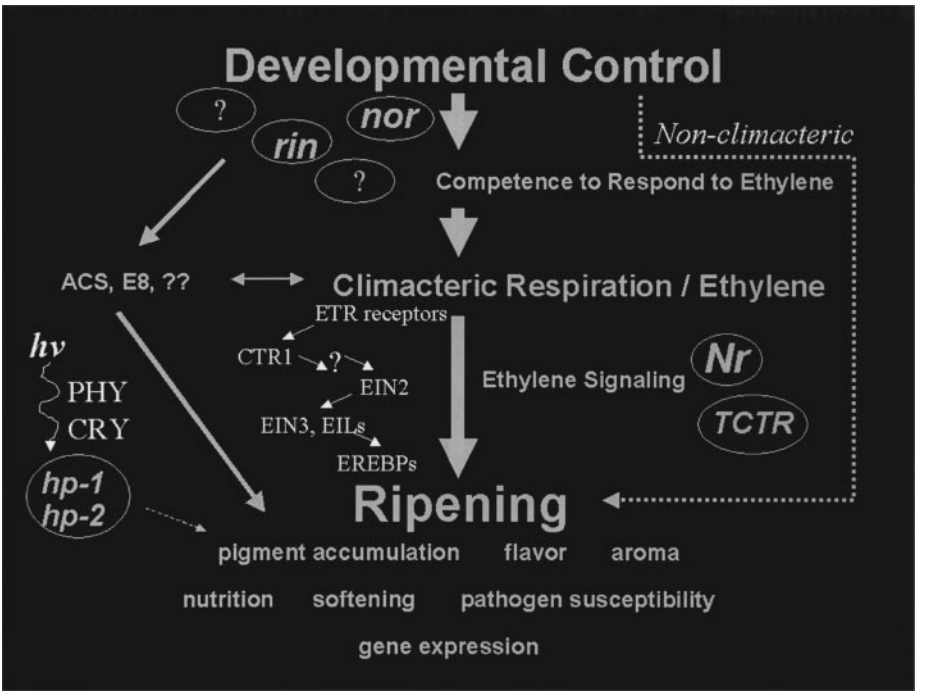

Figure 5 Model for interactions among developmental, hormonal, and light signaling systems that impact ripening. Developmental cues as represented via available tomato ripening mutants are required for climacteric ethylene biosynthesis and response. Studies in tomato also suggest that light is critical in normal pigment accumulation. A key question (dotted line) is whether common developmental mechanisms control climacteric and non-climacteric ripening.

assessment of whether such genes represent regulatory mechanisms common to both climacteric and non-climacteric fruit species.

Cell Wall Metabolism and Softening

Within the context of fruit ripening, tomato PG has been the most widely studied cell wall hydrolase. This is due in large part to initial observations of a high-level extractable endo-PG activity that increased in parallel with the ripening process. These observations led to the pursuit of the tomato endo-PG gene and the hypothesis regarding the role of PG in ripening-related textural modification (reviewed in 50). Gene isolation, and the subsequent functional characterization of tomato fruit PG in transgenic plants, indicated that PG activity alone is not sufficient to significantly impact texture (49, 129, 131); thus, it is likely to function in concert with additional factors. Kramer et al suggested that fruit PG may also play a role in mediating the fruit ripening–associated increase in susceptibility to opportunistic pathogens (73).

Enzymes in addition to PG that are involved in cell wall metabolism have been identified in ripening fruit and, in some cases, have been tested for function. Pectin-methyl-esterase (PME) shows activity throughout fruit development and may increase accessibility of PG to its pectin substrate. Antisense repression of a tomato fruit PME resulted in decreased pectin degradation, but consistent with PG repression, it did not alter additional ripening characteristics, including softening (145). Two tomato β-glucanases (hemicellulases) that show differential expression in ripening fruit and are designated CEL1 and CEL2 were repressed via antisense without observable impact on fruit ripening and softening (20, 79). It is interesting to note that CEL1 repression inhibited pedicel abscission (79), whereas CEL2 repression inhibited fruit abscission (20). Expression of these genes during fruit ripening is suggestive of a function in fruit cell wall metabolism; however, the lack of observable ripening phenotypes in the available transgenic lines indicates that the roles they play are functionally redundant and/or components of a more complicated metabolic process. Repression of additional ripening-related cell wall metabolism enzymes, such as members of the β-galactosidase gene family (132), in addition to pyramiding of multiple cell wall metabolism antisense genes through crosses of available transgenic lines, may shed additional light on the genetic regulation of this complicated metabolic process.

Some of the most definitive results concerning ripening-related texture modification have emerged from analysis of tomato expansins. Expansins are cell wall proteins associated with numerous tissues and developmental stages undergoing (often rapid) changes in size and shape (for review, see 29). Tomato and strawberry expansin genes upregulated during fruit ripening have been isolated (26, 124), and repression of a fruit ripening-specific expansin (Exp1) in tomato resulted in reduced softening. Overexpression of Exp1 resulted in enhanced softening, including softening of mature green fruit owing to ectopic expression via the CaMV35s promoter (21). These results suggest that, although the activity of fruit cell wall hydrolases

may well be important for in vivo textural modifications associated with ripening, fruit expansins contribute significantly and definitively to softening effects. It is important to keep in mind that methods for measuring softening do not reflect all of the nuances associated with this process and are approximate at best. Nevertheless, the transgenic lines described in this section, when combined with sexual hybridization and assessed via more comprehensive genomics approaches, represent a powerful reservoir of genetic tools that will shed considerable insight into ripening associated textural changes.

Light Signal Transduction and Fruit Carotenoid Accumulation

To date, molecular regulation of the role of light in fruit ripening has been studied most thoroughly in tomato, and available evidence suggests that light has its greatest impact on pigmentation, with apparently little effect on additional ripening phenomena (4).

The green to red color transition typical of ripening tomato fruit is largely due to the developmental transition of chloroplasts to chromoplasts; as photosynthetic membranes are degraded, chlorophyll is metabolized, and carotenoids, including β-carotene and lycopene, accumulate (54). The regulation of carotenoid biosynthesis during ripening is due, at least in part, to ripening-related and ethylene-inducible gene expression in both tomato (45, 52, 86, 88, 123) and melon (63). Although numerous tomato mutants that are altered in pigment accumulation have been reported (117, 118), few that result in net carotenoid accumulation have been identified. Nevertheless, a combination of elegant biochemical and genetic approaches has resulted in the isolation of a key gene, *lycopene-ε-cyclase*, responsible for the relative levels of β-carotene and lycopene in tomato fruit (122). Discovery of this gene also led to the elucidation of the molecular basis of the tomato β (*Beta*) and *cr* (*crimson*, often referred to as *og*) mutants, which result in fruit that has shifted toward accumulation of either β-carotene or lycopene, depending on enhanced or reduced expression of the cyclase gene, respectively. Genetic analysis of pepper suggests that numerous loci responsible for tomato fruit pigmentation may be conserved in pepper (144) and thus might be conserved among a wide range of species.

A particularly interesting mutation from the standpoint of fruit carotenoid accumulation is the recessive *high pigment-1* (*hp-1*) mutation. In contrast to most tomato carotenoid mutations, *hp-1* results in increased accumulation of both lycopene and β-carotene during fruit development. It is also responsible for heightened levels of chlorophyll in leaves and green fruit at all stages of development in lines homozygous for the mutant allele (152). A mutation similar in phenotype to *hp-1*, named *hp-2*, was described by Soressi (133) and is non-allelic with *hp-1* (151).

Key to understanding the basis of the *hp-1* mutation is the fact that tomato seedlings homozygous for the *hp-1* allele demonstrate an exaggerated photomorphogenic de-etiolation response (112). In short, *hp-1/hp-1* seedlings are characterized by inhibition of hypocotyl elongation and intense anthocyanin pigmentation,

relative to seedlings of normal NILs, with maximal phenotypic expression in response to red light (111). Tomato seedling de-etiolation is a phytochrome (red light) response, which can be enhanced by blue light, suggesting that *hp-1* may influence phytochrome and blue light receptor action and/or signaling. Overexpression of oat phytochrome A in tomato resulted in phenotypes similar to those observed in the *hp-1* mutant, including increased carotenoid accumulation in ripe fruit (18). Furthermore, Peters et al (111) showed that the *hp-1* phenotype was repressed when associated with the phytochrome deficient *aurea* mutant, confirming the role of *hp-1* in phytochrome responses. Quantification of phytochrome levels in normal and *hp-1/hp-1* seedlings indicates that the amplified phytochrome responses observed in the *hp-1* mutant occur within the context of normal phytochrome concentration and stability, suggesting that the normal *HP-1* gene product acts as a negative regulator of phytochrome signal transduction in tomato (1, 111).

Arabidopsis is the most widely studied plant system for analysis of the genetic basis of light signal transduction, and a number of mutations have been identified and hypothesized to represent genes that function as negative regulators of light signaling (24, 25, 115). Such genes may be similar in function to the normal *Hp-1* allele. Indeed, researchers recently found that the tomato *hp-2* mutation represents a tomato homologue of *Arabidopsis DE-ETIOLATED1* (100, 110). This result confirms the role of general light signaling in fruit pigment accumulation and suggests that a greater understanding of these processes may lead to successful efforts in fruit quality and nutrient modification. Efforts toward the isolation of the *hp-1* locus via a positional cloning strategy are ongoing (160).

Regulation of Gene Expression

The isolation of fruit ripening–related genes has resulted not only in tools for studying the direct effects of specific gene products on ripening but also in opportunities to isolate and study gene regulatory elements that may illuminate regulatory mechanisms. Ripening-related genes have been isolated from a number of species in addition to tomato (53, 89, 103, 137); however, most attempts to study ripening gene regulatory sequences have focused on tomato genes. Genes responding to ethylene and non-ethylene signals have been identified (33, 130). Sequences directing fruit, and in some cases ripening-specific, expression have been localized via promoter-reporter constructs for the PG (99, 104), E8 (31), 2A11 (150), and ACO1 (16) genes, whereas the ripening-induced (but not fruit-specific) E4 (98) gene revealed the presence of regulatory sequences likely associated with more general ethylene regulatory mechanisms that are shared with additional fruit-specific and ripening-related genes (15, 150). The fact that both ethylene and additional developmental factors regulate several of these genes enhanced the possibility that the relationship between both signaling systems could be examined at the molecular level. Indeed, *cis*-elements that impact fruit specificity, in addition to those that mediate ripening-associated developmental and ethylene-mediated regulation, could be separated. Furthermore, *trans*-factors that bind to corresponding sequences were identified (31, 99, 150). Genes corresponding to the factors that result in the

observed promoter binding activities remain unknown, thus limiting knowledge that is relative to specific genetic regulatory mechanisms that controll expression of fruit-specific and ripening-related genes. However, as many of the ripening-related genes that have undergone promoter analysis are impacted by the *rin* and *nor* mutations, the recent cloning of these putative transcription factors will provide opportunities to test for specific interactions of the RIN and NOR proteins with functionally characterized regulatory sequences.

Tomato has also been utilized as a heterologous system to test the function of putative promoter sequences that are isolated from fruit species, such as apple (10) and pepper (76), which are not as easily transformed and (in the case of apple) require a much longer time to reach maturity. Apple ACO and PG promoter-reporter constructs demonstrated upregulation during ripening, confirming that a complement of sufficient regulatory sequences to control expression during ripening had been recovered (10). Perhaps more significant is the fact that these results demonstrated that common regulatory mechanisms are conserved at the molecular level among widely different species that exhibit climacteric ripening of fleshy fruit. Equally significant, if not more intriguing, was the observation by Kuntz et al (76) that promoters from two ripening-induced genes (capsanthin/capsorubin synthase and fibrillin) from non-climacteric pepper were induced in transgenic tomato fruit in parallel with ripening. Expression of both genes was enhanced by application of ethylene, suggesting that climacteric and non-climacteric ripening may share common molecular underpinnings.

OPPORTUNITIES IN FRUIT DEVELOPMENT RESEARCH

The molecular investigations into fruit development and ripening reviewed here, in addition to the advent of recent technologies facilitating functional and comparative genomics (2, 75, 78), have put the field in a position to make significant advances in coming years. The last decade has seen the unraveling of many of the mysteries regarding ethylene biosynthesis and perception, in addition to significant inroads into the control of cell wall metabolism and textural changes associated with fruit ripening. Major genes regulating fruit carotenoid biosynthesis have been discovered, and tantalizing observations regarding the role of light in fruit ripening may lead to opportunities for modification of fruit quality and nutrient content. A number of pioneering attempts in this regard have been undertaken (47, 68, 121), though greater impact is likely to result following a more complete understanding of the regulatory processes influencing such factors (47, 68). Regulation and synergy of the multiple processes contributing to the ripe phenomena remain unknown and may be addressed in coming years with genomic and proteomic approaches. Finally, insights into early regulation of fruit development, and common regulatory mechanisms among climacteric and non-climacteric ripening, represent avenues through which future research activities will follow for the dissection of common regulatory control systems, in addition to identification of discrete molecular

mechanisms specific to unique fruit development traits that differentiate fruiting species.

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LITERATURE CITED

1. Adamse P, Peters JL, Jaspers PAPM, van Tuinen A, Koornneef M, Kendrick RE. 1989. Photocontrol of anthocyanin synthesis in tomato seedlings: a genetic approach. *Photochem. Photobiol.* 50:107–11
2. Aharoni A, Keizer LCP, Bouwmeester HJ, Sun Z, Alvarez HM, et al. 2000. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. *Plant Cell* 12:647–61
3. Akhtar MS, Goldschmidt EE, John I, Rodoni S, Matile P, Grierson D. 1999. Altered patterns of senescence and ripening in gf, a stay-green mutant of tomato (*Lycopersicon esculentum* Mill.). *J. Exp. Bot.* 50:1115–22
4. Alba R, Cordonnier-Pratt MM, Pratt LH. 2000. Fruit-localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato. *Plant Physiol.* 123:363–70
5. Alonso JM, Chamaro J, Granell A. 1995. Evidence for the involvement of ethylene in the expression of specific RNAs during maturation of the orange, a non-climacteric fruit. *Plant Mol. Biol.* 29:385–90
6. Alpert KB, Grandillo S, Tanksley SD. 1995. Fw2.2: A major QTL controlling fruit weight is common to both red- and green-fruited tomato species. *Theor. Appl. Genet.* 91:994–1000
7. Alpert KB, Tanksley SD. 1996. High-resolution mapping and isolation of a yeast artificial chromosome contig containing fw2.2: a major fruit weight quantitative trait locus in tomato. *Proc. Natl. Acad. Sci. USA* 93:15503–7
8. Alvarez-Buylla ER, Pelaz S, Liljegren SJ, Gold SE, Burgeff C, et al. 2000. An ancestral MADS-box gene duplication occurred before the divergence of plants and animals. *Proc. Natl. Acad. Sci. USA* 97:5328–33
9. Arumuganathan K, Earle E. 1991. Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.* 9:208–18
10. Atkinson RG, Bolitho KM, Wright MA, Iturriagagoitia BT, Reid SJ, Ross GS. 1998. Apple ACC-oxidase and polygalacturonase: ripening-specific gene expression and promoter analysis in transgenic tomato. *Plant Mol. Biol.* 38:449–60
11. Azpiroz LR, Feldman KA. 1997. T-DNA

insertion mutagenesis in Arabidopsis: going back and forth. *Trends Genet.* 13:152–56
12. Barry CS, Llop-Tous I, 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.* 123:979–86
13. Bassett CL, Artlip TS. 1999. Isolation of an ETR1 ethylene receptor homologue from peach (*Prunus persica* (L.) Batsch). *HortScience* 34:542
14. Bishop GJ, Harrison K, Jones JDG. 1996. The tomato Dwarf gene isolated by heterologous transposon tagging encodes the first member of a new cytochrome P450 family. *Plant Cell* 8:959–69
15. Blume B, Barry CS, Hamilton AJ, Bouzayen M, Grierson D. 1997. Identification of transposon-like elements in non-coding regions of tomato ACC oxidase genes. *Mol. Gen. Genet.* 254:297–303
16. Blume B, Grierson D. 1997. Expression of ACC oxidase promoter-GUS fusions in tomato and *Nicotiana plumbaginifolia* regulated by developmental and environmental stimuli. *Plant J.* 12:731–46
17. Bonnema G, Hontelez J, Verkerk R, Zhang YQ, Van Daelen R, et al. 1996. An improved method of partially digesting plant megabase DNA suitable for YAC cloning: application to the construction of a 5.5 genome equivalent YAC library of tomato. *Plant J.* 9:125–33
18. Boylan MT, Quail PH. 1989. Oat phytochrome is biologically active in transgenic tomatoes. *Plant Cell* 1:765–73
19. Browleader MD, Jackson P, Mobasheri A, Pantelides AT, Sumar S, et al. 1999. Molecular aspects of cell wall modifications during fruit ripening. *Crit. Rev. Food Sci. Nutr.* 39:149–64
20. Brummell DA, Hall BD, Bennett AB. 2000. Antisense suppression of tomato endo-1,4-beta-glucanase Cel2 mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. *Plant Mol. Biol.* 40:615–22
21. Brummell DA, Harpster MH, Civello PM, Palys JM, Bennett AB, Dunsmuir P. 1999. Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. *Plant Cell* 11:2203–16
22. Bucheli P, Voirol E, DeLaTorre R, Lopez J, Rytz A, et al. 1999. Definition of volatile markers for flavor of tomato (*Lycopersicon esculentum* Mill.) as tools in selection and breeding. *J. Agric. Food Chem.* 47:659–64
23. Budiman MA, Mao L, Wood TC, Wing RA. 2000. A deep-coverage tomato BAC library and prospects toward development of an STC framework for genome sequencing. *Genome Res.* 10:129–36
24. Chamovitz DA, Deng XW. 1996. Light signaling in plants. *Crit. Rev. Plant Sci.* 15:455–78
25. Chory J. 1993. Out of darkness: mutants reveal pathways controlling light-regulated development in plants. *Trends Genet.* 9:167–72
26. Civello PM, Powell ALT, Sabehat A, Bennett AB. 1999. An expansin gene expressed in ripening strawberry fruit. *Plant Physiol.* 121:1273–79
27. Clough SJ, Bent AF. 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16:735–43
28. Cohen JD. 1996. In vitro tomato fruit cultures demonstrate a role for indole-3- acetic acid in regulating fruit ripening. *J. Am. Soc. Hortic. Sci.* 121:520–24
29. Cosgrove DJ. 2000. New genes and new biological roles for expansins. *Curr. Opin. Plant Biol.* 3:73–78
30. Davies C, Robinson SP. 1996. Sugar accumulation in grape berries: cloning of two putative vacuolar invertase cDNAs and their expression in grapevine tissues. *Plant Physiol.* 111:275–83
31. Deikman J, Kline R, Fischer RL. 1992.

Organization of ripening and ethylene regulatory regions in a fruit- specific promoter from tomato (*Lycopersicon esculentum*). *Plant Physiol.* 100:2013–17
32. Deikman J, Xu R, Kneissl ML, Ciardi JA, Kim KN, Pelah D. 1998. Separation of *cis* elements responsive to ethylene, fruit development, and ripening in the 5′-flanking region of the ripening-related E8 gene. *Plant Mol. Biol.* 37:1001–11
33. DellaPenna D, Lincoln JE, Fischer RL, Bennett AB. 1989. Transcriptional analysis of polygalacturonase and other ripening associated genes in Rutgers, *rin*, *nor*, and *Nr* tomato fruit. *Plant Physiol.* 90:1372–77
34. Doganlar S, Tanksley SD, Mutschler MA. 2000. Identification and molecular mapping of loci controlling fruit ripening time in tomato. *Theor. Appl. Genet.* 100:249–55
35. Ecker JR. 1995. The ethylene signal transduction pathway in plants. *Science* 268: 667–75
36. Eshed Y, Zamir D. 1994. A genomic library of *Lycopersicon pennellii* in *L. esculentum*: a tool for fine mapping of genes. *Euphytica* 79:175–79
37. Ferrandiz C, Liljegren SJ, Yanofsky MF. 2000. Negative regulation of the SHATTERPROOF genes by FRUITFULL during Arabidopsis fruit development. *Science* 289:436–38
38. Ferrandiz C, Pelaz S, Yanofsky MF. 1999. Control of carpel and fruit development in Arabidopsis. *Annu. Rev. Biochem.* 99:321–54
39. Ficcadenti N, Sestili S, Pandolfini T, Cirillo C, Rotino GL, Spena A. 1999. Genetic engineering of parthenocarpic fruit development in tomato. *Mol. Breed.* 5:463–70
40. Filipecki MK, Sommer H, Malepszy S. 1997. The MADS-box gene CUS1 is expressed during cucumber somatic embryogenesis. *Plant Sci.* 125:63–74
41. Fillatti J, Kiser J, Rose B, Comai L. 1987. Efficient transformation of tomato and the introduction and expression of a gene for herbicide tolerance. In *Tomato Biotechnology*, ed. D Nevins, R Jones, pp. 199–210. New York: Liss
42. Fluhr R, Mattoo AK. 1996. Ethylene: biosynthesis and perception. *Crit. Rev. Plant Sci.* 15:479–523
43. Folkertsma RT, Spassova MI, Prins M, Stevens MR, Hille J, Goldbach RW. 1999. Construction of a bacterial artificial chromosome (BAC) library of *Lycopersicon esculentum* cv. Stevens and its application to physically map the Sw-5 locus. *Mol. Breed.* 5:197–207
44. Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, et al. 2000. fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. *Science* 289:85–88
45. Fray RG, Grierson D. 1993. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation, and cosuppression. *Plant Mol. Biol.* 22:589–602
46. Fray RG, Grierson D. 1993. Molecular genetics of tomato fruit ripening. *Trends Genet.* 9:438–43
47. Fray RG, Wallace A, Fraser PD, Valero D, Hedden P, et al. 1995. Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. *Plant J.* 8:693–701
48. Giovannoni JJ. 1993. Molecular biology of fruit development and ripening. *Methods Plant Mol. Biol.* 10:253–87
49. Giovannoni JJ, DellaPenna D, Bennett AB, Fischer RL. 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
50. Giovannoni JJ, DellaPenna D, Bennett A, Fischer R. 1991. Polygalacturonase and tomato fruit ripening. *Hortic. Rev.* 13:67–103
51. Grandillo S, Ku HM, Tanksley SD. 1999. Identifying the loci responsible for natural variation in fruit size and shape in tomato. *Theor. Appl. Genet.* 99:978–87

52. Gray JE, Picton S, Giovannoni JJ, Grierson D. 1994. The use of transgenic and naturally occurring mutants to understand and manipulate tomato fruit ripening. *Plant Cell Environ.* 17:557–71
53. Gray JE, Picton S, Shabbeer J, Schuch W, Grierson D. 1992. Molecular biology of fruit ripening and its manipulation with antisense genes. *Plant Mol. Biol.* 19:69–87
54. Grierson D, Purton M, Knapp J, Bathgate B. 1987. Tomato ripening mutants. *Developmental Mutants in Higher Plants*, ed. H Thomas, D Grierson, pp. 73–94. London: Cambridge Univ. Press
55. Gu Q, Ferrandiz C, Yanofsky MF, Martienssen R. 1998. The fruitfull mads-box gene mediates cell differentiation during Arabidopsis fruit development. *Development* 125:1509–17
56. Hall AE, Chen QG, Findell JL, Schaller GE, Bleecker AB. 1999. The relationship between ethylene binding and dominant insensitivity conferred by mutant forms of the ETR1 ethylene receptor. *Plant Physiol.* 121:291–99
57. Hamilton A, Lycett G, Grierson D. 1990. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. *Nature* 346:284–87
58. Hamilton CM, Frary A, Xu Y, Tanksley SD, Zhang HB. 1999. Construction of tomato genomic DNA libraries in a binary-BAC (BIBAC) vector. *Plant J.* 18:223–29
59. Hua J, Meyerowitz EM. 1998. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell* 94:261–71
60. Ishida BK, Jenkins SM, Say B. 1998. Induction of AGAMOUS gene expression plays a key role in ripening of tomato sepals in vitro. *Plant Mol. Biol.* 36:733–39
61. Jenkins J, Mackinney G. 1955. Carotenoids of the apricot tomato and its hybrids with yellow and tangerine. *Genetics* 40:715–20
62. Johnson PR, Ecker JR. 1998. The ethylene gas signal transduction pathway: a molecular perspective. *Annu. Rev. Genet.* 32:227–54
63. Karvouni Z, John I, Taylor JE, Watson CF, Turner AJ, Grierson D. 1995. Isolation and characterisation of a melon cDNA clone encoding phytoene synthase. *Plant Mol. Biol.* 27:1153–62
64. Kerr E. 1981. Linkage studies of *green ripe* and *never ripe*. *Rep. Tomato Genet. Co-op.* 31:7
65. Kerr E. 1982. *Never ripe-2* (*Nr-2*) a slow ripening mutant resembling *Nr* and *Gr*. *Rep. Tomato Genet. Co-op.* 32:33
66. Kieber JJ. 1997. The ethylene response pathway in Arabidopsis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:277–96
67. Kita M, Hisada S, Endo IT, Omura M, Moriguchi T. 2000. Changes in the levels of mRNAs for putative cell growth-related genes in the albedo and flavedo during citrus fruit development. *Plant Cell Rep.* 19:582–87
68. Klann EM, Hall B, Bennett AB. 1996. Antisense acid invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit. *Plant Physiol.* 112:1321–30
69. Klee HJ, Hayford MB, Kretzmer KA, Barry GF, Kishore GM. 1991. Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. *Plant Cell* 3:1187–93
70. Knapp S, Larondelle Y, Rossberg M, Furtek D, Klaus T. 1994. Transgenic tomato lines containing Ds elements at defined genomic positions as tools for targeted transposon tagging. *Mol. Gen. Genet.* 243:666–73
71. Koornneef M, Alonso BC, Peeters AJM. 1997. Genetic approaches in plant physiology. *New Phytol.* 137:1–8
72. Kopeliovitch E, Rabinowitch HD, Mizrahi Y, Kedar N. 1981. Mode of inheritance of alcobaca, a tomato fruit ripening mutant. *Euphytica* 30:223–25
73. Kramer M, Sanders R, Sheehy R, Melis M,

Kuehn M, Hiatt W. 1990. Field evaluation of tomatoes with reduced polygalacturonase by antisense RNA. In *Horticultural Biotechnology*, ed. A Bennett, S O'Neill, pp. 347–55. New York: Liss

74. Ku HM, Doganlar S, Chen KY, Tanksley SD. 1999. The genetic basis of pear-shaped tomato fruit. *Theor. Appl. Genet.* 99:844–50
75. Ku HM, Vision T, Liu J, Tanksley SD. 2000. Comparing sequenced segments of the tomato and Arabidopsis genomes: large-scale duplication followed by selective gene loss creates a network of synteny. *Proc. Natl. Acad. Sci. USA* 97:9121–26
76. Kuntz M, Chen HC, Simkin AJ, Romer S, Shipton CA, et al. 1998. Upregulation of two ripening-related genes from a non-climacteric plant (pepper) in a transgenic climacteric plant (tomato). *Plant J.* 13:351–61
77. Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ. 1994. The *Never Ripe* mutation blocks ethylene perception in tomato. *Plant Cell* 6:521–30
78. Lander ES, Weinberg RA. 2000. Genomics: journey to the center of biology. *Science* 287:1777–82
79. Lashbrook CC, Giovannoni JJ, Hall BD, Fischer RL, Bennett AB. 1998. Transgenic analysis of tomato endo-beta-1,4-glucanase gene function. Role of cel1 in floral abscission. *Plant J.* 13:303–10
80. Lashbrook CC, Tieman DM, Klee HJ. 1998. Differential regulation of the tomato ETR gene family throughout plant development. *Plant J.* 15:243–52
81. Lelievre JM, Latche A, Jones B, Bouzayen M, Pech JC. 1997. Ethylene and fruit ripening. *Physiol. Plant.* 101:727–39
82. Liljegren SJ, Ditta GS, Eshed Y, Savidge B, Bowman JL, Yanofsky MF. 2000. SHATTERPROOF MADS-box genes control seed dispersal in Arabidopsis. *Nature* 404:766–70
83. Liljegren S, Ferrandiz C, Alvarez-Buylla E, Pelaz S, Yanofsky M. 1998. Arabidopsis MADS-box genes involved in fruit dehiscence. *Flower. Newsl.* 25:9–19
84. Lincoln JE, Campbell AD, Oetiker J, Rottmann WH, Oeller PW, et al. 1993. LE-ACS4, a fruit ripening and wound-induced 1-aminocyclopropane-1-carboxylate synthase gene of tomato (*Lycopersicon esculentum*): expression in *Escherichia coli* structural characterization, expression characteristics, and phylogenetic analysis. *J. Biol. Chem.* 268:19422–30
85. Lincoln JE, Cordes S, Read E, Fischer RL. 1987. Regulation of gene expression by ethylene during *Lycopersicon esculentum* (tomato) fruit development. *Proc. Natl. Acad. Sci. USA* 84:2793–97
86. Lois LM, Rodriguez CM, Gallego F, Campos N, Boronat A. 2000. Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. *Plant J.* 22:503–13
87. Lukowitz W, Gillmor CS, Scheible WR. 2000. Positional cloning in Arabidopsis. Why it feels good to have a Genome Initiative working for you. *Plant Physiol.* 123:795–805
88. Mann V, Pecker I, Hirschberg J. 1994. Cloning and characterization of the gene for phytoene desaturase (Pds) from tomato (*Lycopersicon esculentum*). *Plant Mol. Biol.* 24:429–34
89. Manning K. 1998. Isolation of a set of ripening-related genes from strawberry: their identification and possible relationship to fruit quality traits. *Planta* 205:622–31
90. Martienssen RA. 1998. Functional genomics: probing plant gene function and expression with transposons. *Proc. Natl. Acad. Sci. USA* 95:2021–26
91. Martin GB, Ganal MW, Tanksley SD. 1992. Construction of a yeast artificial chromosome library of tomato and identification of cloned segments linked to two disease resistance loci. *Mol. Gen. Genet.* 233:25–32

92. Martineau B, Houck CM, Sheehy RE, Hiatt WR. 1994. Fruit-specific expression of the *A. tumefaciens* isopentenyl transferase gene in tomato: effects on fruit ripening and defense-related gene expression in leaves. *Plant J.* 5:11–19
93. Mathews H, Wagoner W, Kellogg J, Bestwick R. 1995. Genetic transformation of strawberry: stable integration of a gene to control biosynthesis of ethylene. *In Vitro Cell. Dev. Biol. Plant* 31:36–43
94. Maunder M, Holdsworth M, Slater A, Knapp J, Bird C, et al. 1987. Ethylene stimulates the accumulation of ripening-related mRNAs in tomatoes. *Plant Cell Environ.* 10:177–84
95. Mazzucato A, Taddei AR, Soressi GP. 1998. The parthenocarpic fruit (pat) mutant of tomato (*Lycopersicon esculentum* Mill.) sets seedless fruits and has aberrant anther and ovule development. *Development* 125:107–14
96. Meissner R, Chague V, Zhu Q, Emmanuel E, Elkind Y, Levy AA. 2000. A high throughput system for transposon tagging and promoter trapping in tomato. *Plant J.* 22:265–74
97. Meyerowitz EM. 1998. Genetic and molecular mechanisms of pattern formation in Arabidopsis flower development. *J. Plant Res.* 111:233–42
98. Montgomery J, Goldman S, Deikman J, Margossian L, Fischer RL. 1993. Identification of an ethylene-responsive region in the promoter of a fruit ripening gene. *Proc. Natl. Acad. Sci. USA* 90:5939–43
99. Montgomery J, Pollard V, Deikman J, Fischer RL. 1993. Positive and negative regulatory regions control the spatial distribution of polygalacturonase transcription in tomato fruit pericarp. *Plant Cell* 5:1049–62
100. Mustilli AC, Fenzi F, Ciliento R, Alfano F, Bowler C. 1999. Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. *Plant Cell* 11:145–57
101. Nakata K, Tanaka H, Ito T, Sasagawa N, Chung IK, et al. 1993. Construction and some characterization of a yeast artificial chromosome library from DNA of a tomato line having four disease resistance traits. *Biosci. Biotechnol. Biochem.* 57:1790–92
102. Nakatsuka A, Murachi S, Okunishi H, Shiomi S, Nakano R, et al. 1998. Differential expression and internal feedback regulation of 1-aminocyclopropane-1-caboxylate synthase, of 1–aminocyclopropane-1-caboxylate oxidase, and ethylene receptor genes in tomato during development and ripening. *Plant Physiol.* 118: 1295–305
103. Nam YW, Tichit L, Leperlier M, Cuerq B, Marty I, Lelievre JM. 1999. Isolation and characterization of mRNAs differentially expressed during ripening of wild strawberry (*Fragaria vesca* L.) fruits. *Plant Mol. Biol.* 39:629–36
104. Nicholass FJ, Smith Christopher JS, Schuch W, Bird CR, Grierson D. 1995. High levels of ripening-specific reported gene expression directed by tomato fruit polygalacturonase gene-flanking regions. *Plant Mol. Biol.* 28:423–35
105. Oeller PW, Wong LM, Taylor LP, Pike DA, Theologis A. 1991. Reversible inhibition of tomato fruit senescence by antisense 1-aminocyclopropane-1-carboxylate. synthase. *Science* 254:427–39
106. O'Neill SD. 1997. Pollination regulation of flower development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:547–74
107. Osborne B, Corr C, Prince J, Hehl R, Tanksley S, et al. 1991. *Ac* transposition from a T-DNA can generate linked and unlinked clusters of insertions in the tomato genome. *Genetics* 129:833–44
108. Parinov S, Sundaresan V. 2000. Functional genomics in Arabidopsis: Large-scale insertional mutagenesis complements the genome sequencing project. *Curr. Opin. Biotechnol.* 11:157–61

109. Payton S, Fray RG, Brown S, Grierson D. 1996. Ethylene receptor expression is regulated during fruit ripening, flower senescence and abscission. *Plant Mol. Biol.* 31:1227–31
110. Pepper A, Delaney T, Washburn T, Poole D, Chory J. 1994. DET1, a negative regulator of light-mediated development and gene expression in Arabidopsis, encodes a novel nuclear-localized protein. *Cell* 78:109–16
111. Peters JL, Schreuder MEL, Verduin SJW, Kendrick RE. 1992. Physiological characterization of a high-pigment mutant of tomato. *Photochem. Photobiol.* 56:75–82
112. Peters JL, van Tuinen A, Adamse P, Kendrick RE, Koornneef M. 1989. High pigment mutants of tomato exhibit high sensitivity for phytochrome action. *J. Plant Physiol.* 134:661–66
113. Picton S, 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.* 3:469–81
114. Pnueli L, Hareven D, Rounsley SD, Yanofsky MF, Lifschitz E. 1994. Isolation of the tomato AGAMOUS gene TAG1 and analysis of its homeotic role in transgenic plants. *Plant Cell* 6:163–73
115. Quail PH, Boylan MT, Short TW, Xu Y, Wagner D. 1995. Phytochrome: photosensory perception and signal transduction. *Science* 268:675–80
116. Rhodes MJC. 1980. The maturation and ripening of fruits. In *Senescence in Plants*, ed. KV Thimann, pp. 157–205. Boca Raton, FL: CRC Press
117. Rick CM. 1956. New mutants. *Rep. Tomato Genet. Coop.* 6:22–23
118. Rick CM. 1980. Tomato linkage survey. *Rep. Tomato Genet. Coop.* 30:2–17
119. Rick CM, Butler L. 1956. Cytogenetics of the tomato. *Adv. Genet.* 8:267–382
120. Riechmann JL, Krizek BA, Meyerowitz EM. 1996. Dimerization specificity of Arabidopsis MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA, and AGAMOUS. *Proc. Natl. Acad. Sci. USA* 93:4793–98
121. Romer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, et al. 2000. Elevation of the provitamin A content of transgenic tomato plants. *Nat. Biotechnol.* 18:666–69
122. Ronen G, Carmel GL, Zamir D, Hirschberg J. 2000. An alternative pathway to beta-carotene formation in plant chromoplasts discovered by map-based cloning of Beta and old-gold color mutations in tomato. *Proc. Natl. Acad. Sci. USA* 97:11102–7
123. 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.* 17:341–51
124. Rose JKC, Lee HH, Bennett AB. 1997. Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proc. Natl. Acad. Sci. USA* 94:5955–60
125. Rosin FM, Hannapel D. 1999. RNA localization of a strawberry MADS-box gene (SAG1) involved in fruit development. *HortScience* 34:457
126. Sato NK, Yuhashi KI, Higashi K, Hosoya K, Kubota M, Ezura H. 1999. Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiol.* 120:321–29
127. Schaller GE, Bleecker AB. 1995. Ethylene-binding sites generated in yeast expressing the Arabidopsis ETR1 gene. *Science* 270:1809–11
128. Seymour GB, Taylor JE, Tucker GA, eds. 1993. *Biochemistry of Fruit Ripening*. London: Chapman & Hall. 442 pp.
129. Sheehy R, Kramer M, Hiatt W. 1988. Reduction of polygalacturonase activity in tomato fruit by antisense RNA. *Proc. Natl. Acad. Sci. USA* 85:8805–9

130. Sitrit Y, Bennett AB. 1998. Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: a re-examination. *Plant Physiol.* 116:1145–50
131. Smith C, Watson C, Ray J, Bird C, Morris P, et al. 1988. Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. *Nature* 334:724–26
132. Smith DL, Gross KC. 2000. A family of at least seven beta-galactosidase genes is expressed during tomato fruit development. *Plant Physiol.* 123:1173–83
133. Soressi GP. 1975. New spontaneous or chemically-induced fruit ripening tomato mutants. *Tomato Genet. Coop. Rep.* 25:21–22
134. Stepanova AN, Ecker JR. 2000. Ethylene signaling: from mutants to molecules. *Curr. Opin. Plant Biol.* 3:353–60
135. Sung SK, An G. 1997. Molecular cloning and characterization of a MADS-Box cDNA clone of the Fuji apple. *Plant Cell Physiol.* 38:484–89
136. Sung SK, Yu GH, Nam J, Jeong DH, An G. 2000. Developmentally regulated expression of two MADS-box genes, MdMADS3 and MdMADS4, in the morphogenesis of flower buds and fruits in apple. *Planta* 210:519–28
137. Suyama T, Yamada K, Mori H, Takeno K, Yamaki S. 1999. Cloning cDNAs for genes preferentially expressed during fruit growth in cucumber. *J. Am. Soc. Hortic. Sci.* 124:136–39
138. Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, et al. 1992. High density molecular linkage maps of the tomato and potato genomes. *Genetics* 132:1141–60
139. Tattersall DB, Van Heeswijck R, Hoj PB. 1997. Identification and characterization of a fruit-specific, thaumatin-like protein that accumulates at very high levels in conjunction with the onset of sugar accumulation and berry softening in grapes. *Plant Physiol.* 114:759–69
140. Tesniere C, Verries C. 2000. Molecular cloning and expression of cDNAs encoding alcohol dehydrogenases from *Vitis vinifera* L. during berry development. *Plant Sci.* 157:77–88
141. Theissen G, Saedler H. 1999. The Golden Decade of molecular floral development (1990–1999): a cheerful obituary. *Dev. Genet.* 25:181–93
142. Theologis A, Oeller PW, Wong LM, Rottmann WH, Gantz DM. 1993. Use of a tomato mutant constructed with reverse genetics to study fruit ripening, a complex developmental process. *Dev. Genet.* 14:282–95
143. Thompson AJ, Tor M, Barry CS, Vrebalov J, Orfila C, et al. 1999. Molecular and genetic characterization of a novel pleiotropic tomato-ripening mutant. *Plant Physiol.* 120:383–89
144. Thorup TA, Tanyolac B, Livingstone KD, Popovsky S, Paran I, Jahn M. 2000. Candidate gene analysis of organ pigmentation loci in the Solanaceae. *Proc. Natl. Acad. Sci. USA* 97:11192–97
145. 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* 4:667–79
146. Tieman DM, Taylor MG, Ciardi JA, Klee HJ. 2000. The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. *Proc. Natl. Acad. Sci. USA* 97:5663–68
147. Tigchelaar E, McGlasson W, Buescher R. 1978. Genetic regulation of tomato fruit ripening. *Hortic. Sci.* 13:508–13
148. Tucker GA, Brady CJ. 1987. Silver ions interrupt tomato fruit ripening. *J. Plant Physiol.* 127:165–69
149. Tucker GA, Schindler CB, Roberts JA. 1984. Flower abscission in mutant tomato Lycopersicon plants. *Planta* 160:164–67
150. Van Haaren MJJ, Houck CM. 1993. A

functional map of the fruit-specific promoter of the tomato 2A11 gene. *Plant Mol. Biol.* 21:625–40

151. van Tuinen A, Cordonnier-Pratt MM, Pratt LH, Verkerk R, Zabel P, Koornneef M. 1997. The mapping of phytochrome genes and photomorphogenic mutants of tomato. *Theor. Appl. Genet.* 94:115–22
152. Wann EV, Jourdain EL, Pressey R, Lyon BG. 1985. Effect of mutant genotypes *hp* og^c and *dg* og^c on tomato fruit quality. *J. Am. Soc. Hortic. Sci.* 110:212–15
153. Wilkinson JQ, Lanahan MB, Clark DG, Bleecker AB, Chang C, et al. 1997. A dominant mutant receptor from Arabidopsis confers ethylene insensitivity in heterologous plants. *Nat. Biotechnol.* 15:444–47
154. Wilkinson JQ, Lanahan MB, Conner TW, Klee HJ. 1995. Identification of mRNAs with enhanced expression in ripening strawberry fruit using polymerase chain reaction differential display. *Plant Mol. Biol.* 27:1097–108
155. Wilkinson J, Lanahan M, Yen H, Giovannoni JJ, Klee HJ. 1995. An ethylene-inducible component of signal transduction encoded by *Never-ripe*. *Science* 270:1807–9
156. Yamasaki S, Fujii N, Takahashi H. 2000. The ethylene-regulated expression of CS-ETR2 and CS-ERS genes in cucumber plants and their possible involvement with sex expression in flowers. *Plant Cell Physiol.* 41:608–16
157. Yang SF. 1985. Biosynthesis and action of ethylene. *HortScience* 20:41–45
158. Yao JL, Kvarnheden A, Morris B. 1999. Seven MADS-box genes in apple are expressed in different parts of the fruit. *J. Am. Soc. Hortic. Sci.* 124:8–13
159. Yen H, Lee S, Tanksley S, Lanahan M, Klee HJ, Giovannoni JJ. 1995. The tomato *Never-ripe* locus regulates ethylene-inducible gene expression and is linked to a homologue of the *Arabidopsis ETR1* gene. *Plant Physiol.* 107:1343–53
160. Yen H, Shelton A, Howard L, Vrebalov J, Giovannoni JJ. 1997. The tomato *high pigment* (*hp*) locus maps to chromosome 2 and influences plastome copy number and fruit quality. *Theor. Appl. Genet.* 95:1069–79
161. Yueming J, Jiarui F. 2000. Ethylene regulation of fruit ripening: molecular aspects. *Plant Growth Regul.* 30:193–200
162. Zegzouti H, Jones B, Frasse P, Marty C, Maitre B, et al. 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
163. Zhou D, Kalaitzis P, Mattoo AK, Tucker ML. 1996. The mRNA for an ETR1 homologue in tomato is constitutively expressed in vegetative and reproductive tissues. *Plant Mol. Biol.* 30:1331–38

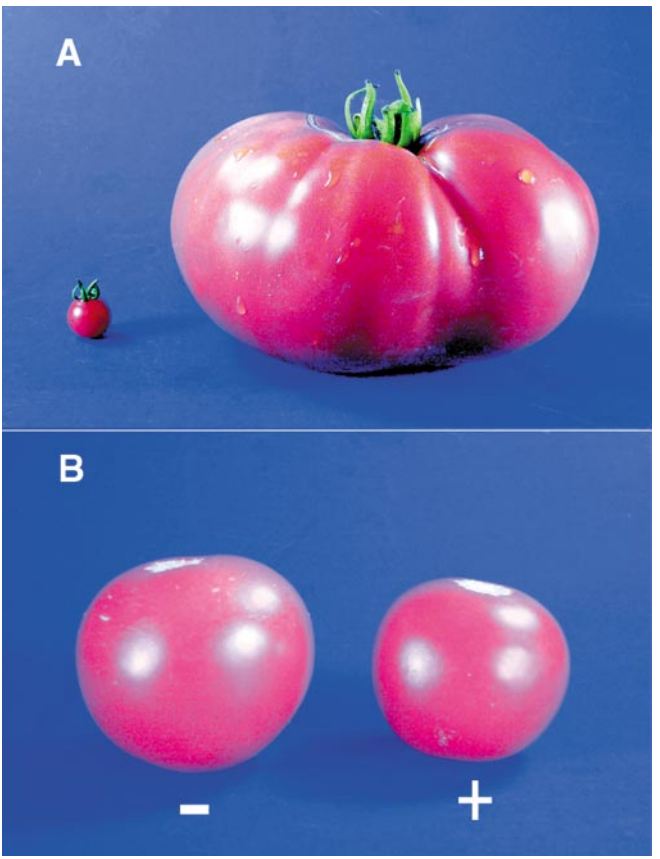

Figure 1 Isolation of an *fw2.2*, a QTL regulating fruit mass. (*A*) Fruit of the wild tomato species *L. pennellii* (left) and a large cultivated variety of *L. esculentum* (right). A gene residing at a major QTL for fruit mass, designated *fw2.2*, was identified and isolated. The dominant *L. pennellii* allele of *fw2.2* was inserted into the genome of a relatively large fruited *L. esculentum* variety via T-DNA transfer resulting in a reduction in fruit weight and confirmation of isolation of the target gene (*B*). From Frary et al (44) with permission.

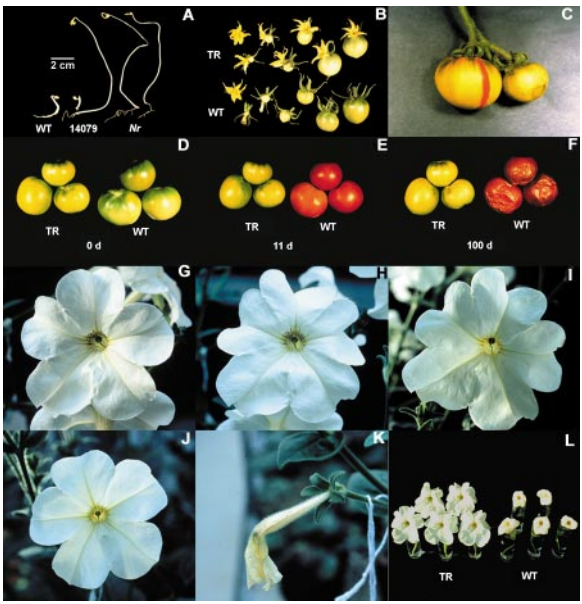

Figure 3 Ethylene insensitivity in tomato and petunia resulting from expression of a mutant *Arabidopsis* ethylene receptor. Expression of a mutant *Arabidopsis* ethylene receptor (ETR1-1) in tomato resulted in (*A*) seedlings that were insensitive to ACC in the growth medium, (*B*) petals that failed to senescence following pollination, and inhibition of fruit ripening at 0, 10, and 100 days post-mature green (*D*, *E*, *F*, respectively). Non-uniform expression of the transgene correlated with sectored ripening (C). Expression of the same gene in petunia resulted in delayed petal senescence. *G*, *H*, and *I* are transgenic petunia flowers at 0, 3, and 8 days post pollination, respectively. *J* and *K* are non-transformed controls at 0 and 3 days, respectively. Treatment of transgenic petunias with exogenous ethylene resulted in reduced senescence as compared to wild-type controls (*L*). WT, wild type; TR, transgenic. Reproduced from Wilkinson et al (153) with permission.

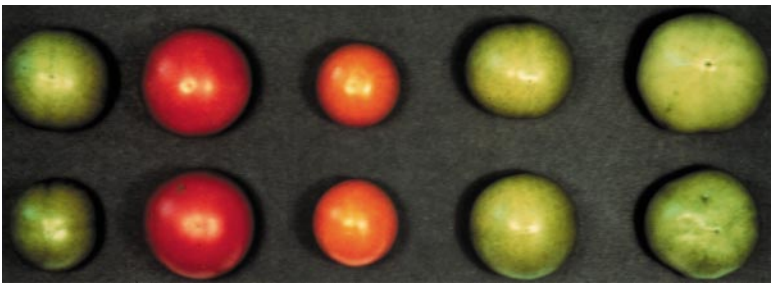

Figure 4 Tomato fruit ripening mutants. From left to right are mature green and ripe (mature green + 7 days) fruit from tomato *cultivar Ailsa Craig*. Following are fruit of identical age as the ripe control and from nearly isogenic lines homozygous for the *Nr* (*Never-ripe*), *rin* (*ripening-inhibitor*), and *nor* (*non-ripening*) mutations, respectively.