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Molecular biology of ethylene during tomato fruit development and maturation

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ABSTRACT

Important traits for complete ripening and consumer fruit quality preferences include development of aroma, flavor, color, texture, and nutritional quality. These attributes are influenced by the endogenously produced hormone ethylene in many fleshy fruits such as apple, avocado, banana, mango, pear and tomato. Even in species where endogenous ethylene seems to play little if any role as an endogenous regulator, exogenous ethylene will often promote ripening characteristics and can be the target of post-harvest strategies designed to accelerate, synchronize or delay ripening. In recent decades the YANG cycle for ethylene biosynthesis has been revealed and characterized at the molecular level with much of this important work done via the analysis of fruit systems. However, the genetic regulation that controls ethylene production at different developmental stages of fruits has only recently begun to be studied. Tomato has emerged as the primary model plant to further understand the molecular biology that controls ethylene synthesis and additional ripening regulators during fruit development. Here we summarize data pertaining to ethylene biology specifically as related to fruit maturation and including recent insights into genetic control of the ripening process prior to and controlling ethylene.

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1. Regulation of ethylene synthesis in tomato fruit

The phytohormone ethylene plays critical roles in many developmental events and environmental responses of plants. Climacteric fruits such as tomato, apple or pears, are characterized by a ripening-related increase in respiration and elevated ethylene synthesis to rapidly coordinate and synchronize ripening. In contrast, non-climacteric fruits such as strawberries, grapes or citrus, lack the respiratory peak associated with ripening. The reason for a respiratory climacteric is still poorly understood as non-climacteric fruit manage ripening absent this change in physiology. Similar biochemical events often take place during ripening in both climacteric and non-climacteric fruits including color change, altered starch/sugar metabolism, fruit softening, textural modification, synthesis of aroma volatiles and increased susceptibility to pathogens. In addition, common genes regulating ripening in both types of fruits often show altered expression supporting the hypothesis that ethylene-dependent and ethylene-independent gene regulation pathways coordinate fruit maturation processes with primary regulators possibly conserved through evolution [1–5].

As reviewed elsewhere in this issue, the biochemical synthesis of ethylene was defined by the pioneering work leading to definition of the YANG cycle [6]. To summarize, the enzyme S-adenosyl-methionine (SAM) synthase catalyzes adenosylation of the sulphur atom of methionine. SAM is then metabolized to 5′-methylthioadenosine (MTA), which is incorporated into the methionine cycle to recover the sulphur atom and 1-aminocyclopropane-1-carboxylic acid (ACC), the first compound of the pathway committed to ethylene biosynthesis. The enzyme catalyzing this reaction is ACC synthase (ACS) which is pyridoxal phosphate-dependent. Finally, in the presence of oxygen, ACC is converted to ethylene by ACC oxidase (ACO), originally defined as the ethylene-forming enzyme (EFE). Genes encoding ACS and ACO were originally identified via elegant studies employing maturing fleshy fruit [7,8].

Tomato has proven a highly useful model system for fruit development and ripening and is the system in which the role of ethylene during fruit ripening has been most thoroughly studied (for reviews see refs. [4–5]). Although several genes in the methionine biosynthesis pathway are responsive to ethylene during tomato fruit ripening [9], the *ACS* and *ACO* genes have been characterized most extensively. In tomato plants, nine genes encoding ACS (*LeACS1A*, *LeACS1B*, and *LeACS2*-8) have been described to date [10–18], and four are differentially expressed during fruit ripening: *LeACS1A*, *LeACS2*, *LeACS4* and *LeACS6* [19,11]. Barry et al. [19] proposed a model that explains the differential regulation of these genes during pre-climacteric (System 1) and climacteric (System 2) ethylene

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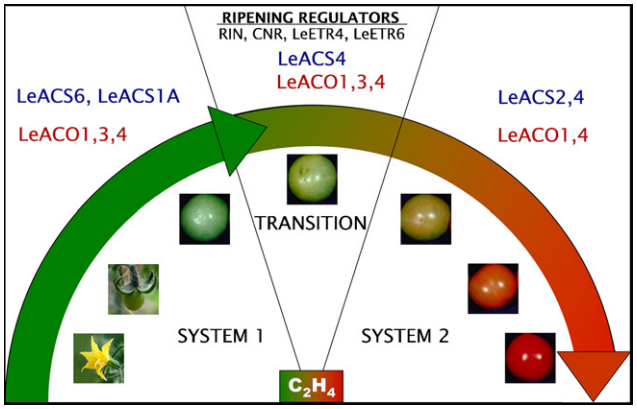

Fig. 1. Regulation of ethylene biosynthesis in tomato fruit development and ripening. During development (System 1) lower and auto-inhibitory ethylene is synthesized by LeACS1A,6 and LeACO1,3,4. At the transition stage, the ripening regulators indicated play critical roles. *LeACS*4 is induced and a large increase of auto-catalytic ethylene starts, resulting in negative feedback on System 1. LeACS2,4 and LeACO1,4 are then responsible for the high ethylene production through System 2.

production of maturing tomato fruit (Fig. 1). Briefly, *LeACS*6 has been shown to be the main gene responsible for ACS and subsequent ethylene synthesis in green fruit (System 1), although expression of *LeACS*1*A* is also observed in these tissues. At the transition to ripening, expression of *LeACS*1*A* and *LeACS*4 is induced and is further dependent on the RIN MADS-box transcription factor [20]. As a result of increased climacteric ethylene synthesis due to *LeACS*1*A* and *LeACS*4 activation, *LeACS*2 expression is also induced resulting in the auto-catalytic ethylene evolution characteristic of System 2 ethylene. High ethylene production occurs in the ripening fruit, resulting in negative feedback on the System 1 pathway and reduced *LeACS*1*A* and *LeACS*6 expression.

Five genes encoding the ACO enzyme have been defined in tomato (*LeACO*1-5) and three of them (*LeACO*1, *LeACO*3, *LeACO*4) have been shown to be differentially expressed in fruit [10,11,13,14,19,21,22]. *LeACO*1 and *LeACO*4 accumulate in immature green stages and their expression levels were shown to increase dramatically at the onset of the climacteric burst and ripening. The expression of *LeACO*3 is induced but transitory at the breaker stage while *LeACO*1 and *LeACO*4 expression is sustained during ripening. The ripening-related induction of *LeACO*1 and *LeACO*4 expression is ethylene-dependent as defined in experiments where fruit where treated with the ethylene perception inhibitor 1-methylcyclopropene (1-MCP).

ACS and *ACO* genes have been characterized in many other fruits including but not limited to melon [23,24], apple [25–27], banana [28,29], pear [30], kiwifruit [31], peach [32,33] and persimmon [34]. In all cases examined at depth, these two families of enzymes have been shown to belong to multigene families and with members displaying unique expression patterns depending on developmental and environmental factors.

2. Transcriptional control of ethylene responsive genes in ripening fruit

From the mid 1980s to early 1990s numerous investigators demonstrated that ethylene regulates ripening in climacteric fruits by stimulating changes in gene expression. The hypothesis that ethylene mediated its ripening effects via regulated gene expression was both based on and shown through experiments where differential expression of mRNA transcripts and proteins was evaluated under conditions of exogenous ethylene addition or inhibition in wild-type fruits, natural ripening mutants and transgenic plants altered in expression of ethylene synthesis or response genes. Examples of the best characterized ethylene regulated fruit genes reported in these studies include the previously described ethylene synthesis enzyme encoding genes *ACS* [35] and *ACO* [8]; the fruit-specific polygalacturonase (*PG*), which is involved in depolymerization of cell wall pectin during ripening [36,37], yet has little effect on fruit softening [38]; pectin methylesterase (*PME*), which provides accessibility to pectin by PG [39–41]; phytoene synthase (*PSY*), which catalyzes the rate limiting and highly regulated reaction from geranylgeranyl diphosphate to phytoene in the carotenoid biosynthesis pathway responsible for the pigmentation of many fruits and flowers including those of tomato [42]; or the ripening induced genes E4 and E8, whose functions are still uncertain, though the predicted peptides encoded by these genes show similarity to methionine sulphoxide reductase proteins and a dioxygenase with similarity to ACC oxidase, respectively [43,44]. These and many other ethylene responsive genes were recovered in multiple screens of ripening, ethylene treated and mutant fruits (reviewed in ref. [45]).

To better understand the mechanisms that control the expression of ethylene responsive genes during tomato ripening, the promoter regions of several of these genes were isolated and analyzed with the aim to identify functional regulatory motifs. The structure of the *LeACO*1 gene promoter is well characterized [21]. The −1855 to −396 region of the promoter confers ethylene-dependent expression. It contains two repeat regions (RPT) with homology to ethylene responsive promoters of the ripening-specific genes 2A11 and E4. Several ethylene responsive regions (ERE; the 8 bp motif A(A/T)TTCAAA) and stress-related motifs (TCA; the 10 bp motif TCATCTTCTT) are also present in this promoter region. In contrast, the −396 region confers ethylene-independent expression.

Specific regulatory elements controlling the expression of *ACS*2 and *ACS*4 genes during tomato development and ripening were reported by Lincoln et al. It was shown that both promoters share a wound response element and the *LeACS*4 promoter contains a sequence with similarities to an anaerobiosis-responsive element (ARE) found in the alcohol dehydrogenase genes of maize. An analysis of the *LeACS*6 promoter has been recently reported [46]. The aim of this study was to identify the *cis*-elements responsible for the negative feedback control of ethylene at the transition from System 1 to System 2 during fruit ripening. The results localized putative *cis*-elements required for negative ethylene-response between −347 and −266 upstream from the translation start. Several *LeACS*6::*GUS* stable lines containing internal deletion of this region showed loss of response of the promoter to exogenous ethylene and provide a molecular explanation for the System 1 repression phenotype of this gene. Further analyses of the *cis*-elements and the proteins that interact with them are needed to better understand the transcriptional regulation by ethylene of this gene. Furthermore, there is considerable evidence that regulation of ACS activity, through protein phosphorylation and turnover, also plays a critical role in the function of this enzyme suggesting that ethylene synthesis is regulated at several steps in the path from transcription to activity (for review, see ref. [47]).

The contrasting expression profiles of the *E*4 and *E*8 genes in response to ethylene make them attractive for analysis. Ethylene stimulates the transcription of the *E*4 gene in tomato fruit in response to both System 1 and System 2 ethylene. Indeed, every tissue analyzed for *E*4 expression results in expression upon exposure to ethylene and conversely, *E*4 can be found in virtually all tissues producing exogenous ethylene suggesting this gene is responsive to ethylene irrespective of tissue and developmental stage. In contrast, *E*8 is only induced in mature fruit (System 2-specific), indicating that ethylene regulation of this gene is both tissue-specific and developmentally regulated [48,49]. Analysis of the *E*4 promoter has shown that ethylene responsiveness of this