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Fruits: A Developmental Perspective

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"Fruit: the edible product of a plant or tree, consisting of the seed and its envelope, especially the latter when juicy and pulpy."
(Oxford English Dictionary)

"But this [a fruit's] beauty serves merely as a guide to birds and beasts in order that the fruit may be devoured and the manured seeds disseminated."
(Charles Darwin)

INTRODUCTION

Embryonic development in many angiosperms occurs concomitantly with the development of the ovary into a specialized organ, the fruit, which provides a suitable environment for seed maturation and often a mechanism for the dispersal of mature seeds, as Darwin observed. Despite centuries of intensive genetic selection of agriculturally valuable fruit, we still lack most information about how fruits develop, how this development is coordinated with embryonic development and seed formation, and the molecular, cellular, and physiological events that control fruit growth and differentiation. The last 10 years have seen a rapid surge of information on one commercially important aspect of fruit development, fruit ripening, including the genetic control of temporal events during the ripening phase (Theologis, 1992; Theologis et al., 1992). However, fewer advances have been made on temporal and spatial controls of fruit set and growth, although from the agricultural point of view, these aspects are of equally critical importance. We will provide a perspective on the molecular, cellular, and physiological mechanisms that must be considered as integral parts of the fruit developmental process. The discussion below will illustrate that fruit development is a potentially useful system to learn more about complex regulatory mechanisms that control the division, growth, and differentiation of plant cells.

ONTOGENIC RELATIONSHIPS BETWEEN FRUIT AND LEAF

Most fruits develop from a gynoecium that contains one or more carpels. Although the term fruit development is used in this review, it is not strictly precise in embryological terms, and fruit development would be more correctly defined as the differentiation of a preexisting organ. In pseudocarpic fruit, organs other than the gynoecium (e.g., receptacle bracts, the floral tube, or the enlarged axis of the infloresence) participate in the formation of the fruit, but these will not be considered here. Because of the morphological diversity of fruits, the tomato fruit was selected as a primary example to emphasize ontogenic relationships and developmental aspects. Tomato fruit is classified as a berry fruit because the thick pericarp encloses many seeds. In some varieties of *Lycopersicon esculentum* (e.g., cherry tomato), the gynoecium consists of two carpels, as shown in Figure 1, but the number increases to three or four in larger fruit, and the distinction between carpels becomes difficult.

When an ovary develops into a fruit, the ovary wall becomes the pericarp. It appears that the size (i.e., the number of cells) of the internal layer (L3) in the shoot apical meristem determines the floral meristem size and carpel number in tomato (and other fruit; Satina and Blakeslee, 1943). Studies with interspecific chimeras between tomato and *L. peruvianum*, which differ in number of carpels per flower, demonstrated that the size of the floral meristem during carpel initiation and final carpel number are determined by the genotype of L3 but not L1 or L2 (Szymkowiak and Sussex, 1992; Huala and Sussex, 1993, this issue). This is interesting because the bulk of the tissue in the pericarp appears to be derived from cells in L3, whereas L1 and L2 contribute to the outer and inner epidermal cell layer and to a layer of small cells immediately adjacent to the epidermal cell layers, respectively (Figure 1). Thus, L3 can directly determine the eventual sink size and strength (i.e., activity of nutrient import) of the developing fruit, but it is presently unclear how this is genetically controlled in the context of the size and activities of source tissues (i.e., leaves).

The ontogenic relationship between fruits and leaves is evident from the cytological appearance of the cells in a cross-section of the carpel and fruit pericarp (Figure 1), as well as from the expression of several genes whose expression is usually confined to leaves, as shown in Figure 2. In the flower, the ovary wall consists of undifferentiated parenchyma cells,

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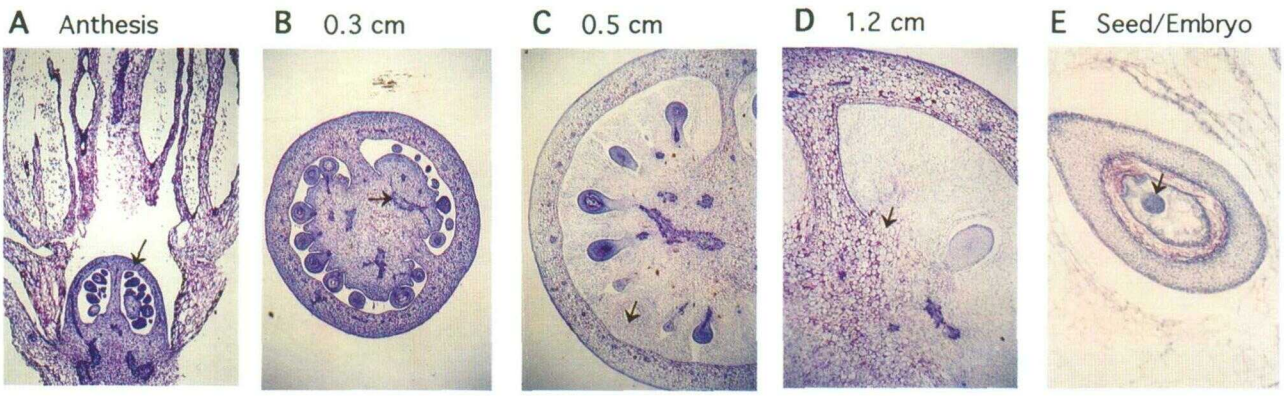

Figure 1. Tomato Fruit Development.

Tissue from VFNT cherry tomato was fixed in formalin plus acetic acid, embedded in paraffin, cut into 10-μm sections, and stained with toluidine blue. Sections were photographed with bright-field illumination at a magnification of ×6.25 **(A)** to **(D)** and ×400 **(E)**.

(A) Longitudinal section through the ovary within the flower at anthesis. Arrow indicates the pericarp.

(B) Cross-section of a fruit 0.3 cm in diameter. Arrow points to vascular tissue within the placenta.

(C) Cross-section of a fruit 0.5 cm in diameter. Arrow indicates the presence of locular tissue, which has differentiated from the placenta.

(D) Part of a cross-section through a fruit 1.2 cm in diameter. Arrow points to the gradient zone of differentiation between placenta and locular tissue.

(E) Cross-section through a developing seed from a fruit 1.2 cm in diameter. Arrow points to the developing embryo within the seed.

vascular bundles, and inner and outer epidermal cell layers (Figure 1A). During fruit development, the ovary wall becomes the pericarp, which consists of three distinct layers: the endocarp, mesocarp, and exocarp (Figure 1D). The septa of the carpels divide the ovary and fruit into two or more locules. An elongated axial placenta, to which the seeds are attached, is highly parenchymous and later gives rise to the tissue that fills the locular cavity. A distinct concentric vascular system radiates through the pericarp but is more diffuse in other parts of the fruit (Figures 1B to 1D).

The pericarp is covered on the outside by a thin cuticle that thickens as the fruit ages. The skin of the pericarp further consists of an epidermal layer and three to four layers of collenchymous tissue. The outer epidermal cells contain little to no starch and no stomata, but the inner pericarp cells contain many starch grains (Esau, 1953; Spurr, 1959; Varga and Bruinsma, 1986). In tomato, the cells that contribute to most of the carpel and fruit pericarp are large and vacuolated, and they are morphologically similar to leaf palisade cells. They contain most of the chloroplasts that give the developing fruit its green appearance. Cells in the outer and inner epidermal layer are small and have fewer chloroplasts. Thus, the carpel can be viewed as a modified leaf that has folded into a tubular structure that encloses the ovules. The fusion of two or more carpels in fruits such as tomato results in complex morphological structure in which it is difficult to discern the ontogenical relationships of cells in the fusion zones (see Gasser and Robinson-Beers, 1993 this issue).

Detailed genetic analysis of flower development has demonstrated that the antagonistic action of homeotic genes is required for normal carpel formation (Bowman et al., 1991). In Arabidopsis, double recessive mutant strains of *apetela-2* and *agamous* display a homeotic conversion with the reduction of carpels to leaflike structures, suggesting that homeotic genes have been recruited during evolution for the modification of leaves into the specialized carpel structure that develops into the fruit. The tissues that later contribute to the growing fruit differentiate from cells in the fourth whorl primordium early during normal carpel development. In contrast to organ specification, little is known about cellular differentiation or about the genes that act downstream of homeotic genes that determine carpel development (Yanofsky et al., 1990; Bradley et al., 1993; see Gasser and Robinson-Beers, 1993, this issue).

Cells in developing fruit often contain photosynthetically active chloroplasts and express nuclear and plastid genes for photosynthetic proteins (Piechulla et al., 1986, 1987). This is consistent with the above discussed ontogenic relationship between cells in leaf and fruit. However, the expression pattern of genes for photosynthetic proteins in fruit can vary from that in leaves, as has been shown for the gene family that encodes the small subunit of ribulose-1,5-bisphosphate carboxylase (*rbcS*) in tomato. Only two of the five *rbcS* genes are expressed during fruit development (Sugita and Gruissem, 1987), and this pattern is controlled at the transcriptional level (Wanner and Gruissem, 1991). However, the interaction of DNA binding proteins with the promoter regions of all five tomato *rbcS* genes is similar in leaf and developing fruit (Manzara et al., 1991), suggesting that the inactivation of a subgroup of *rbcS* genes in fruit must be regulated at a level other than DNA–protein interactions alone, presumably through signal

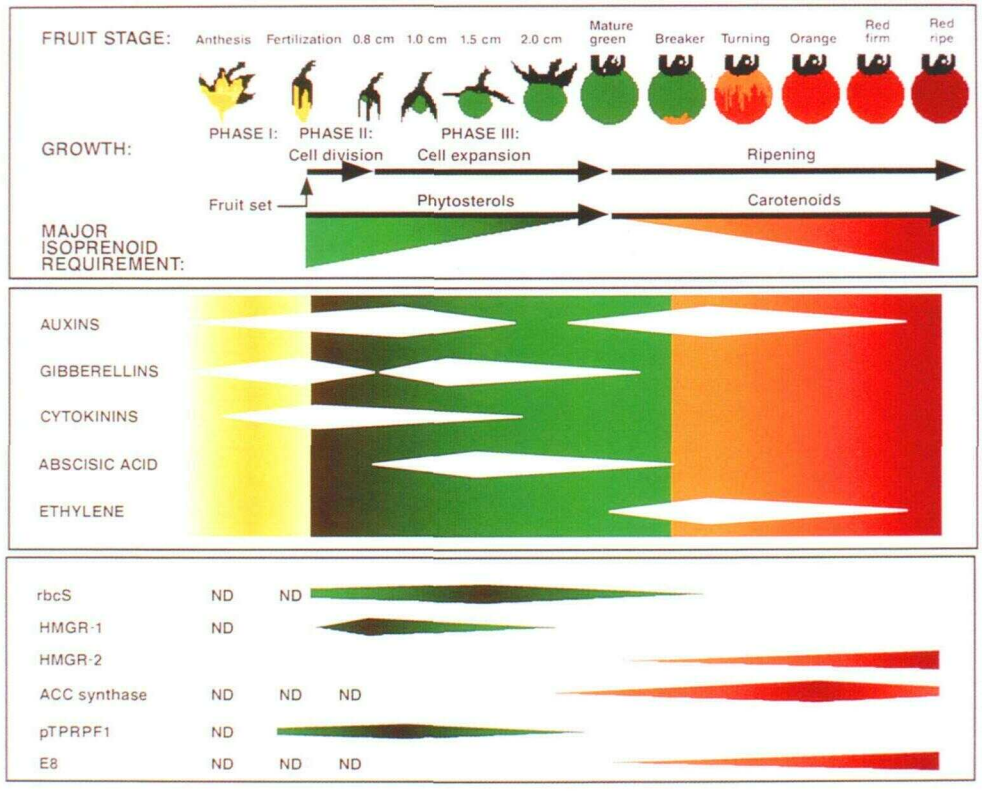

Figure 2. Hormonal Changes and Differential Gene Expression during Fruit Development.

Changes in hormone levels throughout tomato fruit development are indicated by white diamonds. Changes in steady state levels of selected mRNAs are indicated by green (phase II and III) and red (fruit ripening) diamonds.

transduction pathways that are specific to fruit cells (Carrasco et al., 1993; Manzara et al., 1993). It is tempting to speculate that the altered *rbcS* expression pattern in fruit is regulated by the physiological sink state of fruit cells (see below) rather than by a fruit developmental program.

Compared to the photosynthetically active young fruit, most of the DNA–protein interactions are undetectable in ripening fruit, as shown in Figure 3, which is consistent with the transcriptionally inactive state of all *rbcS* genes during this phase of development (Wanner and Gruissem, 1991). Presumably, the reduction in *rbcS* promoter–protein interactions is a developmentally regulated event that is not reflective of a general disappearance of DNA binding proteins, because new genes are activated during ripening. The expression of genes for proteins such as TPRF-F1 (a proline-rich protein; Salts et al., 1991) and 2A11 (a protein of unknown function; Pear et al., 1989) is high in carpels and during early tomato fruit growth but is very low or undetectable in other organs (Figure 2). These examples demonstrate that, although ontogenic relationships exist between cells in leaf and fruit that can be demonstrated at the genetic level, fruit cells have also evolved a unique gene expression program that reflects their difference in function. Nothing is known about the mechanisms that control the expression of genes that are uniquely and highly transcribed in carpels and growing fruit.

Ripening is an aspect of development that is unique to fruit and that is initiated after seed maturation has been completed. Tissue softening and, in several cases, conversion of chloroplasts into carotenoid-accumulating chromoplasts are controlled by the action of new gene products that are expressed prior to, or concomitant with, the first visible color changes (Briggs et al., 1986; Schuch et al., 1989). Ripening is different from the senescence process in leaves because metabolites are not mobilized and recovered but are converted to accumulate high levels of sugars and acids that give the fruit an appealing taste. Although it has been well established that ethylene activates the transcription of several genes early in the ripening phase (Figure 2; Lincoln et al., 1987; Rothman et al., 1991), this is not true for all genes that encode ripening-related proteins, some of whose activation precedes the surge in ethylene synthesis (Della-Penna et al., 1986). Thus, it is presently unclear how the transcription of ethylene-independent genes or the initiation of the ripening process itself are controlled by the fruit developmental program. The ripening aspect,

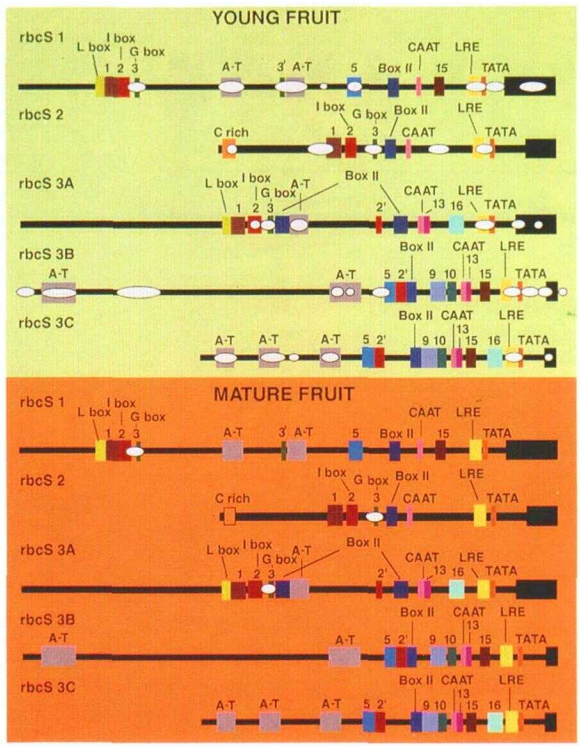

Figure 3. Developmental Changes in *rbcS* Promoter–Protein Interactions.

Promoter regions of the five tomato *rbcS* genes are diagrammed schematically. Heavy solid black lines and solid black rectangles represent the upstream sequences and the transcribed regions of each gene, respectively. Colored boxes indicate conserved DNA sequence motifs; the numbers above these boxes refer to the terminology used in previous publications (Manzara and Gruissem, 1988; Manzara et al., 1991). White ovals represent protected regions detected by in vitro DNAase I footprint analysis (Manzara et al., 1991, 1993; Carrasco et al., 1993) and in vivo genomic footprinting using ligation-mediated PCR (*P. Carrasco, R. Ach, and W. Gruissem, unpublished results*). The protected DNA sequences indicate that multiple DNA binding proteins are involved in the coordinate and differential regulation of this gene family during fruit development.

which has been reviewed in detail (Grey et al., 1992), will not be considered here in the context of fruit development.

FRUIT DEVELOPMENT PHASES

In most plants, early fruit development can be divided into three phases. The earliest phase involves the development of the ovary and the decision to abort or to proceed with further cell division and fruit development, which is generally referred to as fruit set. In the second phase, fruit growth is due primarily to cell division. The third phase begins after cell division ceases. During this phase, fruit growth continues, mostly by cell expansion, until the fruit reaches its final size. This growth phase is the most visible and physiologically most significant because of the strong sink activity exerted by the expanding cells. Different types of fruit display variations of this general developmental program. For instance, in some cases, such as avocado (Schroeder, 1953), cell division in the pericarp continues until shortly before ripening.

Phase I: Ovary Development, Fertilization, and Fruit Set

The molecular nature of the signal(s) that control ovary development are not known. It is now well established that the precise spatial and temporal synthesis and action of auxin, cytokinin, and gibberellins are required for most or all of the normal (and visible) fruit developmental program, but their function during ovary development prior to fertilization is not known. It appears that factor(s) produced by the sporophytic tissue surrounding the developing ovary are required for triggering and maintaining cell division in the fruit primordia until the ovary has reached its mature size. At this time, cell division activity is reduced temporarily until fertilization has been completed. In tissue culture, these factors cannot be replaced by any known combination of cytokinins, auxins, and gibberellins (Nitsch, 1970). This would suggest that communication between cells in the developing flower is essential for normal ovary development.

In normal development, the decision to set fruit is dependent on successful completion of pollination and fertilization. Fertilization after pollination requires pollen germination and penetration and growth of the pollen tube in the stylar tissue toward the ovule and into the embryo sac for fusion with the egg cell. These aspects of fertilization are described in more detail in other reviews in this issue (Dumas and Mogensen, 1993, this issue; Mascarenhas, 1993, this issue). The presence of fertilized ovules generally triggers the development of the ovary into a fruit. The commitment to proceed with fruit development (fruit set) is therefore dependent on one or more positive growth signals generated during or after pollination, and possibly as late as fertilization.

Positive growth stimuli are produced by pollen during germination and pollen tube growth and during or after fusion of the nuclei. Growth factors by which pollen influence fruit set most likely include the plant hormones auxin and gibberellins (Nitsch, 1970). Gibberellins stimulate pollen germination and pollen tube growth, and exogenous application of gibberellins to flowers can result in fruit set in the absence of fertilization (Gustafson, 1960). Application of gibberellins to unpollinated tomato flowers causes an increased auxin level in the ovary (Sastry and Muir, 1963). In tobacco, appreciable quantities of auxin start to diffuse from the base of the style 14 hr after pollination, from the base of the ovary 21 hr later, and from the pedicel 7 hr after the increased ovary auxin levels are detectable

(Muir, 1942). These waves of auxin have been explained as new auxin synthesis induced by enzymes or cofactors supplied by the pollen, although no direct evidence for enzymatic activities or regulatory proteins has been reported. Gibberellins produced by the pollen may thus play a role in increasing auxin production in the ovary, which in turn may act as a signal (or may amplify a signal) for fruit set and subsequent activation of cell division. A direct causal relationship or signal transduction pathway from gibberellin to auxin to fruit set, however, remains to be established.

From the above observations, it follows that poor pollination would result in incomplete fruit set and undersized fruits or ovary abortion. In fact, unless pollination and fertilization are completed, most fruit primordia that have been growing normally within the flower bud will cease cell division and the flowers will abscise. Arrest of fruit set and subsequent growth or abscission of ovaries are not restricted to unpollinated flowers, however, because they can also occur in ovaries after pollination. In this case, either syngamy (leading to embryo formation; see Dumas and Mogensen, 1993, this issue), triple fusion (leading to endosperm formation; see Lopes and Larkins, 1993, this issue), or both fail to take place; alternatively, the endosperm and the embryo degenerate. The results from many studies illustrate that fruit set involves regulatory interactions between the sporophyte and the gametophytic cells. It is unlikely that hormones alone mediate these regulatory interactions but rather that they are important in executing and amplifying the information provided by developmental and gametophytic signals. Mutants in which the failure to set and develop fruit cannot be rescued by hormone application may provide important new insights into control factors that are currently elusive.

Parthenocarpy, i.e., the formation of seedless fruits, has contributed much information about the role of hormones in early stages of fruit development (George et al., 1984). Parthenocarpic fruit development can be genetically controlled or artificially induced by exogenous application of hormones. It usually results from lack of pollination, pollination that does not lead to fertilization (such as pollination with dead pollen or pollen from incompatible species), or successful fertilization that is followed by embryo abortion. Several lines of evidence suggest a correlation between increased auxin and gibberellin levels in the ovary before fertilization and parthenocarpic fruit development. For example, the endogenous levels of auxins and gibberellins are higher in ovaries of parthenocarpic tomato lines than in normal (i.e., seed-producing) lines (Gustafson, 1939b; Nitsch et al., 1960; Mapelli et al., 1979; Mapelli and Lombardi, 1982). In addition, the application of auxins or gibberellins to the outside of the ovary before fertilization often results in parthenocarpic fruit development. Similarly, the auxin transport inhibitor chloroflurenol, when applied to flowers, can induce parthenocarpic fruit development (Robinson et al., 1971). It is likely, therefore, that application of auxin to the ovary or blocking its outward flow from the ovary results in its accumulation within the ovary to concentrations sufficient to establish fruit set and activate cell division in the absence of fertilization.

Together, these observations suggest that parthenocarpy may be a direct consequence of incorrect temporal and/or spatial regulation of auxin synthesis. This conclusion further implies that signal transduction pathways must exist during ovary development that control the temporal synthesis of auxin in the tissues surrounding the ovules to coordinate cell division activity with gametophyte development and fertilization. As discussed above, the signal transduction pathway that leads to increased auxin synthesis after fertilization may involve gibberellins. It has been found that ovaries of parthenocarpic fruits contain a different set of gibberellins than those of normal, seed-producing ovaries at anthesis (Talon et al., 1990). Also, as discussed above, pollen produces gibberellins, and parthenocarpy can be induced in tomato by applying pollen extracts to the sides of the ovary (Gustafson, 1937).

In view of these and other observations, it appears that the sequential or cooperative action of gibberellins and auxin is part of a signal transduction chain that leads to fruit set and subsequent activation of cell division. In ovaries that develop parthenocarpic fruits, this signal transduction pathway is altered such that one or more signals now act constitutively or independently of other regulatory factors to produce elevated hormone levels prior to, or in the absence of, the normal fertilization event. Although recent studies have been targeted at identifying the molecular basis for parthenocarpy (Corella et al., 1986; Barg et al., 1990; Carrasco and Carbonell, 1990), no conclusive answers have yet been obtained to explain pathenocarpic fruit development.

Phase II: Cell Division, Seed Formation, and Early Embryo Development

Following fertilization in tomato, cell division is activated in the ovary and continues for ~7 to 10 days (Mapelli et al., 1978; Varga and Bruinsma, 1986; Bohner and Bangerth, 1988a). As the cell division phase ends, individual cells enlarge, as does the entire fruit, for the next 6 to 7 weeks. Before the cell enlargement phase, dividing cells in the developing fruit are small, tightly compressed, and rich in cytoplasmic substances and have small vacuoles. As cells enlarge, the primary cell wall and the cytoplasmic layer become relatively thinner, and vacuoles occupy a greater proportion of the cell volume (Smith, 1935).

It is generally concluded that during phase II, cell division activity is highest in pericarp and placental tissues (e.g., Spurr, 1959; Varga and Bruinsma, 1986). Primary data to support this conclusion, however, are difficult to obtain from the literature. Two novel experimental approaches using an antibody to DNA labeled with 5-bromo-deoxyuridine and an antibody against proliferating cell nuclear antigen ([PCNA], a subunit of DNA polymerase; Suzuka et al., 1989; Daidoji et al., 1992) to probe thin sections from tomato fruit of different developmental stages

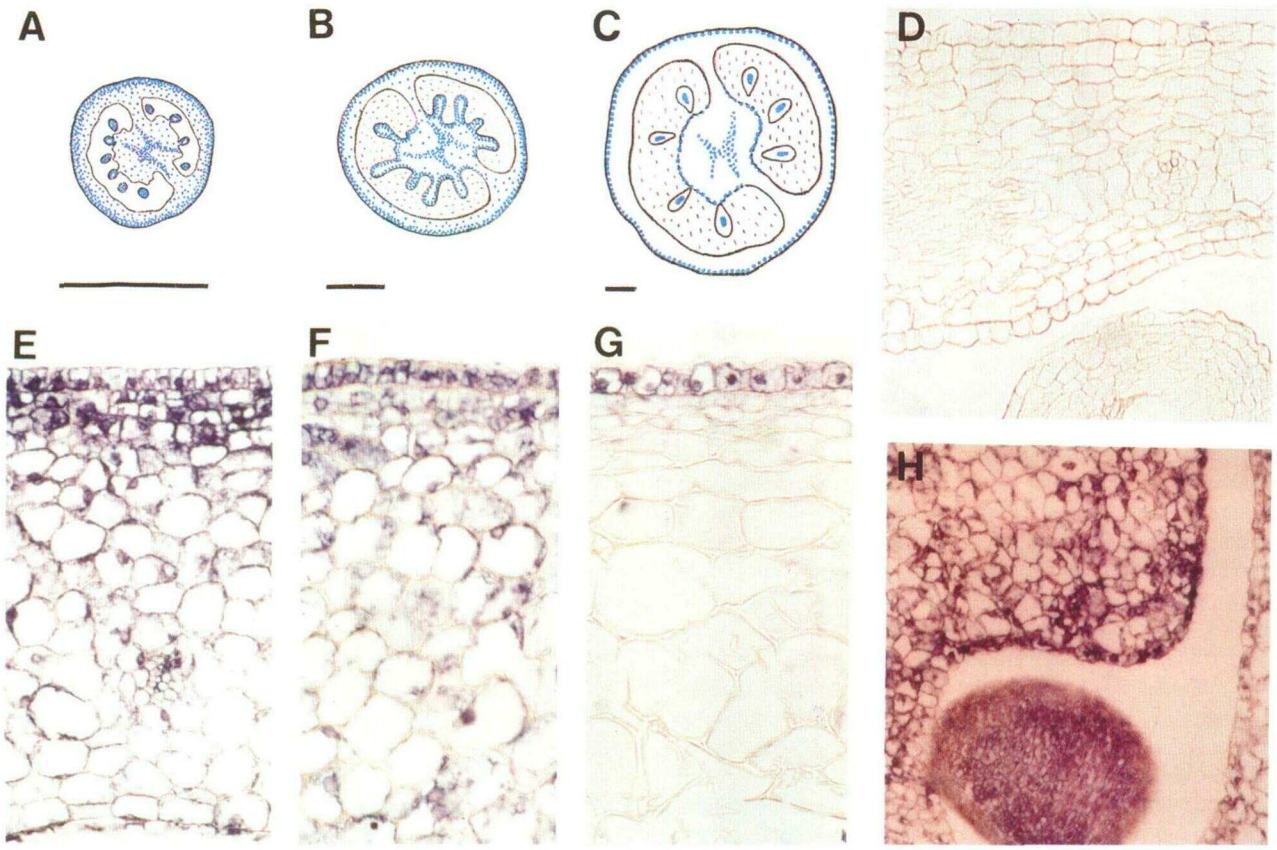

Figure 4. Mitotic Activity in Phase II and III of Tomato Fruit Development.

(A) to **(C)** Schematic summary of tomato fruit cell division; blue dots represent areas of mitotic activity. Bars = 0.2 cm.
(A) Cross-section of an early phase II fruit.
(B) Cross-section of a later phase II fruit.
(C) Cross-section of an early phase III fruit.
(D) to **(H)** Data from PCNA immunocytochemistry experiments. Tissues were prepared as described in Figure 1 and reacted with a monoclonal antibody that recognizes PCNA (Santa Cruz Biotechnology, Inc.). After washes and subsequent incubation with a secondary antibody conjugated to alkaline phosphatase (Oncogene Science), sections were reacted with substrates and washed, and coverslips were mounted with an aqueous medium (Polysciences). Sections were photographed under bright-field illumination.
(D) A cross-section from a fruit 0.3 cm in diameter reacted with secondary antibody alone (control). Under these conditions, no staining of nuclei is detectable in the pericarp, vascular tissues, or seeds. Magnification ×200.
(E) Pericarp from fruit 0.3 cm in diameter. Cross-section was incubated with both primary and secondary antibodies. Magnification ×200.
(F) Pericarp from fruit 0.5 cm in diameter. Cross-section was incubated with both primary and secondary antibodies. Magnification ×200.
(G) Pericarp from fruit 1.2 cm in diameter. Cross-section was incubated with both primary and secondary antibodies. Magnification ×200.
(H) Placenta and seed tissue from a fruit 0.5 cm in diameter. Cross-section was incubated with both primary and secondary antibodies. Magnification ×100.

have provided detailed information about the temporal and spatial distribution of mitotic activity in fruit tissues. Both approaches have given similar results, and a schematic summary with primary data from PCNA studies is shown in Figure 4.

In the very early stages of phase II, mitotic activity is higher in the outer pericarp than in the inner pericarp (Figures 4A and 4E). Cell divisions in the developing seeds occur at the peripheral integument layers rather than in the embryos. Cells within the columellar and placental tissue, which most likely represent vascular cells, also show high mitotic activity. Four to six days after anthesis (Figures 4B and 4F), cell division is still occurring in the fruit. At this time, mitotic activity in the pericarp is confined mostly to the outer layer, and mitotic activity within the placenta is localized to cell layers peripheral to the seeds. Vascular tissues and developing seeds also show mitotic activity at this time. At the end of phase II and overlapping with phase III, mitotic activity is restricted to the outer pericarp cell layer and the outer placental cell layer, from which

the cells in the locule are derived (Figures 4C, 4G, and 4H). Vascular tissue continues to show mitotic activity, and increased mitotic activity is now detectable in the developing embryo.

It is generally accepted that in normal fruit development, the developing embryo or seed controls the rate and sustenance of cell division in the surrounding fruit tissue. This view is consistent with observations that the number of fertilized ovules generally determines the initial growth rate of the ovary, that is, the rate of cell division (Varga and Bruinsma, 1986). If ovules do not develop seeds in part of a fruit, lopsided fruits are formed in which normal and retarded organ development coincide closely with the presence or absence of seeds, respectively (Roberts, 1946; Nitsch et al., 1960). A positive correlation also exists between the number of developing seeds and sustained fruit growth (Gustafson, 1939a; Hobson and Davies, 1970). Thus, the genetic parameters that control the final size and weight of the fruit can be influenced by the number of developing seeds (Nitsch, 1970).

There is currently very little information about the mechanisms by which genetically controlled growth parameters and the number of developing seeds regulate the number of cell divisions that occur in phase II. Final fruit size is in part the result of a defined number of cell divisions that occur within the developing fruit after fertilization. It is also, however, a function of the number of cells within the ovary prior to fertilization, the number of successful fertilizations that have occurred within the ovary, and the extent of cell enlargement (Bohner and Bangerth, 1988a). For example, the presence of multiple fertilized ovules usually induces rapid growth of the ovary into a fruit. This rapid growth may be a reflection of the rate and number of cell divisions or the rate of cell enlargement. To understand the mechanisms that regulate early events in fruit development, it is important, therefore, to distinguish between the genetic and hormonal controls that regulate cell division and the physiological and biochemical parameters that control cell growth.

Information on the biochemical and molecular interplay between the developing embryo/seed and fruit cells is sparse, but bioassays generally indicate a correlation between high cytokinin levels in developing seed and cell division activity in the surrounding tissue (Figure 3; Abdel-Rahman, 1977; Bohner and Bangerth, 1988b). In tomato, most of the cytokinin is found in the developing seed, with very little in the pericarp and placental tissues. How cytokinin accumulates to high levels in the seed and how it can regulate cell division in the surrounding tissues are currently unknown. Cytokinins are most probably transported into the seeds and not produced there (Bohner and Bangerth, 1988b), and seeds are believed to control the rate of cytokinin transport. This suggestion is supported by the observation that parthenocarpic fruits, which lack seeds, contain low levels of cytokinin (Mapelli, 1981), and also by experiments in which substrates for cytokinin synthesis were applied to extracts from developing seeds but which failed to demonstrate enzyme activity. It is possible that an outward flow of cytokinin from the developing seed regulates cell division activity but that the hormone is rapidly degraded in the surrounding tissues. Alternatively, cytokinin in the developing seed may control the synthesis of a positive regulator that diffuses into surrounding cells, which are developmentally programmed to divide.

The molecular mechanisms by which temporal and spatial cell division patterns and cellular differentiation are regulated in plants are not understood. In animals, induction of cell proliferation and differentiation is mediated by a cascade of protein phosphorylations in response to a variety of growth factors or differentiation signals (Cohen, 1992; Pazin and Williams, 1992). As a consequence, cells enter the mitotic cycle, which results either in cellular proliferation or in specific differentiation events that are often coupled to gene activation (Rubin, 1991; Sternberg and Horvitz, 1991; Hunter and Karin, 1992).

Fruit development serves as a good model system for molecular and genetic studies of these processes in plants, because mitotic activity is required in the fruit for both proliferation and differentiation of fruit cells. In tomato, mitotic activity in the pericarp after fertilization results in both the proliferation of cells in the exo-, meso-, and endocarp and the differentiation of a set of mesocarp cells into vascular cells. Activation of mitosis in the peripheral placental cells caused by the developing embryo or seed gives rise to locular cells. These cells are morphologically distinct from the placental cells, although there appears to be a differentiation gradient from the columellar cells to the locular cells adjacent to the pericarp (Figure 1D). Cell proliferation and differentiation in the fruit tissue are temporally coordinated with the mitotic activity in the developing seed and in the developing embryo. Research on signals that coordinate cell division and differentiation in the developing fruit with that in the seed and embryo will benefit further from the increased availability of plant genes for cell cycle regulatory proteins such as $p34^{cdc2}$ (Feiler and Jacobs, 1990; Colasanti et al., 1991), cyclins (Hata et al., 1991), MAP kinase (Duerr et al., 1993), and the nuclear GTP binding RAN protein (R. Ach and W. Gruissem, unpublished results). It will be interesting to determine how these genes and their products are regulated in response to the developmental program and embryonic signals.

Phase III: Cell Expansion and Embryo Maturation

After the period of cell division, fruit growth is due mostly to an increase in cell volume (Figures 1D and 4). The number and timing of cell divisions can vary significantly in different fruits, and both contribute to its final size; in most plants, however, the increase in cell volume makes by far the greatest contribution to the final size of the fruit. Cell expansion commonly increases fruit size by a factor of 100-fold or more (Coombe, 1976). In tomato fruit, the volume of cells in the placenta, locular tissue, and mesocarp tissue can increase

by more than 10-fold (Figures 1B to 1D), but cells that comprise the exo- and endocarp, which continue to divide, expand less (Figure 4). This cell expansion in the fruit tissues is not paralleled by developmental events in the seed, which does not show a comparable increase in size. During this period of rapid fruit cell expansion, the embryo develops from a globular structure to a bilateral embryo that shows well-developed cotyledons and an established root–shoot axis (Smith, 1935).

It is generally accepted that auxins are responsible for the increase in cell expansion in fruit tissues (for a general discussion, see Rayle and Cleland, 1992), although in most fruits, the auxin concentrations are higher in the seed than in the surrounding fruit cells. Auxins presumably cause an increase in the extensibility of cell walls and induce uptake and retention of water and solutes (Hackett and Thimann, 1952). The auxin content of the tomato fruit peaks twice during development (Figure 2). The first surge in auxin level occurs ~10 days postanthesis and coincides with the initiation of cell expansion (Iwahory, 1967). Several observations indicate, however, that cell expansion may not be caused directly by seed-produced auxins alone but rather by an ill-defined sink activity exerted by the developing seeds. For example, cell expansion in parthenocarpic fruit or fruit with a small number of seeds is reduced, and auxins were found to be unable to replace developing seeds as stimulants of in vitro fruit growth by cell expansion (Asahira and Hosoki, 1977).

It is also possible that the developing seed or embryo produces a signal molecule other than auxin that regulates cell expansion and sink activity of the surrounding fruit cells. Such a molecule could be transported passively by diffusion or actively through plasmodesmata-connecting cells in the pericarp and placental tissues. Alternatively, a certain threshold level of seed-produced auxins might be necessary for sustained cell wall expansion in the fruit tissues, but these auxins might be rapidly consumed during the expansion process and would, therefore, be difficult to detect. In addition, a regulatory molecule other than auxin that is produced by the seed or embryo may direct the sink activity of cells in the pericarp, placenta, and locular tissues. The concerted action of both molecules could then give rise to the increase in cell volume. This would explain why exogenous auxins alone are unable to sustain fruit cell expansion in vitro.

Regardless of which scenario better describes how the seed influences cell expansion, it is apparent that the fruit will be a useful model system to dissect the interaction of sporophytic and gametophytic cells and to define the signals produced by the developing embryo that direct cell activities in the surrounding tissues. Several cDNAs for auxin binding proteins have been identified (Palme, 1993), and it will be interesting to determine whether they could provide a molecular basis for the seed-directed transport of auxin. Mutants such as tomato *diageotropica*, which shows auxin-deficient growth characteristics (Zobel, 1973) and lacks high specific activity auxin binding sites (Hicks et al., 1989) but has normal fruit, may be useful to gain further insights into the role of the hormone in fruit development.

In tomato, the second peak in auxin accumulation occurs late in fruit development and coincides with the final phase of embryo development. During this time, fruit cells have reached their maximum volume, but growth of the embryo is rapid and due primarily to cell enlargement. The auxin peak is attributable mainly to high levels of the hormone in the seed, because auxin levels are low or undetectable in the pericarp, placenta, and locular tissues. This is consistent with a role of auxin in cell expansion during embryo development and raises further questions about the role of the hormone in directing the sink activity and volume increase of the surrounding fruit cells. In parthenocarpic fruit, the second auxin peak is undetectable (Mapelli et al., 1978), as would be expected if the seeds are its source.

Although detailed information is now available on the microscopic structure and composition of cell walls, less is known about the developmental control of cell wall formation and expansion. The rapid and signficant cell expansion that occurs during this period of fruit development could serve as a useful model system to dissect the mechanisms by which synthesis, transport, and integration of cell wall proteins and carbohydrates are regulated. Considering the large increase in cell volume during fruit development (10-fold or more), it is likely that this process cannot be accomplished by cell wall extension alone but that synthesis of new cell wall material is necessary. The expression of genes such as those represented by pTPRPF1 (Figure 3), which likely encodes a proline-rich cell wall protein (Salts et al., 1991), is consistent with new synthesis of cell wall molecules to support cell expansion.

Recent work in mammalian and yeast cells has identified a group of RAS-like GTP binding proteins (RAB, SEC, YPT; Balch, 1990) that have a regulatory role in the vesicular trafficking of proteins through the exocytic and endocytic pathways. Several RAB- and YPT-like proteins have now been reported from higher plants (Anai et al., 1991; Dallmann et al., 1992), and at least one member of this family is highly expressed in tomato fruit during phase III but not at later stages of fruit development (A. Loraine and W. Gruissem, unpublished results). It will be interesting to determine whether the temporal expression of this protein directs aspects of the cell expansion program. Recently identified proteins that induce cell wall extension (McQueen-Mason et al., 1992) may represent other potential factors that could be part of hormonal and/or developmental signal transduction networks controlling cell expansion in fruit.

The role of gibberellins in fruit development is not well understood, but it is generally assumed that they are necessary to stimulate cell division and to maintain cell expansion. During tomato fruit development, there are two peaks of gibberellin accumulation, which coincide with activation of cell division early in phase II and cell expansion in phase III (Figure 3). The increase in gibberellins in phase III occurs during maximal fruit growth, when auxin levels decrease. This profile is consistent with a model in which auxin-stimulated gibberellin synthesis and accumulation are required for subsequent expansion and/or sink activity of the fruit cells. Parthenocarpic

fruit show a similar temporal accumulation of gibberellins in phase II, although the level is several fold higher than in normal fruit (Ho and Hewitt, 1986). This correlates with an increase in the rate of cell expansion during early phase II, which does not occur in seeded fruit. The temporal accumulation of the hormone during phase III is less pronounced in parthenocarpic fruit than in normal fruit (Mapelli et al., 1978), which could explain the reduced growth rate of parthenocarpic fruit at this stage of development.

Normal seed development is also dependent on the developmental stage of the sheath and locular tissues with which the seed is in contact (Figures 1C through 1E). It has been suggested that changes in abscisic acid (ABA) concentration and osmotic potential in these tissues regulate seed desiccation and induce dormancy of the embryo to prevent precocious germination in the locular environment (Berry and Bewley, 1992; see Thomas, 1993, this issue). During tomato seed development, the endogenous ABA concentration peaks at the stage of cell enlargement and then declines at later stages of fruit development (Figure 1G). The ABA concentration in the sheath tissue immediately surrounding the seed increases with time of development, whereas that of the locular tissue declines. The water content of the seed and locular tissues is similar during early development (i.e., during the rapid cell division stage), but the water potential and osmotic potential of the embryo are lower than those of the locular tissue at the cell expansion stage. ABA-deficient mutants often show a high frequency of precocious germination of the embryo, but at present it is difficult to discern whether this is the result of reduced ABA levels in the locular tissue or in the developing seed.

METABOLIC CONTROL OF FRUIT GROWTH

Fruit development and growth are dependent on photosynthetic carbon dioxide fixation in leaves and the translocation of sucrose, amino acids, and organic acids to the fruit cells (Ho, 1988). Thus, fruit cells need to monitor continuously the import rate of photoassimilates required to sustain cell division and growth in both the fruit tissue and the embryo. During the early phase of development, most fruits, like meristems, can be classified as utilization sinks because of their high metabolic activities and rapid cell division. During the later phase of development, which is characterized by cell expansion and seed development and maturation, most fruits accumulate high levels of carbohydrates in the form of either sugars or starch and, therefore, are more typical of storage sinks (Ho, 1988). Most fruit cells have functional chloroplasts during their development, but it does not appear that photosynthetic carbon dioxide fixation in fruit contributes significant levels of photoassimilates to fruit growth. Because of their importance for crop yield, sink strength and activity during later phases of fruit development have been investigated extensively from the aspects of enzyme activities, source–sink relationships, and physiological and environmental factors that affect sink functions (reviewed in Ho, 1988; Guan and Janes, 1991; Yelle et al., 1991; Sun et al., 1992; Wang et al., 1993). These studies, however, usually fail to address the role of sink function from the developmental point of view.

The number of cells in the preanthesis ovary and cell division activity following pollination are critical factors for the determination of the actual sink strength of the developing fruit and are genetically determined (reviewed in Coombe, 1976). As discussed above, the molecular mechanisms that control cell division and cell number in ovaries after pollination and during early fruit development are not known. In tomato, a fruit growing at the distal position contains fewer cells than a fruit at a proximal position of the same truss. The differences in fruit cell number appear to be determined at the initiation of the floral primordium and are first evident in the carpels. During normal truss development, the sink strength of a proximal fruit is greater than that of a distal fruit, consistent with a direct correlation between cell number and sink strength. By altering the sequence of fruit set, however, fruit in the distal position, although they contain fewer cells, develop an increased sink strength relative to later set proximal fruit (Bangerth and Ho, 1984). These observations suggest that actual sink strength is only partially determined by cell number and that it may also be regulated by the temporal and metabolic activities of cells during early fruit development as a function of fruit position and efficiency of photoassimilate import. At present, the genetic and physiological factors that control the metabolic activities that influence fruit growth are not well understood, but novel molecular approaches to sink–source interactions may provide new insights into this problem (Sonnewald and Willmitzer, 1992).

Recent work on phytosterol biosynthesis in tomato has provided new insights into factors other than photoassimilate import that may control processes early in fruit development. Inhibition of hydroxymethyl glutaryl CoA reductase (HMGR), which is highly expressed during the early stages of tomato fruit development (Figure 2), by injection of the inhibitor mevinolin effectively arrests normal development. The resulting fruits are small, although viable and metabolically active, and have altered tissue morphologies (e.g., reduced pericarp thickness and locular tissue; Narita and Gruissem, 1989; J. Narita and W. Gruissem, unpublished observations). Coinjection of phase II fruit with mevinolin and mevalonic acid (MVA) results in mature fruits that are phenotypically normal because the exogenously applied MVA can be utilized to sustain cell proliferation and expansion. These results show that fruit cells are autonomous for the synthesis of MVA, which is the precursor of phytosterols and many other isoprenoid compounds.

It is possible that growth inhibition and morphological differences caused by mevinolin are the direct result of depletion of phytosterols, which are critical for membrane synthesis and function. Alternatively, these phenotypic effects could result from a failure to synthesize cytokinin and gibberellins, which are derived from MVA via isopentenyl phosphate and geranylgeranyl pyrophosphate (GGPP), respectively. It is also possible

that synthesis of MVA (a C_5 isoprenoid) or other phytosterol intermediates may be strictly monitored by the cells and that alterations in the flow of such intermediates could be relayed directly to molecules that regulate cell division and growth. It is now well established in yeast and animal cells that the two sterol intermediates farnesyl pyrophosphate (FPP, C_{15}) and GGPP (C_{20}) are required for the modification and biological activity of growth regulators and signal transduction proteins such as RAS and receptor-coupled G-proteins, as well as nuclear lamins and GTP binding proteins that control secretory functions (Schafer and Rine, 1992). Inhibition of MVA synthesis in mammalian cells often results in a specific arrest of the cell cycle in late G1 or early S phase that can be released by low concentrations of MVA (Langan and Salter, 1991a, 1991b). The enzymatic activities that catalyze the modification of regulatory and signal transduction proteins by FPP or GGPP are also present in plants (D. Schmidt, J. Narita and W. Gruissem, unpublished results), and the β subunit of the farnesyltransferase has recently been cloned from pea (Yang et al., 1993) and tomato (J. Narita, K. Callan, and W. Gruissem, unpublished results). As these and other molecular probes become available, it will be possible to study the mechanisms that regulate early fruit development in more detail.

FRUIT DEVELOPMENT AS A MODEL SYSTEM FOR TEMPORAL CONTROL OF CELL PROLIFERATION AND DIFFERENTIATION

It is presently unclear how regulatory networks operate in plant cells to control cell division, growth, and differentiation, and it is not known whether membrane-bound receptors and signal transduction pathways exist in plants that activate kinases and transcription factors to direct new developmental programs. Many aspects of fruit development make this a uniquely valuable plant organ in which to address these problems. First, early fruit growth is one of the few developmental stages in which cell division is activated rapidly upon one or more specific signals. Initiation of fruit development can be manipulated by external application of hormones that may, directly or indirectly, control events that lead to activation of cell division. In many plants, inhibitors or hormones can also be applied to early fruit stages by injection into the pedicel, from which diffusible substances are transported rapidly throughout the fruit (Narita and Gruissem, 1989). Second, mitotic activity in fruit tissues during phase II results in restricted cell proliferation that can be altered genetically. Third, proliferation of cells in fruit tissues is coupled to specific differentiation events (e.g., of vascular and locular cells), and because of the accessibility of the developing fruit, it may be subjected to cellular or biochemical manipulations. For example, molecular manipulations to alter developmental sequences or mitotic activities are feasible using promoters from genes that are exclusively or highly expressed during early fruit development to control the expression of cell cycle regulatory genes.

The dependence of cell proliferation during fruit development on nutrient import (i.e., sink activity) is another area in which the fruit can serve as an important model system for the dissection of potential regulatory interactions between metabolic pathways and signal transduction pathways in the control of cell proliferation and differentiation. Together, these few examples illustrate that fruits may not only "serve as a guide to birds and beasts in order to be devoured" but also have the potential as a (little explored) biological system to understand problems central to plant development.

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