Chapter 21

The Role of Plastids in Ripening Fruits

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Summary

Although integrated into a sink tissue, fruit plastids play a key role in plant productivity because lowering fruit plastid metabolism decreases crop yield. Unlike leaf chloroplasts, the capacities of fruit plastids for photosynthetic electron transfer and carbon dioxide assimilation are low and decline dramatically during the ripening stage. However, during this developmental transition hexoses derived from plastid starch hydrolysis and metabolites imported from the cytosol are actively used for the biogenesis and accumulation of carotenoids, prenyl- and acyl- lipids and amino acids. These activities are sustained by non-photosynthetic generation of ATP and reducing power within the organelle. Here we summarize the function of plastids during fruit ripening in relation to recent advances in biochemistry and molecular biology.

I. Introduction

One of the most prominent changes during fruit ripening is the breakdown of plastid thylakoids concomitantly to the degradation of chlorophylls and the down-regulation of photosynthetic gene expression (Piechulla *et al.*, 1985). Although these changes are reminiscent of senescent or aging processes (Rhodes, 1980), they are not deteriorative *per se*. Indeed, during fruit ripening, plastids gradually acquire new biosynthetic capabilities and in most cases the chloroplasts differentiate into non-photosynthetic chromoplasts.

Plastid starch present in unripe fruit is progressively converted to hexoses during the ripening period (Robinson *et al.*, 1988) and in parallel diverse metabolites are imported into the plastid or exported to the cytosol *via* specific plastid translocators (Fischer and Weber, 2002). The resulting carbon skeletons are used in the organelle for the generation of non-photosynthetic ATP and reducing power and for the biogenesis of diverse products. The latter includes carotenoids that give the yellow to orange colors characteristic of many fruits (Camara *et al.*, 1995). Although the biological significance of this phenomenon

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is unknown, it is assumed that the accumulation of massive amount of carotenoids constitutes part of the signals favoring seed dispersal by animals (Goodwin, 1986). In a similar vein, in ripening fruit plastids play a key role in the *de novo* biosynthesis of acyllipids as shown by the fleshy oil palm mesocarp which produces palm oil, that ranks second among consumed vegetable oil (Salas *et al.*, 2000). Along with the utilization of the carbon skeleton, the plastidial glutamine synthetase-glutamate synthase, a main route for nitrogen assimilation, is subject to a ripening-specific regulation (Gallardo *et al.*, 1988, 1993). Beyond the fact that these events are developmentally regulated, little is known about the molecular mechanisms inducing these changes. This review focuses on the progress made using biochemical and molecular approaches to better understand the function of plastids during fruit ripening.

II. Plastid Differentiation

A. Evolution of Photosynthetic Genes and Plastid Differentiation

Fruits are largely considered as sink organs and as such, their photosynthetic capacity is usually low. In the absence or reduced presence of stomata (Willmer and Johnston, 1976; Blanke, 1986), $C0_2$ used for fruit photosynthesis is derived mainly from respiration (Blanke and Lenz, 1989). In tomato fruit, fruit photosynthesis contributes 10 to 15% of the fruit carbon gain (Tanaka *et al.*, 1974). On a protein basis ribulose-1,5,-*bis*phosphate carboxylase (Rubisco) of tomato pericarp is approximatively 35% that of tomato leaves (Piechulla *et al.*, 1987). In pepper fruit the Rubisco activity is about one-fifteenth the activity found in leaves (Steer and Pearson, 1976). This feature is obviously consistent with the sink characteristic of pepper fruit (Hall, 1977). Along with the steady decrease of Rubisco during fruit ripening, the 33-kD oxygen evolving protein, cytochrome b559, the chlorophyll a/b binding proteins the D1 protein of photosystem II also decline similarly to their corresponding transcripts (for a review see Camara *et al.*, 1995). These changes are generally followed by chloroplast to chromoplast differentiation which involves the disintegration of the chlorophyllous thylakoid and the appearance of new membrane or lipoprotein structures which sequester excess carotenoids and other lipophilic derivatives produced during the ripening process (Camara *et al.*, 1995).

B. Hormonal and Nutritional Control of Fruit Chromoplast Differentiation

Attempts to understand the cellular mechanisms underlying differentiation of chloroplasts to chromoplasts have revealed hormonal (Coggins *et al.*, 1980; Gemmrich and Kayser, 1984; Goldschmidt, 1988; Trebitsh *et al.*, 1993; Alexander and Grierson, 2002) and nutritional determinants (Huff, 1984; Iglesias *et al.*, 2001).

In contrast to research on the hormonal effect, which has focussed largely on the predominant role of ethylene (Alexander and Grierson, 2002), the nutritional aspect has received limited attention. According to this hypothesis, the chloroplast to chromoplast development in *Citrus* fruit is regulated by the carbon to nitrogen ratio (C/N), i.e., a high ratio induces chromoplast differentiation while a low ratio favors the reversion process (Huff, 1983, 1984; Mayfield and Huff, 1986).

The glutamate-oxoglutarate aminotransferase (GOGAT) cycle, which represent the main route of nitrogen assimilation in plants, could play a key role in the C/N ratio. It has been shown that in tomato 70% of the total free amino acid of the pericarp belongs to the glutamate family (Valle *et al.*, 1998) especially glutamine and glutamate (Boggio *et al.*, 2000). It has also been established that tomato mutant *rin* (for ripening inhibitor) accumulates half the normal level of glutamate of wild fruit (Nagata and Saito, 1992). However, the contribution of tomato fruit plastids in the synthesis of glutamine and glutamate is limited because total GS and GOGAT activities are drastically

Abbreviations: ACCase – acetyl-CoA carboxylase; AOS – allene oxide synthase; Ccs – capsanthin-capsorubin synthase; CrtB – bacterial phytoene synthase; CrtHb – non-heme diiron monooxygenases; CrtIso – carotenoid isomerase; DMAPP – dimethylallyl diphosphate; FAS – fatty acid synthetase; FBPase – fructose-1,6-*bis*phosphatase; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; GGPP – geranylgeranyl diphosphate; GGPPS – geranylgeranyl diphosphate synthase; GOGAT – glutamate-oxoglutarate aminotransferase cycle; GS– glutamine synthetase; G6PDH – glucose 6-phosphate dehydrogenase; Hggt – homogentisate geranylgeranyl diphosphate transferase; HPL – hydroperoxide lyase; IPP – isopentenyl diphosphate; KAS I, II, III – β-keto acyl-ACP synthases I, II, III; Lcyb – lycopene β-cyclase; Lcye – lycopene ε-cyclase; MACP – malonyl-acyl carrier protein; Nsy – neoxanthin synthase; OPP – oxidative pentose phosphate pathway; Pds – phytoene desaturase; pGlcT – plastid glucose transporter; PPO – polyphenol oxidase; PSY – phytoene synthase; PII – a signal transduction protein involved in monitoring cellular C and N status; Rubisco – ribulose-1,5,-*bis*phosphate carboxylase; TE – thioesterase; Zds – ζ-carotene desaturase; Zep – zeaxanthin epoxidase.

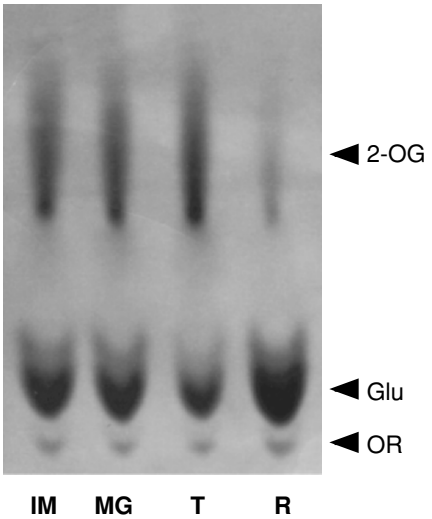


Fig. 1. Glutamic acid synthesis in isolated pepper plastids. Glutamate synthesis from 2-keto (1-^{14}C) glutaric acid in plastids isolated from pepper fruit at different stages (IM, immature green; MG, mature green; T, turning; R, red). The reaction products were separated using a Cellulose thin layer chromatography plate developed with butanol/formic acid/water: 70/12/10, v/v and visualized by autoradiography. The abbreviations refer to: Glu, Glutamic acid, 2-OG, 2-Oxoglutarate, OR, origin.

reduced during the ripening stage (Gallardo *et al.*, 1988, 1993; Boggio *et al.*, 2000). Therefore, these amino acids must be actively translocated from the leaves during the ripening period according to an unknown mechanism. Alternatively, the reversible reaction catalyzed by mitochondrial glutamate deshydrogenase which is induced during tomato ripening (Boggio *et al.*, 2000) could be involved. Clearly the down regulation of chromoplast GS and GOGAT during tomato ripening is a diagnostic feature of the elevation of the C/N ratio. Whether this mechanism could be generalized to other fruit plastids is debatable. Using an assay based on the use of radioactive 2-oxoglutarate, we observed in pepper fruit chromoplasts, a labeling pattern consistent with the synthesis of glutamic acid by transamination (Fig. 1). 2-Oxoglutarate is synthesized in the cytosol and mitochondria (Hodges, 2002) and is transported into the plastid by a malate-coupled, two-translocator system which involves a 2-oxoglutarate/malate translocator and a glutamate/malate translocator (Weber and Flugge, 2002; see Chapter 14). In addition to NADPH derived from the oxidation of glucose 6-phosphate via the plastidial pentose phosphate pathway, the redox equivalent for GOGAT could be provided by specific ferredoxin isoforms synthesized during chloroplast to chromoplast differentiation (Green *et al.*, 1991).

Obviously, further studies are required to test the C/N ratio and to analyze its potential relationship with the PII signal transduction protein which monitors the cellular status of C (oxoglutarate) and N (glutamine) and has been previously identified as a carbon/nitrogen sensor in bacteria (Stadtman, 2001) and plants (Hsieh *et al.*, 1998; Moorhead and Smith, 2003; Smith *et al.*, 2003).

III. Plastid Biogenesis and Molecular Regulation

A. Carbohydrate Metabolism and Cytosolic Interactions

During fruit ripening starch stored in plastids is totally or progressively transformed into hexoses by amylase, ADP Glucose pyrophosphorylase and phosphorylase (Fig. 2). In tomato fruits, the plastid starch content starts declining between 14 to 50 days after anthesis (Yelle *et al.*, 1988), according to a phosphorolytic pathway (Robinson *et al.*, 1988).

Although it has been shown that glucose resulting from the amylolytic degradation of starch is exported to the cytosol through a specific plastid glucose transporter (pGlcT) in leaves (Weber *et al.*, 2000), the situation in non-green tissue might different. This is based on the fact that the pGlcT transcript is highly expressed in the non-photosynthetic albedo tissue of *Citrus* fruit, apricot and tomato fruits (Fischer and Weber, 2002). A detailed analysis reveals that in tomato, a starch- and sugar-storing fruit and in olive, a lipid-storing fruit, the expression pattern of pGlcT is highest during the ripening period (Butowt *et al.*, 2003). This led to the suggestion that pGlcT could be involved in the plastidial import of glucose from the cytosol in non-green tissues and especially during chloroplast to chromoplast differentiation (Butowt *et al.*, 2003) (Fig. 2). This hypothesis is reinforced by the fact that unlike in leaves, the olive fruit pGlcT gene does not display diurnal expression (Butowt *et al.*, 2003) and also by the recent characterization of a plastid stromal hexokinase (Olsson *et al.*, 2003).

The role of plastid fructose-1,6-*bis*phosphatase (FBPase) could also be important for providing hexoses in non-green tissues. FBPase, a key enzyme of the Calvin-Benson cycle, is involved in the conversion of triose phosphates into hexose phosphates and is present in tomato leaves and green fruits, whereas

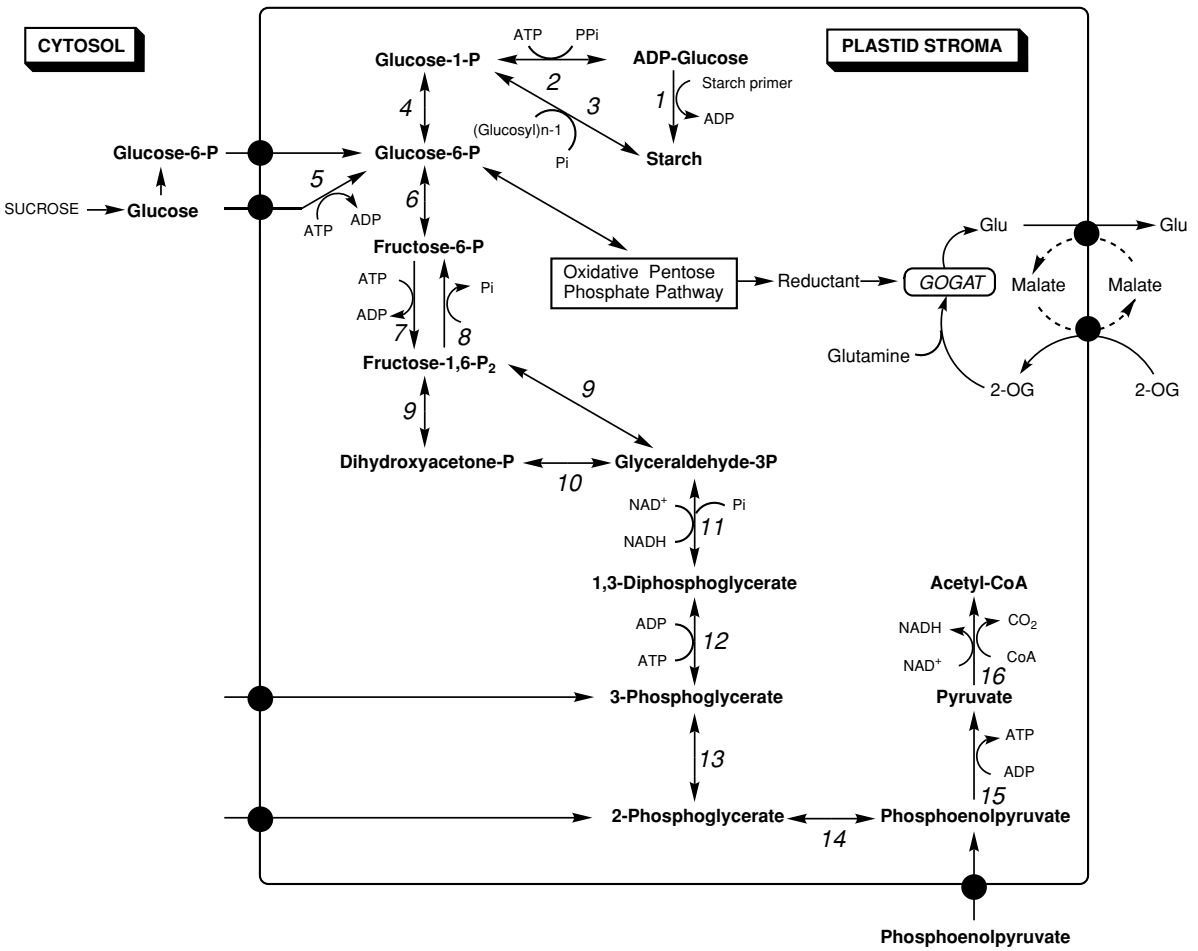


Fig. 2. Pathway of the utilization of sucrose-derived metabolites in plastids from ripening fruits. Numbers refer to the following enzymes : 1, starch synthase; 2, ADP-glucose pyrophosphorylase; 3, α-glucan phosphorylase; 4, phosphoglucomutase; 5, hexokinase; 6, hexose phosphate isomerase; 7, ATP-phosphofructokinase, 8; fructose,1, 6-*bis*phosphatase; 9, aldolase; 10, triose-P isomerase; 11, glyceraldehyde 3-P dehydrogenase; 12, phosphoglycerate kinase; 13, phosphoglycerate mutase; 14, enolase; 15, pyruvate kinase; 16, pyruvate dehydrogenase. Abbreviations refer to: GOGAT, glutamine-2-oxoglutarate aminotransferase; Glu, glutamic acid; 2-OG, 2-oxoglutarate. Plastid translocators are shown as solid, black circles.

red fruits contain only the cytosolic form (Büker *et al.*, 1998). This suggests that hexose phosphates are imported from the cytosol during chloroplast to chromoplast differentiation in tomato. In pepper fruits during the ripening process the decrease of Rubisco (Ziegler *et al.*, 1983) is paralleled by an increase in plastidial FBPase (Thom *et al.*, 1998).

Whatever their origin, the carbon skeletons are used in different biosynthetic pathways such as transient starch, fatty acids and isoprenoids. They are also used in the oxidative pentose phosphate (OPP) pathway which is a main source of reducing power in the absence of photosynthetic electron transport (Fig. 2). In this context, it is worth noting that tomato chromoplast glucose 6-phosphate dehydrogenase (G6PDH), a main enzyme of the OPP pathway, is 48.7 and 7.4 more active than leaf and green fruit chloroplast G6PDH from the same plant (Aoki *et al.*, 1998).

The capacity of fruit chromoplasts to oxidize hexoses to pyruvate (Fig. 2) has been reviewed previously (Camara *et al.*, 1995). In this context it is interesting to note that a chromoplast-specific, NAD^+-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) named GapCp has been characterized recently from pepper fruits (Petersen *et al.*, 2003). The expression of *GapCp* is restricted to the ripe fruits and roots. On the other hand the chloroplast $NADP^+$-dependent GAPDHs, *GapA* and *GapB*,

are down-regulated during pepper fruit ripening. This suggests that in chromoplasts or in a more general context, non-green plastids, GapCp is specifically engaged in the production of energy in the absence of photosynthesis (Petersen *et al.*, 2003). In relation to the energy requirements in nongreen plastids, it is interesting to note that heterotropic daffodil chromoplasts can direct part of the NADP(H) generated by plastid glycolysis to the generation of ATP (Morstadt *et al.*, 2002).

The plastid glycolytic pathway is interconnected with the cytosolic glycolytic pathways by several membrane translocators (Fig. 2). It has been suggested that in relation to their metabolic activity plastid translocators could play specific roles (Heldt *et al.*, 1991). This contention is supported by the fact that the plastid triose phosphate translocator is more highly expressed in green tomato fruit than in the red fruit (Schünemann *et al.*, 1996), while the reverse situation is observed in red tomato fruits (Schünemann and Borchert, 1994). In a similar vein, the phosphoenolpyruvate transporter gene is more expressed in non-green tissue (Fischer *et al.*, 1997). Based on the fact that the initial phase of respiratory climacteric is correlated (at least in banana fruit) with a reduced level of PEP and an increased level of pyruvate (Beaudry *et al.*, 1989; Ball *et al.*, 1991) this suggests that during the ripening period fruit plastids may exert indirect control over cytosolic glycolytic flux.

B. Acyllipid Metabolism

Although most commercial oils are derived from seeds, the ripe fleshy pericarp of oil palm and olive fruits represent an important source of vegetable oil. For instance, palm oil ranks second after soybean (Salas *et al.*, 2000). Palm oil contains about 45% palmitic acid and 40% oleic acid, while olive oil is 60 to 70% enriched in oleate (Salas *et al.*, 2000). In both fruits, the initial control of the lipid synthesis is exerted at the level of the *de novo* synthesis of fatty acid in the plastid, before the modification steps in the endoplasmic reticulum and eventual triacylglycerol accumulation (Daza and Donaire, 1982; Sambanthamurthi *et al.*, 2000) (Fig. 3).

Two routes have been established for the synthesis of the acetyl-CoA, the initial precursor (Ohlrogge and Browse, 1995). These include the plastid glycolytic degradation of hexoses via the pyruvate dehydrogenase complex. Alternatively, acetyl-CoA produced from the mitochondrial pyruvate dehydrogenase complex could be imported into the plastid. Both pathways have been demonstrated in olive fruit pericarp (Salas *et al.*, 2000). Acetyl-CoA is sequentially converted to malonyl-CoA by acetyl-CoA carboxylase (ACCase) which is transformed to malonyl-acyl carrier protein (MACP) before the formation of acyl-ACP by individual component enzymes of the fatty acid synthetase pathway (FAS)

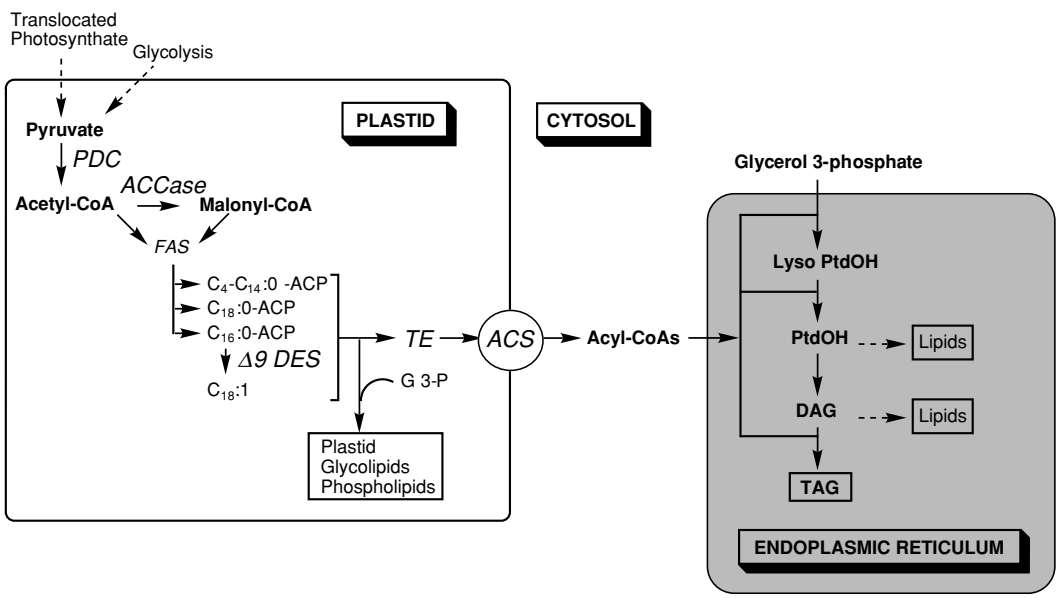


Fig. 3. De novo synthesis of lipids in plastids of ripening fruits and modification in the endoplasmic reticulum. Abbreviations refer to: PDC, pyruvate dehydrogenase complex; ACCase, acetyl-CoA carboxylase; FAS, fatty acid synthetase; ACP, acyl carrier protein; Δ9 DES, Δ9 desaturase; TE, thioesterase; ACS, acyl-CoA synthetase; PdtOH, phosphatidic acid; DAG, diacylglycerol; TAG, triacylglycerol.

(Ohlrogge and Jaworski, 1997) (Fig. 3). FAS comprises the condensing enzymes β-keto acyl-ACP synthases (KAS) I, II and III, β-ketoacyl-ACP reductase and β-hydroxyacyl-ACP dehydratase. The ratio between the final products 16-ACP and 18-ACP is specified by the plastid thioesterase (Fig. 3), which cleaves the acyl-ACP, and also by the KASII activity with elongates palmitoyl-ACP to steraroyl-ACP. Consistent with the regulatory role of plastid thioesterases and KASII, a specific palmitoyl-ACP thioesterase (Othman *et al.*, 2000) is induced during the ripening stage of oil palm fruit, while the KASII activity is decreased (Salas *et al.*, 2000; Sambanthamurthi *et al.*, 2000). Thus, these coupled activities contribute to the profuse plastidial synthesis and export of palmitate to the cytosol and account for the high palmitate content of oil palm fruit. On the other hand, in olive fruit the role of the plastidial C_{18} acyl-ACP thioesterases seems to predominate (Harwood, 1996).

In some plants, specific plastidial KASs and acyl-thioesterases contribute to the synthesis of fatty acids having shorter carbon chains. The latter are probably exported to the cytosol and used for the synthesis of capsaicinoids during pepper fruit ripening (Aluru *et al.*, 2003) (Fig. 4). Alternatively, some early intermediates formed in the plastid could be exported to the cytosol and used for the biogenesis of acylglucose derivatives (van der Hoeven and Steffens, 2000) (Fig. 4).

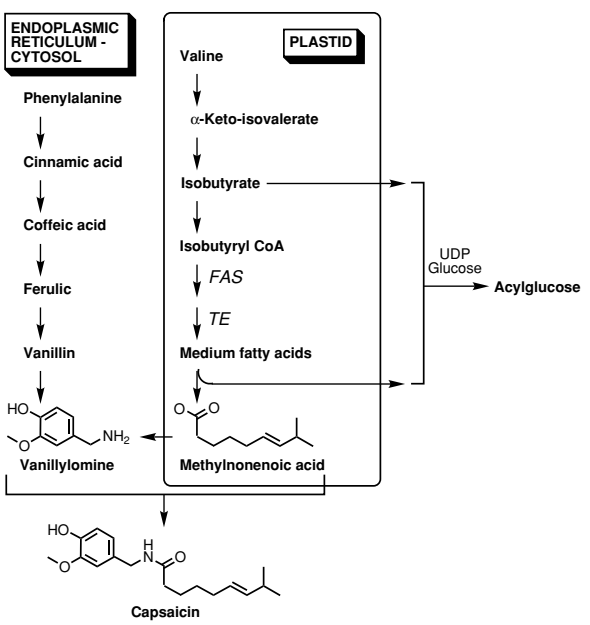


Fig. 4. Channeling of lipid precursors towards capsaicin and acylglucose synthesis. Abbreviations refer to: FAS, fatty acid synthetase; TE, thioesterase.

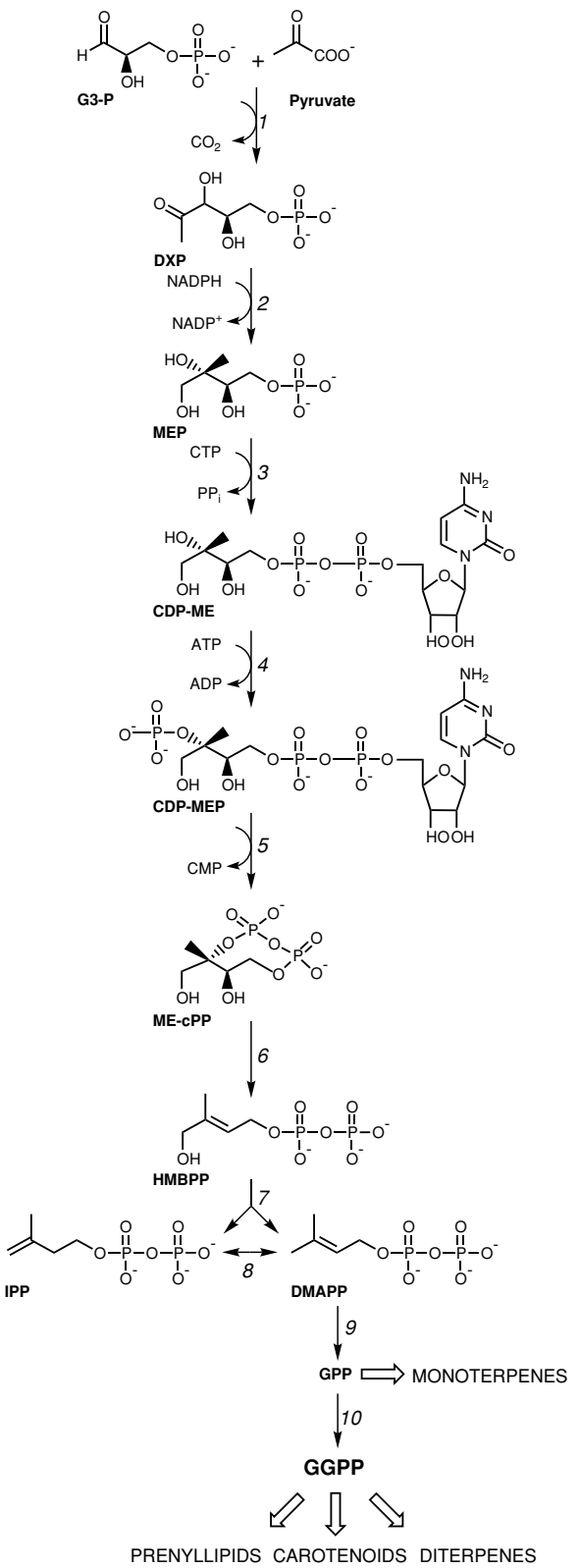
Several lines of evidence suggest the involvement of oxylipins derived from the lipoxygenase pathway in the ripening of fruits. Lipoxygenase catalyzed reactions produce 9- and 13-hydroperoxide derivatives. The 13-hydroperoxide is converted into several products including jasmonic acid and the volatile aldehyde hexenal (Mack *et al.*, 1987), while the 9-hydroperoxide product could be converted under constrained conditions into hexanal with low efficiency (Hatanaka *et al.*, 1992). In tomato, the hydroperoxide lyase (HPL) catalyzing the synthesis of hexenal and the allene oxide synthase (AOS) initiating the jasmonic acid pathway have been characterized and shown to be respectively located in the inner and the outer chloroplast membrane envelopes (Howe *et al.*, 2000; Feild *et al.*, 2001; Froehlich *et al.*, 2001). However, during the ripening period tomato fruit HPL and AOS genes are apparently not induced (Back *et al.*, 2000). A similar trend was observed during the ripening of pepper (Matsui *et al.*, 1997). Thus further studies are required to clarify the involvement of oxylipin metabolism during fruit ripening.

C. Carotenoid Metabolism

The yellow, orange and red colors of many fruits are due to carotenoids which are classified into carotenes and their oxygenated derivatives, xanthophylls. Carotenoids are synthesized as C_{40} isoprenoid derivatives in plastids. In green fruits, they accumulate in photosynthetic chloroplasts which differentiate into chromoplasts during the ripening process (Camara *et al.*, 1995).

Chromoplast-synthesized isoprenoids derive from deoxylulose phosphate (Fellermeier *et al.*, 2003) and not from mevalonate as envisioned previously (Fig. 5). The initial steps involve a transketolase reaction between pyruvate and glyceraldehyde 3-phosphate and the downstream steps lead to dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) (Fig. 5). This leaves unanswered the role of plastid IPP isomerase that catalyzes the isomerization of IPP to DMAPP (Dogbo and Camara, 1987). Further addition of three IPP to DMAPP by plastidial geranylgeranyl diphosphate synthase (GGPPS) (Dogbo and Camara, 1987) yields geranylgeranyl diphosphate (GGPP) (Fig. 5), the immediate precursor of carotenoids and other prenyllipids and diterpenes.

The first committed step of carotenoid biosynthesis is the dimerization of GGPP into phytoene by a bifunctional phytoene synthase (PSY) (Dogbo *et al.*, 1988) (Fig. 6). Phytoene is further desaturated by phytoene



desaturase (Pds) and ζ-carotene desaturase (Zds) to yield neurosporene and lycopene. Recently, the gene encoding carotenoid isomerase (CrtIso) has been characterized and shown to be involved in the isomerization of poly-*cis* carotenoids to all *trans* carotenoids (Isaacson *et al.*, 2002; Park *et al.*, 2002). The cyclization of lycopene catalyzed by lycopene β-cyclase (Lcyb) and/or lycopene ε-cyclase (Lcye), yields α-carotene and β-carotene (Fig. 6). The cyclase step represents a crucial branching point because only β-carotene is converted into zeaxanthin by non-heme diiron monooxygenases (CrtHb) (Bouvier *et al.*, 1998c), while α-carotene is converted to lutein by recently characterized cytochrome p450-type monooxygenases (Tian *et al.*, 2004). Zeaxanthin is further converted to violaxanthin via antheraxanthin by zeaxanthin epoxidase (Zep) and finally violaxanthin is converted to neoxanthin by neoxanthin synthase (Nsy) (Fig. 6). In ripening pepper fruit antheraxanthin and violaxanthin are further converted into the red ketocarotenoids, capsanthin and capsorubin by capsanthin-capsorubin synthase (Ccs) (Bouvier *et al.*, 1994) (Fig. 6).

Xanthophylls accumulating in ripening fruits are generally esterified by medium chain (C_{12}, C_{14}) fatty acids (Breithaupt and Bamedi, 2001) or even C_4 fatty acids (Pott *et al.*, 2003). The physiological significance of this phenomenon may be linked to the fact that acylation enhances the lipophilic character of the xanthophylls thus favoring their massive accumulation or sequestration in specialized chromoplast structures (Camara *et al.*, 1995). In addition to the esterification reaction, fruit carotenoids are cleaved by specific dioxygenases to yield diverse aroma compounds (Winterhalter and Rouseff, 2002).

Fig. 5. Overview of the MEP pathway in plastids. The numbers correspond to the following enzymes: 1, 1-deoxy-D-xylulose 5-phosphate synthase (DXS); 2, 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR); 3, 2C-methyl-D-erythritol 4-phosphate cytidylyltransferase (CMS); 4, 4-diphosphocytidyl-2 C-methyl-D-erythritol kinase (CMK); 5, 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MCS); 6, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase (HDS); 7, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate reductase (HDR); 8, IPP isomerase; 9, geranyl diphosphate synthase; 10, geranylgeranyl diphosphate synthase. Abbreviations refer to: DXP, 1-deoxy-D-xylulose 5-phosphate; MEP: methylerythritol 4-phosphate; CDP-ME, 4-diphosphocytidyl ME; CDP-MEP, CDP ME 2-phosphate; ME-cPP, ME 2,4-cyclodiphosphate; HMBPP, hydroxymethylbutenyl 4-diphosphate; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl diphosphate.

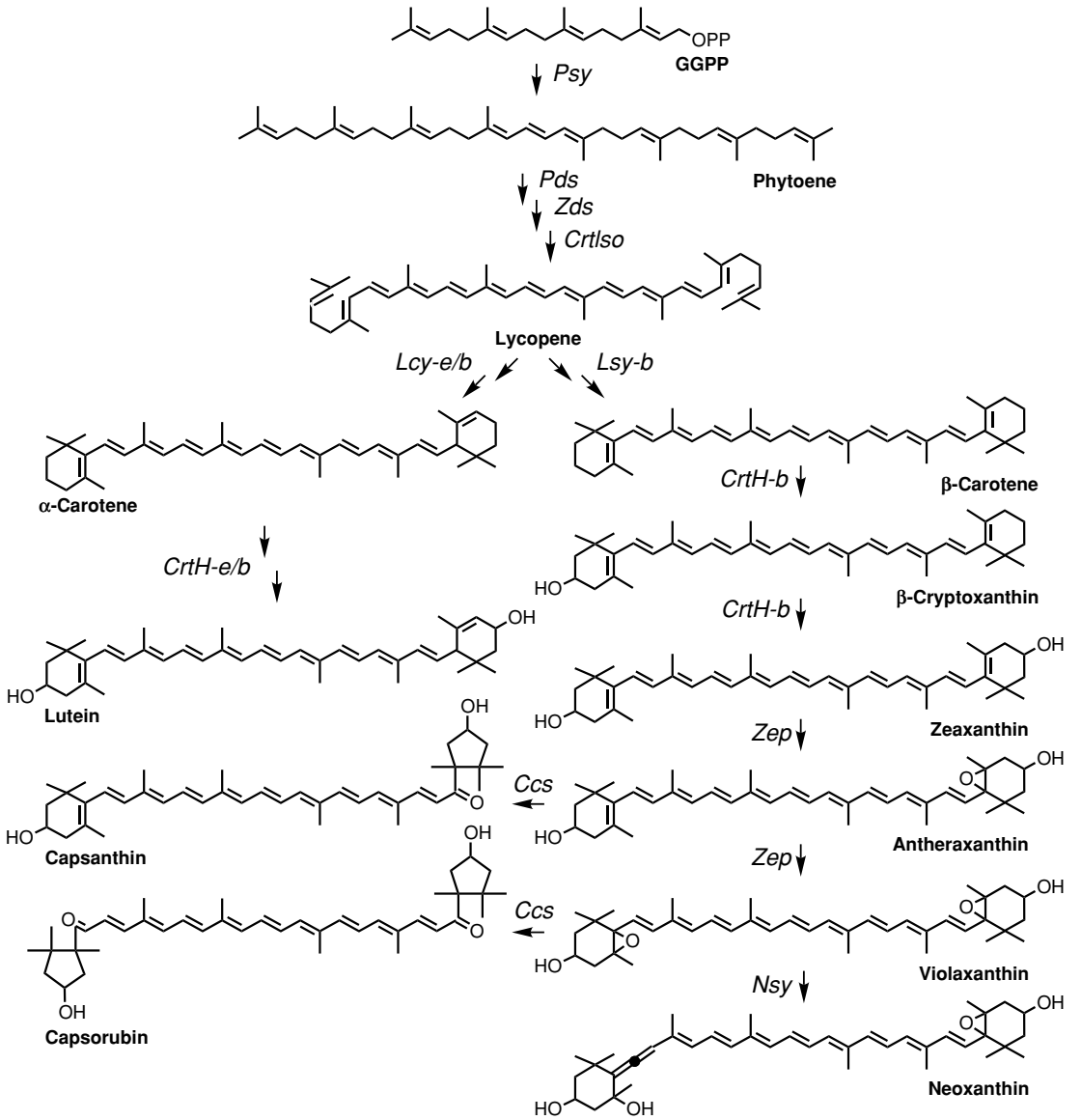


Fig. 6. Typical pathway leading to chromoplast specific carotenoids during fruit ripening. The enzymes are indicated in italics: Psy, phytoene synthase; Pds, phytoene desaturase; Zds, ζ-carotene desaturase; CrtIso, carotenoid isomerase; Lcy-b, lycopene β-cyclase; Lcy-e, lycopene ε-cyclase; CrtH-b, carotenoid β-hydoxylase; CrtH-e, carotenoid ε-hydoxylase; Zep, zeaxanthin epoxidase; Ccs, capsanthin-capsorubin synthase; Nsy, neoxanthin synthase.

Although carotenoid biosynthesis is a highly regulated process, its control is poorly understood. While many developmental and metabolic processes are light regulated in green tissues, this appears not to be the general rule for carotenogenic genes (Corona *et al.*, 1996; von Lintig *et al.*, 1997; Wetzel and Rodermel, 1998; Bugos *et al.*, 1999). In chromoplast-containing tissue the situation is less ambiguous since available data suggest that transcriptional regulation prevails. During tomato fruit ripening, the expression of *DXS* (Lois *et al.*, 2000; Bartley and Ishida, 2002), *Psy* and *Pds* (Giuliano *et al.*, 1993; Fraser *et al.*, 1994) increases, wheras the expression of *DXR* (Rodriguez-Concepcion *et al.*, 2001; Bartley and Ishida, 2002) and *HDS* (Rodriguez-Concepcion *et al.*, 2003) remain constant, while *Lcyb* (Pecker *et al.*, 1996) and *Lcye* (Ronen *et al.*, 1999) are down-regulated, thus leading to massive accumulation of lycopene sequestered in crystal structures. In pepper fruits the ripening is paralleled by an increased of the expression of *DXP*

(Bouvier *et al.*, 1998b), GGPPS (Kuntz *et al.*, 1992), *CrtHb* (Bouvier *et al.*, 1998c) and *Ccs* (Bouvier *et al.*, 1994). In Valencia orange and Satsuma mandarin the intense accumulation of α-cryptoxanthin, zeaxanthin and violaxanthin which occurs during the ripening period, is paralleled by a coordinated increased expression of *Psy*, *Pds*, *Zds*, *Lcyb*, *CrtHb* and *Zep* (Kato *et al.*, 2004).

In contrast to the situation prevailing in chloroplasts, the accumulation of carotenoids in chromoplasts is flexible and readily amenable to genetic manipulation. Transformation of tomato with the bacterial phytoene synthase (*CrtB*) from *Erwinia* increased 2- to 4-fold the total fruit carotenoid in the ripe fruits (Fraser *et al.*, 2002). As lycopene is an acylic precursor of β-carotene, introduction of heterologous *Lcyb* in tomato fruit via a specific promoter induces partial conversion of lycopene into β-carotene (Rosati *et al.*, 2000). In a similar vein, the introduction of *Lcyb* and *CrtHb* in tomato resulted in the accumulation of β-cryptoxanthin and zeaxanthin during the ripening stage (Dharmapuri *et al.*, 2002). This flexibility of fruit chromoplasts has also been observed by introducing the multifunctional bacterial phytoene desaturase in tomato fruit. Under these conditions, β-carotene represented 45% of the total carotenoid content (Romer *et al.*, 2000). Finally, the loss of function of *CrtIso* leads to the accumulation of prolycopene (Isaacson *et al.*, 2002) which is characteristic of tangerine tomato fruits.

Based on the above evidence one can suggest that the transition of chloroplasts to carotenogenic chromoplasts in ripening fruits involves up-regulation of specific genes in the pathway. How these changed are triggered is presently unknown. Efforts directed toward unraveling the mechanism inducing these changes revealed three facts. First, in tomato, the high pigment-2 (*hp-2*) locus which affects carotene accumulation, is involved in photomorphogenesis signalling (Mustilli *et al.*, 1999). Second, reactive oxygen species act as secondary messengers during the strong induction of carotenoid biosynthesis in pepper chromoplasts (Bouvier *et al.*, 1998a). Third, in nonphotosynthetic tissue, the accumulation of carotenoids is indirectly regulated by the sequestration of excess carotenoid in deposit structures (Deruère *et al.*, 1994; Vishnevetsky *et al.*, 1999). In this context, it is interesting to note that in cauliflower, the *Or* gene, which does not encode a carotenoid biosynthetic enzyme, induces the accumulation of massive amounts of β-carotene in the normally uncolored tissue of cauliflower (Li *et al.*, 2001; Li and Garvin, 2003).

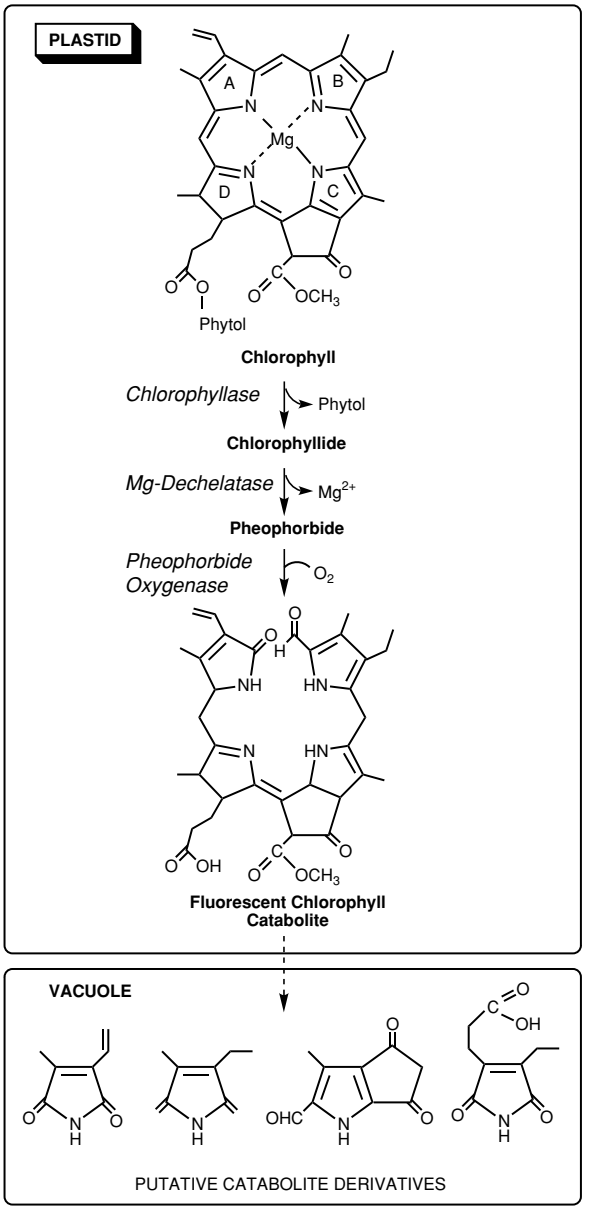


Fig. 7. Chlorophyll catabolism and vacuolar sequestration of final products during fruit ripening.

D. Prenyllipid Metabolism

The breakdown of chlorophyll is a characteristic phenomenon associated with fruit ripening. As such, it is stimulated by ethylene, a regulator of ripening in many fruits (Shimokawa *et al.*, 1978). However, in some tomato mutants (*Green flesh*) and pepper cultivars (Mulato), chlorophyll content is preserved during the ripening process without affecting the accumulation of lycopene in tomato or capsanthin and capsorubin in

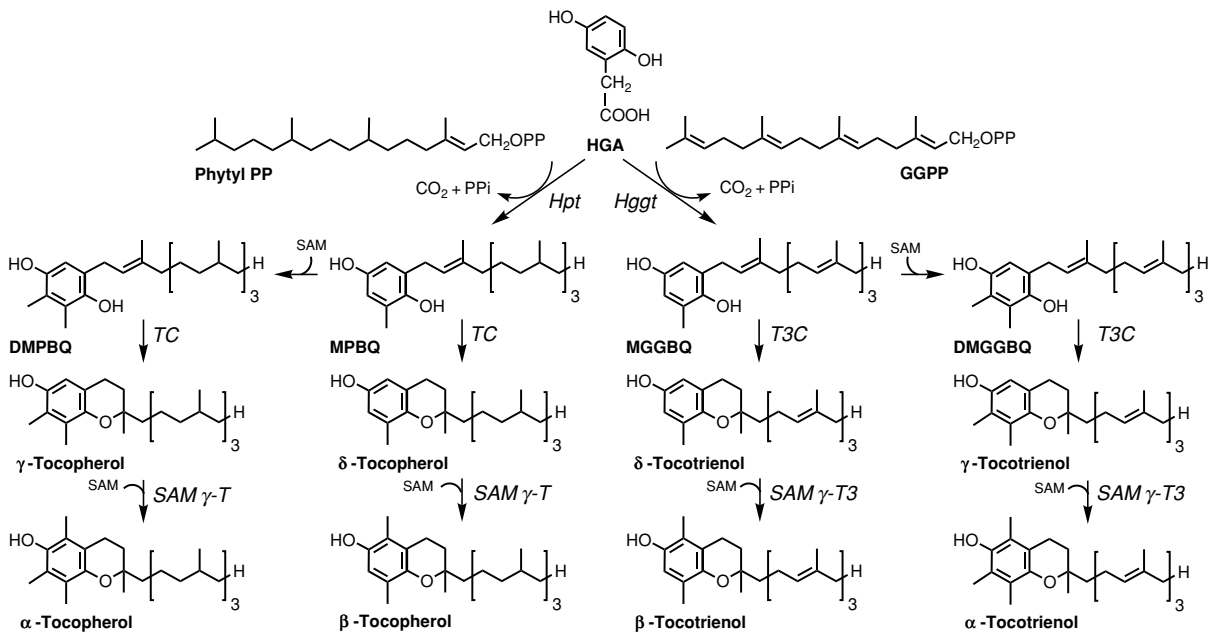


Fig. 8. Tocopherol and tocotrienol biosynthetic pathways. Abbreviations refer to: HGA, homogentisic acid, Hpt, homogentisate phytyl transferase; Hggt, homogentisate geranylgeranyl transferase; GGPP, geranylgeranyl diphosphate; MPBQ, 2-methyl-6-phytylbenzoquinol; DMPBQ, 2,3-dimethyl-5-phytylbenzoquinol; MGGBQ, 2-methyl-6-geranylgeranylbenzoquinol; DMGGBQ, 2,3-dimethyl-5-geranylgeranylbenzoquinol; TC, tocopherol cyclase, T3C, tocotrienol cyclase, SAM, S-adenosyl-L-methionine; SAM γ-T, S-adenosyl-L-methionine γ-tocopherol methyltransferase; SAM γ-T3, S-adenosyl-L-methionine γ-tocotrienol methyltransferase.

pepper. The enzymic breakdown of chlorophyll is initiated in the plastid by chlorophyllase (Jacob-Wilk *et al.*, 1999) which catalyzes the cleavage of cholorophyll into phytol and chlorophyllide (Matile *et al.*, 1999) (Fig. 7). While the fate of phytol is unknown, the chlorophyllide moiety is enzymatically converted into fluorescent catabolites which are further degraded and sequestered in the vacuole as colorless derivatives (Matile *et al.*, 1999; Suzuki and Shioi, 1999) (Fig. 7). The degradation of chlorophylls in ripening fruits is not irreversible. Gibberellin treatments are able to induce the regreening of chlorophyll-free fruits (Devidé and Ljubesic, 1974; Coggins *et al.*, 1980). This is probably linked to the fact that chlorophyll-free chromoplasts maintain the potential for chlorophyll biosynthesis as shown by chlorophyll synthesis during *in vitro* incubation with exogenous chlorophyll precursors (Dogbo *et al.*, 1984; Kreuz and Kleinig, 1984; Lutzow and Kleinig, 1990).

In contrast to chlorophylls, tocopherols are generally actively synthesized in plastids during fruit ripening (Camara *et al.*, 1982; Burns *et al.*, 2003). The pathway involves the prenylation of homogentesic by homogentisate phytyl transferase (Hpt) followed by cyclization (Arango and Heise, 1998) and methylation (d'Harlingue and Camara, 1985) in a reaction sequence probably similar to that operating in leaf chloroplasts (Cheng *et al.*, 2003) (Fig. 8).

With regard to tocotrienols, the initial step involves a homogentisate geranylgeranyl diphosphate transferase (Hggt) (Fig. 8). Usually this enzyme is not active in leaves (Cahoon *et al.*, 2003), but in fruits (Silva *et al.*, 2001; Kallio *et al.*, 2002) and especially the mesocarp of ripening oil palm fruit, the presence of an active chromoplast Hggt leads to an accumulation of α-, β-, δ-, γ-tocotrienols (Sambanthamurthi *et al.*, 2000).

E. Polyphenol Oxidase Activity

It has been established that the browning coloration of several fruits is induced by polyphenol oxidase (PPO) which oxidizes phenolic substrates into reactive quinones that are prone to polymerization and to give brown covalent adduct with reactive amino acids. This visually limits both consumer acceptance and the nutritional quality of fruits. Therefore, effort has been invested to down regulate PPO activity by genetic engineering.

PPOs are nuclear-encoded, plastid-destined proteins associated with thylakoid membranes (Vaughn *et al.*,

1988). In general PPO activity is highest in growing fruits and decreases in ripening fruits (Vamos-Vigyazo, 1981). In some plants like apple (Boss *et al.*, 1995) and pineapple (Stewart *et al.*, 2001), the activation of PPO by mechanical wounding is exerted via transcriptional mechanism. Alternatively, the browning mechanism may be explained or enhanced by the fact that PPO is latent and stable (Dry and Robinson, 1994) and thus any loss of cellular compartmentation due to mechanical disruption may give PPO access to phenolic substrates (Walker and Ferrar, 1998).

IV. Conclusions

Plastids constitute a large family of interconvertible organelles among which fruit plastids reveal high structural and metabolic flexibility. This phenomenon is particularly evident during the ripening process which is paralleled by the mobilization of starch, the import of cytosolic metabolites and the generation of non-photosynthetic ATP or reducing power that are used for organelle biogenesis. This offers the unique opportunity to study the biochemical and genetic function of plastids in the absence of photosynthesis.

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