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The chromoplasts of *Or* mutants of cauliflower (*Brassica oleracea* L. var. *botrytis*)

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Summary. The *Or* mutation in cauliflower (*Brassica oleracea* L. var. *botrytis*) leads to abnormal accumulations of β-carotene in orange chromoplasts, in tissues in which leucoplasts are characteristic of wild-type plants. *Or* chromoplasts were investigated by light microscopy of fresh materials and electron microscopy of glutaraldehyde- and potassium permanganate-fixed materials. Carotenoid inclusions in *Or* chromoplasts resemble those found in carrot root chromoplasts in their optical activity and angular shape. Electron microscopy revealed that the inclusions are made up of parallel, membrane-bound compartments. These stacks of membranes are variously rolled and folded into three-dimensional objects. We classify *Or* chromoplasts as "membranous" chromoplasts. The *Or* mutation also limits plastid replication so that a single chromoplast constitutes the plastidome in most of the affected cells. There are one to two chromoplasts in each cell of a shoot apex. The ability of differentiated chromoplasts to divide in the apical meristems of *Or* mutant plants resembles the ability of proplastids to maintain plastid continuity from cell to cell in meristems of *Arabidopsis thaliana* mutants in which plastid replication is drastically limited. The findings are used to discuss the number of levels of regulation involved in plastid replication.

Keywords: *Brassica oleracea*; β-Carotene; Membranous chromoplast; *Or* mutation; Plastid replication; Ultrastructure.

Introduction

The *Or* mutation in cauliflower (*Brassica oleracea* L. var. *botrytis*) leads to abnormal accumulations of β-carotene in orange chromoplasts, in tissues in which leucoplasts are characteristic of the wild type (WT). This alteration is highly regulated in terms of tissue location and timing during the life history of the plant (Li et al. 2001). Particularly striking is the orange coloration of the curd, which is normally white. However, *Or* chromoplasts also develop in sufficient concentration to impart orange color in the pith and leaf bases from about the sixth foliage leaf onward, and in the vegetative shoot apical meristem from the cotyledonary seedling stage onward. Leaf blades and cortical tissues of the stem of *Or* plants are green, like those in WT. Although greening is somewhat delayed, the difference is noticeable only in very young leaves. Therefore, in an intact *Or* plant the orange color of internal tissues is completely masked by chlorophyll, except for an orange cast at the shoot tip.

Or chromoplasts have several interesting properties that we explore in the present study. (1) At the light microscope level, the exceptionally large, optically active carotene accumulations in mature *Or* chromoplasts resemble those formed in the chromoplasts of orange carrot roots. (2) The *Or* mutation limits replication of plastids so that there is typically one plastid in each affected mature cell, and only one or two relatively large plastids in each cell of the shoot apical meristem (the region above the youngest leaf primordium). (3) The plastids in the mutant meristem are chromoplasts, whereas numerous colorless proplastids are found in each meristem cell in normal plants.

The chromoplasts of the carrot root and their carotene inclusions have been studied at the electron microscope level by Frey-Wyssling and Schwegler (1965) and by Ben-Shaul and Klein (1965), and the expansive literature on chromoplast ultrastructure (see Camara et al. 1995) offers ample opportunity for comparisons. Limitation of plastid replication in vegetative meristems to one or two plastids per cell

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is known from normal lower vascular plants and anthocerotes (Brown and Lemmon 1990) and from mutants of *Arabidopsis thaliana* (Robertson et al. 1995, Marrison et al. 1999). The development of carotenoids to levels detectable as color within the vegetative shoot apical meristem is a phenomenon we have not seen reported elsewhere.

Material and methods

The cauliflower varieties used in our studies include the WT cultivars Rushmore and Stovepipe, and a cauliflower line that serves as our standard *Or* homozygous line (1227). The "mutant plants" (or mutants) examined in this study were all homozygous for *Or*. Methods for glutaraldehyde fixation are those reported by Li et al. (2001). However, glutaraldehyde fixation does not preserve carotenoids, whereas potassium permanganate fixation does (Knoth 1981, Camara et al. 1995). Therefore, potassium permanganate fixation was added to our protocol in spite of its inability to preserve many other components of cells and the grainy images it produces. All materials for electron microscopy were embedded in Spurr (1969) resin mixture using flat embeddings. Ultrathin sections were viewed with a Teenai 12 transmission electron microscope Biotwin equipped with a $\pm 80°$ tilt goniometer stage. Observations with the light microscope were made using 0.5 μm thick sections of the materials embedded in plastic and hand sections of fresh materials. Hand sections were cut to contain numerous cells that could be confirmed as intact by focusing through the sections or by looking for cytoplasmic streaming.

We use the term "optically active" to designate materials that appear bright when viewed in appropriate orientations between crossed polarizers. "Crystalline" materials are optically active. "Dichroism" designates a property of crystalline materials that transmit light differentially when rotated above or below a single polarizing filter in the optical path (Bennett 1950). Pleochroism (Frey-Wyssling and Schwegler 1965) is dichroism in colored materials. With β-carotene the color change of interest is from colorless to orange, the color being perceived to be darkest when the axis of the polarizer is parallel to the principal optical axis of the crystalline material (Frey-Wyssling and Schwegler 1965).

Results

Or chromoplasts resemble orange chromoplasts of carrot root

With glutaraldehyde fixation, *Or* chromoplasts from vegetative tissues showed a relatively dense stroma interrupted by sinuous membranes that were associated with an electron-lucent area within the plastid (Fig. 1). The permanganate-fixed chromoplasts showed an abundance of electron-dense membranes that were not sinuous (Figs. 2–6). This difference between glutaraldehyde and permanganate fixation images parallels that reported by Rosso (1968), Harris and Spurr (1969a, b), and Knoth (1981) for lycopene-containing chromoplasts and by Camara et al. (1995) as a general property of chromoplasts that contain large crystalline deposits of carotene or lycopene.

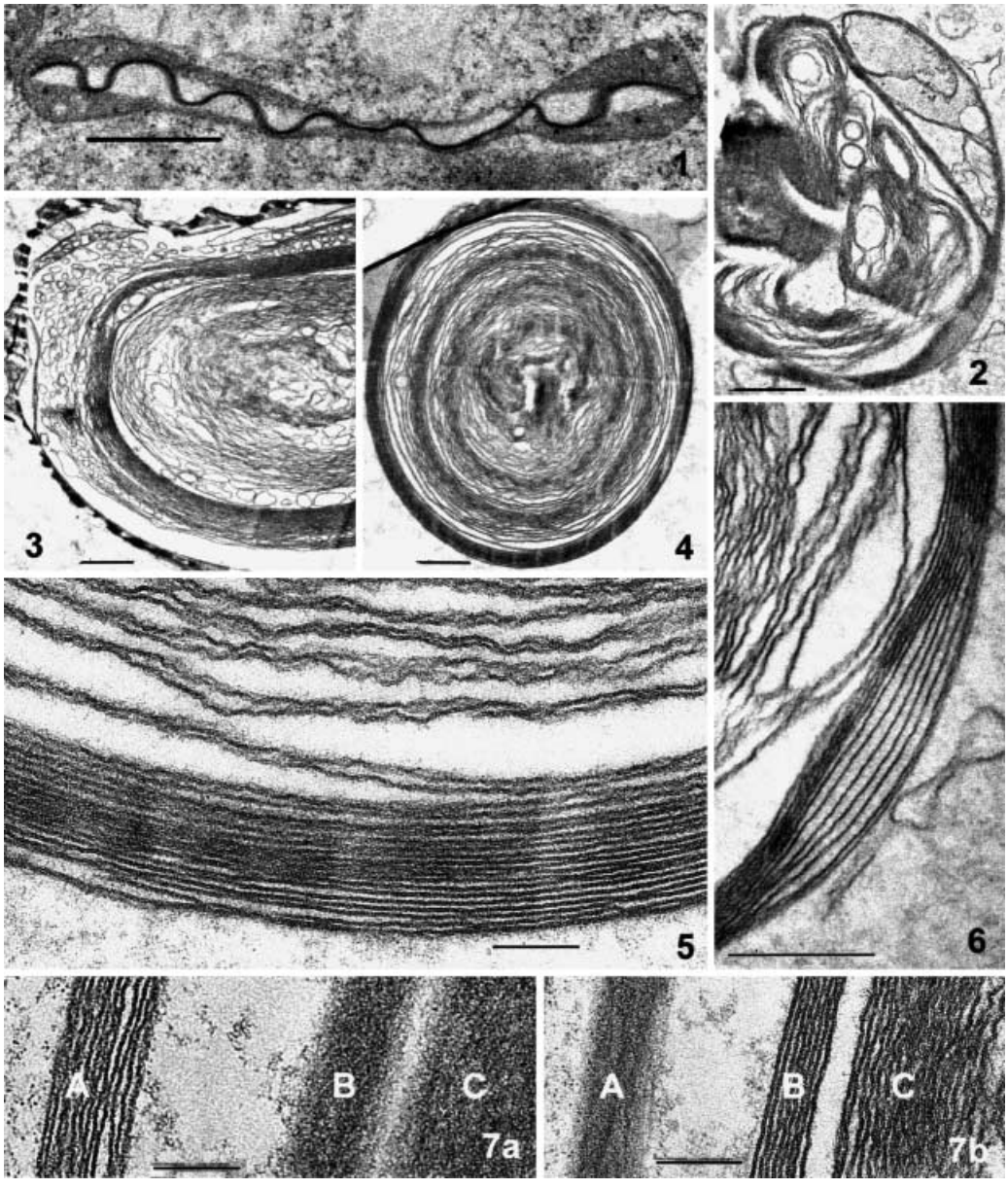
In some areas the membranes were more compactly arranged than in others (Figs. 3–6). Transitional images (Fig. 5) suggested that paired membranes that separate a compartmental (inner) space from the stroma become more closely spaced and aligned with time. The inner space and the intercompartmental stroma space were both reduced where membranes were more nearly parallel, where the almost uniform spacing of successive membranes averaged 15.5 nm (Fig. 5). In oblique sections, a stack of membranes sometimes appeared uniformly dense and even opaque. Tilting of the microscope goniometer stage allowed the visualization of electron-lucent spaces between these membranes (Figs. 6 and 7).

The gross arrangements of membranes in permanganate-fixed *Or* chromoplasts as seen in some of our micrographs strongly resembled those seen in the transmission electron micrographs of permanganate-fixed carrot chromoplasts published by Frey-Wyssling and Schwegler (1965). But as seen in the light microscope, the largest carotene sheets of *Or* chromoplasts were rolled in an irregular fashion, whereas the carotene sheets in carrots tended to wind themselves smoothly into helices (Frey-Wyssling and Schwegler 1965, Li et al. 2001).

In fresh materials, hand sectioning released some of the intensely orange sheets from the chromoplasts into the mounting medium (water) above the sections, whereas others remained within the chromoplasts of intact cells. We concluded that these sheets represented coherent expanses of the compactly stacked membranes. The morphology of the sheets was sometimes complex (Fig. 8), but the smaller sheets with the simplest shapes approached parallelograms (Figs. 9 and 10). The range of measurements of the acute angles we obtained for *Or* carotenoid sheets was 45° to 55°, averaging 48°. Free and in situ the sheets were optically active and strongly dichroic. At the bright setting of a sheet relative to the single polarizer, virtually all aspects of a flattened, face-on view of a carotene sheet disappeared (Figs. 9 and 10), regardless of the complexity of the outline. To obtain the darkest image of the carotenoid sheet, the axis of the polarizer had to be parallel to a line that met the perimeter of the sheet within its acute angle. In these features, the carotenoid sheets resembled those reported for carrots (Frey-Wyssling and Schwegler 1965).

Or limits plastid replication and renders shoot apical meristem and certain tissues orange

When intact mutant seedlings with 4 to 6 emergent leaves were split longitudinally, orange color was evident in and near the shoot tip (Fig. 11). However, the pith at the level of the first foliage leaf was green, as in normal plants, and the cells contained numerous pale green chloroplasts (Fig. 12)



Figs. 1–7. Electron micrographs of *Or* chromoplasts

Fig. 1. Sinuous membranes occupy a translucent space within the relatively dense stroma. From parenchyma cell of a vascular strand. Glutaraldehyde fixation. Bar: 1 μm

Fig. 2. Abundant membranes within chromoplast are rolled and folded in various configurations. From parenchyma of a leaf base. Permanganate fixation. Bar: 1 μm

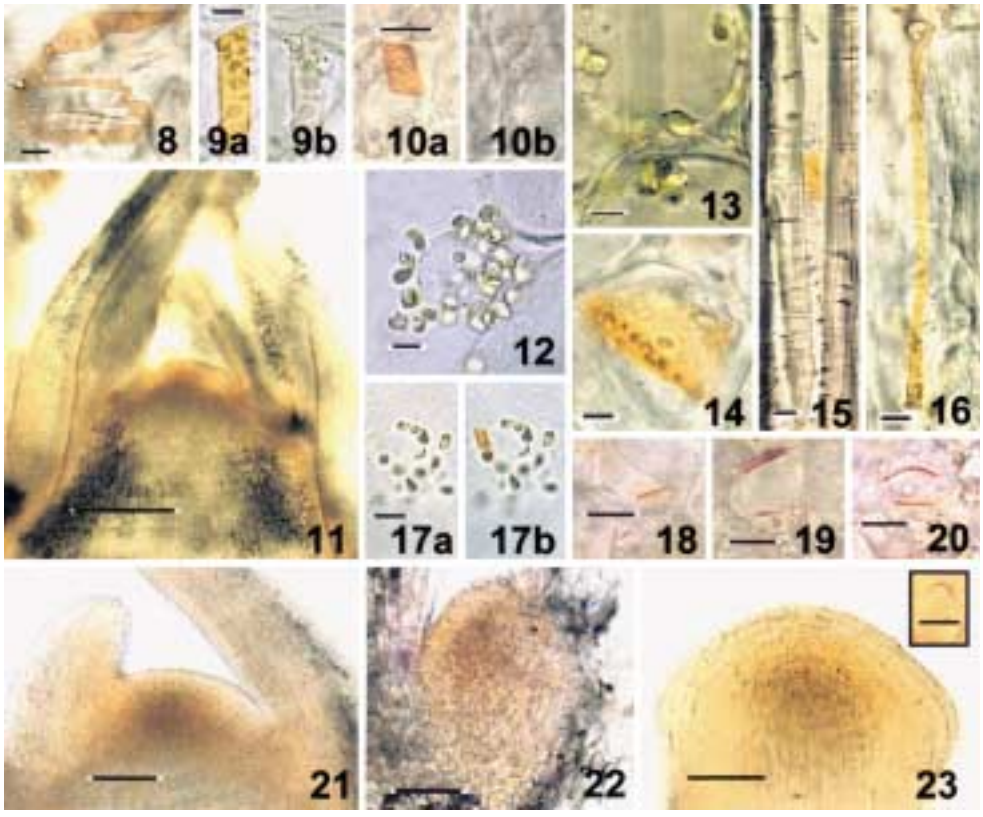
Fig. 3. Similar to Fig. 2, but with tubular or vesicular membranes, also. From parenchyma of the pith. Permanganate fixation. Bar: 1 μm

Fig. 4. Rolled membranes are at different degrees of proximity in different regions of the section. The lower portion of the section appears at higher magnification in Fig. 5. From parenchyma of the pith. Permanganate fixation. Bar: 1 μm

Fig. 5. Higher magnification of lower portion of Fig. 4. The membranes are paired, defining compartments which are closely aligned but not in contact toward the bottom of the figure. Permanganate fixation. Bar: 0.2 μm

Fig. 6. Higher magnification of lower right portion of Fig. 2. Tilted −40°, compared to 0° tilt in Fig. 2. Spacing between the compartments is revealed by the tilt. Permanganate fixation. Bar: 0.5 μm

Fig. 7. Comparison of effect of 0° tilt (**a**) and 40° tilt (**b**) on the clarity of separation of membranes. *A*, *B*, and *C* Equivalent portions of the membrane system in both photographs. Permanganate fixation. Bar: 0.2 μm



Figs. 8–23. Light micrographs of plastids and meristems from *Or* plants. All from fresh materials in aqueous mounts

Fig. 8. Large, complex carotenoid body, freed from a disrupted cell and flattened. The object has "slots" and gaps within it. Flattened objects of this complexity disappear completely when the polarizer is oriented to obtain the brightest image. Bar: 10 μm

Fig. 9. Carotenoid body in situ, with settings of the polarizer for the darkest (**a**) and brightest images (**b**). Except for the rolled edges, the carotenoid body has disappeared at the setting for the brightest image. The starch grains remain visible. Bar: 5 μm

Fig. 10. Flat, rhomboidal carotene body in situ, at settings of the polarizer for the darkest (**a**) and brightest images (**b**). Bar: 5 μm

Fig. 11. Shoot tip from main axis of seedling *Or* plant that had seven emergent leaves. Shoot apex is orange. Stem tissue is most intensely colored where affected by the leaf bases and their vascular tissue. Parenchyma surrounding the vascular trace in the primordium at the left shows more intense orange color than the rest of the leaf tissue. Bar: 300 μm

Fig. 12. Green plastids from parenchyma of pith at the level of node 3. Bar: 5 μm

Fig. 13. Green plastids from cortical parenchyma at the level of node 3. Bar: 10 μm

Fig. 14. Carotenoid body and starch grains in situ, within collenchyma cell of leaf base. The part of the carotenoid body that is more intensely colored is rolled back upon the rest of the object. Bar: 5 μm

Fig. 15. Carotenoid body within sclerenchyma cell of leaf base. Bar: 10 μm

Fig. 16. Elongated carotenoid body within parenchyma cell of leaf base. Bar: 5 μm

Fig. 17. Mixed chloroplast and chromoplast population in pith parenchyma at level of node 3. Carotenoid body invisible at the setting of the polarizer for the brightest image (**a**) is seen at its setting for the darkest image (**b**), with respect to that body. Images of green plastids are not detectably affected by the setting of the polarizer. Bar: 5 μm

Fig. 18. Single chromoplast in first layer of tunica, on flank of shoot apex. Bar: 5 μm

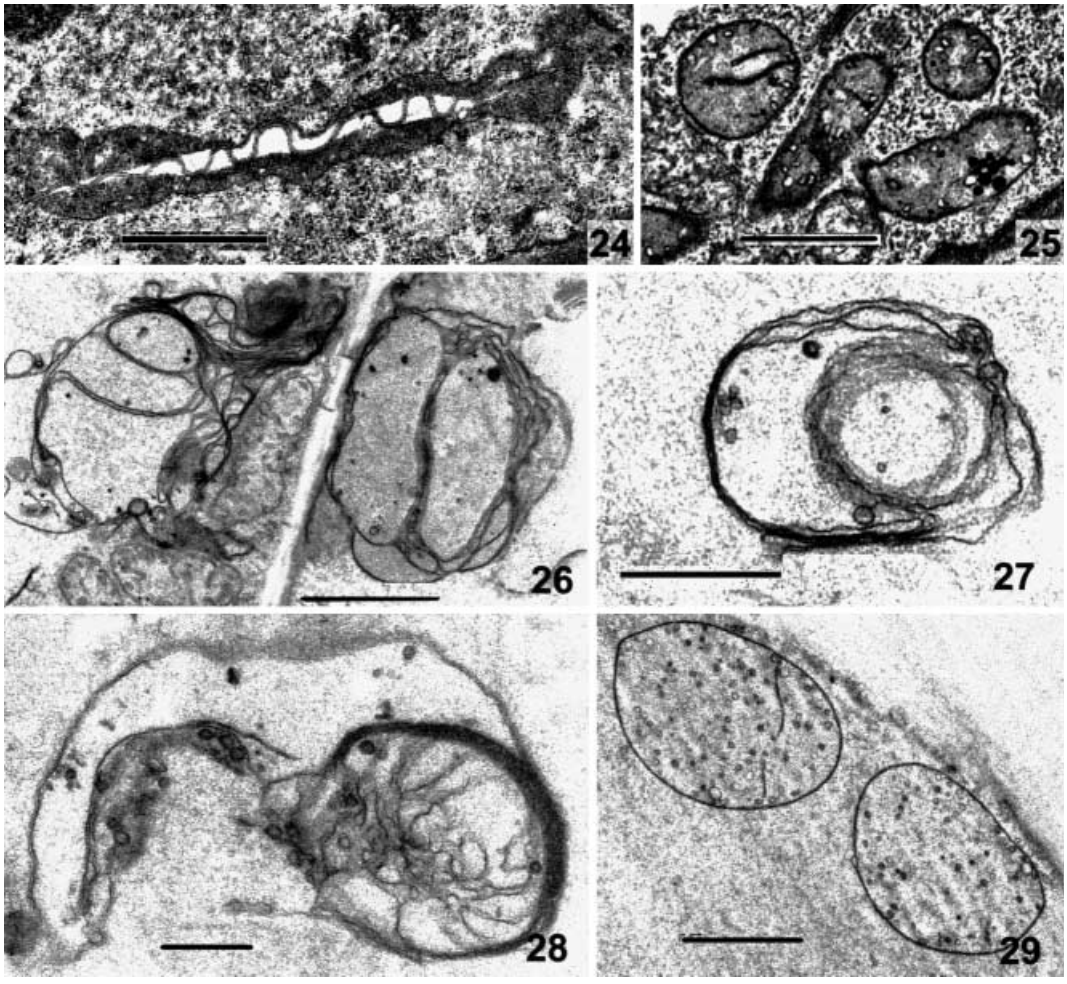
Fig. 19. Two chromoplasts in cell of corpus in shoot apex. Bar: 5 μm

Fig. 20. Similar to Fig. 23. Bar: 5 μm

Fig. 21. Shoot tip of an axillary bud at node 1 of a seedling. In the young leaf primordium at the left, orange color is more obvious on the adaxial side than on the abaxial side. Bar: 50 μm

Fig. 22. Newly organized axillary meristem with no evident leaves, in the axil of the first leaf on the axillary shoot at node 1 of a seedling. Bar: 50 μm

Fig. 23. Curd meristem and chromoplast from curd (**inset**) showing orange color. Bar: 50 μm; inset, 5 μm



Figs. 24–29. Electron micrographs from shoot tip, curd, and flower petal

Fig. 24. Chromoplast from shoot apex of mutant plant. Glutaraldehyde fixation. Note resemblance to Fig. 1. Bar: 1 μm

Fig. 25. Plastid profiles from WT shoot apex. Bar: 1 μm

Figs. 26–28. Chromoplasts in cells of curd meristem of mutant plant showing variability of chromoplast internal structure. Permanganate fixation. Bar: Figs. 26 and 28, 2 μm; Fig. 27, 1 μm

Fig. 29. Chromoplast from yellow petal of normal plant. Only small vesicles and a few scattered membrane profiles are seen in the plastid interior. Bar: 0.5 μm

that showed red fluorescence in blue light. But in contrast to the WT, chromoplasts were present in the parenchyma cells of the vascular strands surrounding the pith. Orange color was most pronounced in the leaf traces and in the vascular bundles of the stem immediately adjacent to the leaf traces. Cortical tissues contained chloroplasts, as in normal plants (Fig. 13). In almost all cases, each intact mature cell affected by *Or* had a single locus of orange color within the cytoplasm, regardless of cell type (Figs. 14–16). The organelle containing this color often contained starch grains, confirming the identity of the organelle as a plastid (Figs. 9 and 14). Cells of the WT from comparable locations contained numerous leucoplasts. Upward in the stem from node one, the pith of the WT changed from green to white. In contrast, mutants exhibited a shift from green to orange coloration. In the transition upward within the pith of mutant plants, there were rare cases of cells with a mixed population of chloro- and chromoplasts (Fig. 17). In the vascular parenchyma and pith parenchyma, there were rare cases of cells with multiple, small chromoplasts. Electron micrographs of *Or*-affected mature cells with single chromoplasts did not show any class of organelles that could be identified as leucoplasts.

Fresh sections of vegetative shoot apices of the main axis and axillary branches revealed an orange meristem (Figs. 11

and 21), with one to two chromoplasts per cell in the tunica and corpus (Figs. 18–20). Leaf primordia showed less concentrated orange color than the corpus of the shoot apical meristem, but developing leaves retained orange coloration, especially within and around the vascular strands (Fig. 11). In young primordia (Fig. 21), *Or* chromoplasts were present in all cell layers. In leaves still folded in the shoot tip, chromoplasts were still evident toward the adaxial side of the leaf base and in the vascular strands.

The shoot apical meristems were orange from their early stages of development. The apical meristem of the main shoot exhibited orange color from the cotyledonary stage. Likewise, the shoot apical meristem of the axillary shoot at the node of the first foliage leaf (with only one expanded leaf of its own) was orange (Fig. 21). Even meristems with no evident leaves were orange (Fig. 22). In meristems that compose the mutant curd, *Or* chromoplasts were present, as revealed by the orange color of the meristems and of individual plastids (Fig. 23 and inset).

Electron microscopy confirmed the presence of chromoplasts in the shoot apex of mutant plants (Fig. 24, cf. Fig. 1). These were much larger than WT proplastids, and they were the only plastids in the mutant cells. In WT shoot apices, no chromoplasts were observed, and cells contained multiple images of proplastids (Fig. 25) with simple interior structure. *Or* chromoplasts in the curd varied in their internal complexity (Figs. 26–28). Those in the more apical region were the simplest, with their profiles sometimes showing only modest development of membranes. As inflorescences developed from the curd, the orange color progressively disappeared from the meristems. Developing petals were yellow, and the chromoplasts in petals of mutant plants were indistinguishable from those in petals of the WT, and the inclusions did not resemble those found in mature *Or* chromoplasts (Fig. 29).

Discussion

Resemblance of Or chromoplasts to other chromoplasts

Parallel membranes rolled into tubes occur as lycopene-containing crystalloids of tomato chromoplasts, with the membrane-to-membrane distance within the roll reported as 15 nm (Harris and Spurr 1969a, b). This resembles the spacing of membranes in *Or* plastids. However, in tomatoes, the stroma space between compartments seems to be eliminated, so that all membrane components except those at the surfaces of a rolled stack are composed of two appressed membranes. We have no evidence that this occurs in *Or* plastids. Unfortunately, the micrographs available for carrots (Frey-Wyssling and Schwegler 1965) leave this feature undetermined. However, the "repeat unit" of the membrane stacks of carrot chromoplasts was reported as 20 nm (Frey-Wyssling and Schwegler 1965).

Parallel, curved compartments separated by stroma are characteristic of chromoplasts of tangerine tomatoes (Rosso 1967) and of *Croton* spp. (Mesquita et al. 1989). The carotenoid stored in the membranes in tangerine tomatoes has been identified as prolycopene (Isaacson et al. 2002), but β-carotene predominates in *Croton* spp., for which the published figures show the compartments more widely spaced than in the present *Or* plastids. However, the images published for *Croton* spp. are from glutaraldehyde fixations.

The angular outline of *Or* carotene bodies, with acute and obtuse angles, and the pronounced optical activity and dichroism provide the strongest similarities to carotene bodies in carrots. The range of measurements of the acute angles we obtained for *Or* β-carotene sheets was 45° to 55°, averaging 48°. Measurements of carotene deposits from other sources fall within this range: (1) for carrots, 48°, as measured on fig. 12 of Ben-Shaul and Klein (1965), and 51 to 53°, as reported by Frey-Wyssling and Schwegler (1965); (2) for sweet potatoes, in which the morphology of carotene bodies strongly resembles that of *Or* carotene bodies, an average of 52° (unpubl. data; also, β-carotene is the overwhelmingly predominant carotenoid in sweet potatoes according to Takahata et al. [1993]), (3) for *Narcissus poeticus*, 50°, as measured for β-carotene crystals, from fig. 24 of Kuhn (1970).

The association of proteins and lipids with carotenoid accumulations is well known and widely reported (Deruere et al. 1994, Vishnevetsky et al. 1999). The models available for tubular and fibrillar carotenoid-bearing structures (Knoth et al. 1986, Deruere et al. 1994) place the lipid and protein in a single layer or in concentric layers around the carotenoids. The carotenoid molecules are thought to crystallize with an optical axis parallel to that of the fibril or tubule. Harris and Spurr (1969b) suggested that the concentrically arranged compartments in red tomato chromoplasts are filled with lycopene. Zsila et al. (2001) report that pure carotenoids are capable of self-assembly into optically active structures. On the basis of these observations, one can argue that lipid and protein assemble to constitute containment structures for the bulk accumulation of free carotenoids in tubular, fibrillar, and lamellar structures. With regard to β-carotene, the crystalline tubules described for high beta tomatoes by Harris and Spurr (1969a) appear to comply with this model. So do the β-carotene crystals

of *Narcissus poeticus* corona, contained within membrane-bound compartments as reported by Kuhn (1970), who showed by chemical analysis that the isolated crystals were free of protein and lipid.

But according to Frey-Wyssling and Schwegler (1965) chemical analyses dictate that the β-carotene bodies of carrots should be considered chromolipoproteins rather than crystals of carotene. By virtue of our electron micrographs, we speculate that the same argument should be applied to carotene bodies of *Or* chromoplasts. Also, the isolated carotene bodies of carrots that are illustrated by Frey-Wyssling and Schwegler (1965) and by Ben-Shaul and Klein (1965) have a structure and dimensions consistent with the presence of multiple membrane-bound compartments in the isolated structure. In contrast, an isolated carotene crystal of *Narcissus poeticus* as reported by Kuhn (1970) represents only the contents (no membranes) of a single compartment. Using x-ray diffraction, Kuhn (1970) concluded that in the *Narcissus poeticus* crystals the alignment of carotene molecules is doubly oblique to the major axis of the crystal and criss-crossed in alternating molecular layers. But Frey-Wyssling and Schwegler (1965) concluded that in carrot chromoplasts there are no carotene crystals with three-dimensional lattices, but only stacks of lamellae, each with carotene stored in a two-dimensional layer lattice. They postulated that in carrots the β-carotene molecules are arranged perpendicular to the surface of the membranes, with the axis of resonance for the molecules aligned with the major axis of the crystal.

Again, the carotene accumulations in *Or* chromoplasts and in the root of orange carrots may be considered crystalline in the sense that they are optically active. As Frey-Wyssling and Schwegler (1965) point out, the orientation of the lamellae with respect to carotenoids must be identical throughout a stack of membranes. Otherwise the carotene bodies would not show the high degree of optical activity and dichroism that they exhibit.

Division of Or chromoplasts

The *Or* mutation renders the shoot apical meristem orange from the cotyledonary stage of seedling development onward (Li et al. 2001) and from the early stages of the organization of axillary shoot apical meristems. The finding that there are only one or two relatively large chromoplasts in each cell of the shoot apical meristem of an *Or* plant is consistent with plastid division occurring at a minimal rate and in coordination with cell growth and division. In WT meristems there are numerous, colorless proplastids per cell. Therefore, even within the meristem of an *Or* plant, plastid division is under severe limitation, and there is a "precocious" differentiation imposed on the plastids.

A comparison with *Arabidopsis thaliana* chloroplast mutants reveals some parallels. In *Arabidopsis thaliana*, the *arc* mutations show that there is one level of genetic regulation for plastid division that allows a characteristic number of proplastids in a cell of the meristem and another level that allows the characteristic number of chloroplasts in a mature green cell (Marrison et al. 1999). Some mutations impose limits at both levels of plastid division (*arc6* and *FtsZ* mutants; Osteryoung et al. 1998, Hashimoto 2003), with one or a few very large chloroplasts per mature cell. In each cell of the shoot apical meristem of an *arc6* plant there are but one or two oversized proplastids (Marrison et al. 1999). However, division of the proplastids in *FtsZ* and *arc6* mutant meristems has to keep pace with cell division, or aplastidic cells would occur, in relatively high frequency (Butterfass 1979, Robertson et al. 1995, Coleman and Nerozzi 1999).

The accumulated observations seem to imply that there is yet a third, uncharacterized level of genetic control of plastid division that assures at least minimal replication in meristem cells by maintaining an environment within the meristem that overrides genetic factors that otherwise prohibit plastid replication. We postulate that this meristem environment (controlled by one or more undescribed genes) maintains plastid replication in meristems of *Or* and the more severe *Arabidopsis thaliana* mutants, so that no cell develops without at least one plastid. This division mechanism is likely to be one that is primitive in land plants, relating to a level of plant evolution in which meristem cells regulate their plastid divisions so that only the minimal number of plastids required to keep up with cell division is maintained in each meristematic cell. Among land plants this arrangement is manifested in normal vegetative meristems of anthocerotes (hornworts) and heterosporous lycopods and in the sporogenous and spermatogenous tissues of mosses and liverworts (Paolillo 1962, Brown and Lemmon 1990, Vaughn et al. 1992). Mechanisms allowing plastids to divide to maintain a larger population of proplastids in meristem cells, and those allowing for division of chloroplasts, are most likely superimposed upon the primitive mechanism but do not replace it. Therefore, in higher plants, the primitive level or mechanism for plastid division becomes evident only in mutants that limit plastid division severely enough to prevent the number of proplastids in a meristem cell from rising above two.

Or, the orange shoot apex, and morphogenesis

The *Or* mutation results in the formation of chromoplasts in the shoot apical meristem. Chromoplasts are highly differ-

entiated plastids. But Camara et al. (1995) argue against the general consensus that chromoplasts do not divide, and Sitte (1987) demonstrated chromoplast division in flower petals of *Forsythia suspensa*. *Or* plants offer clear evidence that chromoplasts can divide in the context of meristematic tissues. But why should such a differentiated state of organelle development occur in the shoot apical meristem? It has long been argued that the distal cells of the shoot apical meristem are partially "differentiated" in that they express some similarities to older, derivative tissues, like the formation of starch grains in their plastids (Nougerède 1967, Buvat 1989). *Or* adds color to the differentiated state in plastids in the meristem in which no developmental program that leads to greening has been activated.

Because *Or* affects shoot apical meristems, *Or* should render the meristem-derived vegetative tissues orange rather than green, posing a potentially lethal condition. However, the meristematic activity of a leaf primordium, which exceeds that of the meristem proper by every measure (Kwiatkowska 2004), continues the replication of chromoplasts as they redifferentiate under the influence of a developmental program leading to greening, and the photosynthetic tissues of the leaf mature with a carotenoid content that is indistinguishable from the WT (Li et al. 2001). The conversion of chromoplasts to chloroplasts is known even in highly differentiated cells (Thomson and Whatley 1980, Schnepf 1980). And in carrot roots the development of chromoplasts is postponed until cells are displaced from the meristem (Ben-Shaul and Klein 1965, Frey-Wyssling and Schwegler 1965), but if an explant is made from the orange tissue, chromoplasts redifferentiate, eventually forming chloroplasts as the explant cells become meristematic (Israel and Steward 1967, Wrisher 1972, Kumar and Neumann 1999).

During the morphogenesis of green tissues in *Or* plants, presumably a conversion of chromoplasts to proplastids or young chloroplasts occurs so that the plastids divide as the cells are redifferentiated. Greening follows as the development of the plastids is normalized. This conversion fails in tissues like the pith above node 5, and in the parenchyma of leaf bases in which cells do not turn green in the WT. Depending on location, chromoplasts also persist in the parenchyma of primary vascular tissues.

Because the curd may be regarded as a proliferation of apical meristems (Sadik 1962), we interpret the orange color of the curd as an extension of the ability of *Or* to impart color to apical meristems. As soon as the curd meristems begin to differentiate into floral meristems, the orange color fades. This change is another case of reversal of the condition imposed by *Or*, here with the morphogenesis of the curd meristem into a flowering meristem (Sadik 1962). Developing petals have a yellow color, and petal chromoplasts in *Or* plants are indistinguishable from those of the WT. The evidence overall indicates that *Or* has its primary effect of chromoplast differentiation in the shoot apical meristem, that its effect is reversed during the formation of tissues that are colored in the normal plant, and that, after reversal, processes that produce normal coloration predominate whether the coloration involves chloroplasts or chromoplasts. Evidently, cells displaced into the pith and leaf bases of the vegetative plant are not capable of overcoming the effect of *Or*, leaving these locations with mature cells that retain and enlarge their *Or* chromoplasts as they continue to accumulate β-carotene in large plastid inclusions.

In closing, we agree with Deruere et al. (1994) that chromoplasts may be regarded as "deposit structures" regardless of any additional functional advantage they might confer. Fortunately, in the case of the *Or* mutation of cauliflower the metabolic "mistake" of overproducing carotenoids has the effect of making the plants attractive as foods that can supply provitamin A in the human diet. If we were to place the *Or* chromoplast in the classification scheme of Camara et al. (1995), we would choose the class "membranous chromoplasts" because of the resemblances of our micrographs to those published for certain varieties of *Narcissus pseudonarcissus* (Mollenhauer and Kogut 1968, Leidvogel et al. 1975, Leidvogel and Falk 1979), which Camara et al. (1995) include in this class. Chromoplasts are difficult to classify because their assorted characteristics overlap. Camara et al. (1995) classify carrot chromoplasts as "crystalline chromoplasts", in a subclass containing "large *crystals*" (our emphasis) of β-carotene. Although the deposits are not crystals of β-carotene alone in either *Or* plants or carrot, we see no problem in characterizing a chromoplast as crystalline when it contains optically active inclusions. But tubular and fibrillar structures, which also have optically active carotenoid inclusions (Falk 1976, Knoth et al. 1986, Deruere et al. 1994) fall outside the crystalline category according to Camara et al. (1995), assumedly because the lipoprotein components of the system give the carotenoid deposits rather specific ultrastructural morphology (Deruere et al. 1994). If ultrastructure takes precedence over optical activity, perhaps carrot chromoplasts too should be regarded as membranous chromoplasts.

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