Isoprenoid Biosynthesis and Stability in Developing Green and Achlorophyllous Leaves of Rye (*Secale cereale* L.)

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Formation of major prenylquinones and carotenoids was investigated by comparing the incorporation of [^{14}C]mevalonate into segments of different age from green and etiolated leaves of 22 °C-grown rye seedlings (*Secale cereale* L.) and from 32 °C-grown rye leaves which contained bleached and proplastid-like ribosome-deficient plastids, due to a heat-sensitivity of 70S ribosome formation. The contents of plastidic isoprenoids were much lower (between 2–30%) in the achlorophyllous than in green leaves. In green leaves [^{14}C]mevalonate incorporation into non-polar lipids and into plastoquinone was partially inhibited in the presence of gabaculin, an inhibitor of chlorophyll synthesis. However, except for β-carotene, [^{14}C]mevalonate incorporation into isoprenoids continuously increased with age also in achlorophyllous etiolated or 32 °C-grown, as in green, leaves and was, except for β-carotene and plastoquinone, higher in etiolated than in green leaves. In bleached 32 °C-grown leaves [^{14}C]mevalonate incorporation into all plastidic isoprenoids was strikingly (up to 45-fold) higher than in green control leaves. While degradation of β-carotene was greatly enhanced in bleached 32 °C-grown leaves, relative to green control leaves, and could thus compensate for a higher apparent synthesis, chase experiments did not reveal any marked differences of the turnover of other isoprenoids. The half times of plastoquinone, phylloquinone and lutein were in the order of 2–3 days. Within a 24 h chase period α-tocopherol degradation did not become apparent. Uptake of [^{14}C]mevalonate and [^{14}C]isopentenyl pyrophosphate by isolated bleached plastids from 32 °C-grown leaves was much more rapid than by chloroplasts and resulted in higher precursor accumulation within the organelle. While mevalonate incorporation into isoprenoid lipids was not detected, isopentenyl pyrophosphate was incorporated into isoprenoid lipids, including plastoquinone. Rates of incorporation by isolated chloroplasts or bleached plastids were of similar order. The results illustrate that divergent types of plastid differentiation are associated with fundamental developmental changes of the metabolic flow of isoprenoid precursors between different products and compartments and, in particular, with changes of import into the plastid compartment.

Introduction

Isoprenoid compounds, such as prenylquinones and carotenoids, are important and general constituents of the thylakoid membranes of chloroplasts. In the photosynthetic tissues they function as accessory pigments in the transfer of excitation energy (carotenoids), as constituents of the photosynthetic electron transport chain (PQ-9, phylloquinone) and as important systems of antioxidative protection (carotenoids, α-T) against photodamage [1–4]. Enzymes of carotenoid and prenylquinone biosynthesis are confined to the chloroplast compartment [2, 5, 6]. However, genes for enzymes of isoprenoid biosynthesis have not been recognized on the chloroplast DNA [7] and the observation that plastidic isoprenoids were synthesized in 70S ribosome-deficient leaves [8] suggested that the biosynthetic enzymes are nuclear-encoded and synthesized on cytoplasmic ribosomes. During leaf development formation of prenylquinones and carotenoids accompanies greening and the differentiation of thylakoids.

Since plastids of 70S ribosome-deficient leaves that were produced by growing rye seedlings at a non-permissive elevated temperature of 32 °C were bleached and did not contain normal thylakoids [9], their contents of carotenoids and prenylquinones remained low, similarly as in etiolated leaves

Abbreviations: PQ-9, plastoquinone-9; α-T, α-tocopherol.

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[8, 10]. In our present work, we have now tried to investigate at which developmental stage and by which mechanisms the accumulation of chloroplast isoprenoids is suppressed in non-green leaves, by comparing rates of synthesis and turnover of individual compounds. Investigations of isoprenoid biosynthesis by radioactive labeling of intact leaf tissues are complicated by the compartmentation of precursor substrates and events of metabolic channelling [see 6]. It is long known that in green leaves CO_2 is more efficiently incorporated into plastidic isoprenoids than exogenously applied mevalonate [11]. For a comparison with non-photosynthetic achlorophyllous leaves we were, however, forced to use only mevalonate as precursor. The results obtained point to the existence of marked differences of transport capacities and metabolic channelling in different stages and situations of leaf differentiation.

Materials and Methods

Plant material and growing conditions

The lower halves of the primary leaves of 6-day-old rye seedlings were used for extraction of prenylquinones and pigments, labeling experiments or plastid isolation. Seeds of winter rye (*Secale cereale* L. *cv.* "Halo") were surface-sterilized by a 10 min vacuum infiltration and about 30 min soaking in a freshly prepared filtered solution of 3% (w/v) of calcium hypochlorite-chloride, thoroughly washed with demineralized H_2O and germinated in glass-covered plastic boxes on filter paper (Schleicher & Schüll, No. 598, or Macherey & Nagel, MN 218) moistened with distilled H_2O. Plants were kept for one day at 22 °C in darkness. During the additional 5 days seedlings were kept either at 22 °C in continuous white light or darkness, or at 32 °C in white light, in order to obtain 70S ribosome-deficient leaves [12]. For the preparation of individual reference compounds from greater leaf quantities, rye seedlings were grown on Vermiculite moistened with a modified Knop's nutrient solution [13] at 22 °C in white light. Continuous irradiation with white light was provided by fluorescent tubes (Osram L 36W/36 Natura and Philips TLD 36W/95 in alternating sequence) giving an incident photon flux density of *ca.* 90 μmol $m^{-2}\,s^{-1}$ (5,000 lux; 20 W m^{-2}).

Preparation of cell-free extracts

Sections from the lower halves of the primary leaves of 6-day-old seedlings were homogenized under ice-cold conditions with mortar and pestle in 0.15 M tricine-KOH, 10 mM KCl, 1 mM $MgCl_2$, 1 mM Na_2-EDTA, 4 mM dithioerythritol. Extracts were centrifuged for 15 min at 20,000 × *g* at 4 °C and supernatants were used for the estimation of enzyme activities.

Isolation of intact plastids

Plastids were isolated from the lower halves of primary leaves of 6-day-old rye seedlings and intact plastids were purified by centrifugation on discontinuous Percoll gradients as described by Höinghaus and Feierabend [14]. The grinding medium consisted of 0.3 M sorbitol, 50 mM tricine-KOH, 1 mM Na_2–EDTA, 10 mM KCl, 1 mM $MgCl_2$, 4 mM dithioerythritol and had a pH of 7.8. The Percoll gradients consisted of layers of 80% and 42% (v/v) Percoll for chloroplasts and of 80% and 26% Percoll for plastids from 32 °C-grown leaves, according to Höinghaus and Feierabend [14].

Incorporation of radioactive precursors into leaf segments

The lower halves of the primary leaves of 6-day-old rye seedlings were divided into three segments of equal length, designated from bottom to top as I, II, III. Ten segments of each type were each further dissected into two pieces and incubated for 6 h with 0.6 ml 0.01% (v/v) Tween 20 containing 444 kBq R-[2-^{14}C]mevalonolactone (2.1 MBq μmol^{-1}) under the respective growing conditions, as previously described for labeling with [^{14}C]acetate [15]. At the end of the incubation period the leaf segments were thoroughly rinsed with distilled H_2O and isoprenoid lipids were extracted, as described below. For measurements of mevalonate incorporation in the presence of inhibitors and for chase experiments only the oldest segment (number III) was used. The leaf sections were for 40 min preincubated on 0.6 ml inhibitor solutions (10 mM 4,6-dioxoheptanoic acid or 1 mM gabaculin) containing 0.01% Tween 20 (v/v) before the addition of radioactive mevalonolactone. For chase experiments leaf segments were at the end of the labeling period thoroughly rinsed with distilled H_2O and,

starting with a short vacuum-infiltration, further incubated under slow rotation with 800 µl each of 37 mM unlabeled mevalonolactone, 10 mM NaOH, 0.01% (v/v) Tween 20; the pH was about 5.0. During the first 3 h the solution was replaced every 30 min. Samples were extracted after chase periods of 3, 6, 12 and 24 h.

Uptake and incorporation of radioactive precursors by isolated plastids

Percoll gradient-purified intact plastids, equivalent to the yield from 250–450 mg leaf tissue, were incubated in darkness for 4 h at either 22 °C or 32 °C (according to the growing conditions used for the leaves) in 0.3 ml grinding medium described for plastid isolation (see above) which, in addition, contained 5 mM ATP, 0.17 mM S-adenosylmethionine, 1 mM *p*-hydroxyphenylpyruvate, and 37 kBq R-[2-^{14}C]mevalonolactone or [4-^{14}C]isopentenyl pyrophosphate (see [16, 17]). The incubation was terminated by the addition of 2 ml 80% acetone and prenylquinones were extracted (see [8]).

Uptake of radioactive precursors was estimated by centrifugation through a layer of silicone oil according to Heldt [18]. A suspension (0.1 ml) of Percoll gradient-purified intact plastids in grinding medium (see above) was incubated after the addition of 5 mM ATP with labeled precursors at 4 °C. At different time intervals after the addition of either 18.5 kBq R-[2-^{14}C]mevalonolactone (2.1 MBq µmol^{-1}) or 15 kBq [4-^{14}C]isopentenyl pyrophosphate (1.8 MBq µmol^{-1}) plastids were centrifuged through a layer of silicone oil (AR 170 for chloroplasts, AR 150 for ribosome-deficient plastids) into a layer of 10% (v/v) $HClO_4$ and the uptake into the sorbitol-impermeable space was determined as described by Höinghaus and Feierabend [19].

Extraction and analysis of prenylquinones and pigments

Prenylquinones were extracted and identified according to Lichtenthaler *et al.* [20] and Lichtenthaler and Pfister [21], as previously described [8]. Plastohydroquinone was oxidized by the addition of ferricyanide.

Prenylquinones and β-carotene were separated by thin-layer chromatography on "Kieselgel 60" (Merck 5721) plates according to Lichtenthaler [1]. (1. Dimension: 16.5% (v/v) diethyl ether in *n*-hexane; after impregnation with paraffin oil 2. dimension: 95% acetone (v/v) in H_2O). In order to improve the separation of β-carotene, chromatography in the 2. dimension was, in addition, repeated two times with 80% acetone (v/v in H_2O). The area of the plate containing the prenylquinones was protected from the additional chromatography in 80% acetone by removing stripes from the silica gel layer and separating the prenylquinones containing portion. The absence of carotene precursors or squalene from the β-carotene spot was assayed by chromatography according to Lütke-Brinkhaus and Kleinig [22]. The amounts of β-carotene were calculated after one-dimensional thin-layer chromatography from measurements of the absorbance at 464 mm according to Hager and Meyer-Bertenrath [23].

For the separation of xanthophylls the partition chromatography system of Hager and Meyer-Bertenrath [23] was used for the 1. direction. For the 2. dimension the plate was impregnated with paraffin oil and chromatographed with 65% acetone (v/v in H_2O).

Determination of radioactivity

Radioactive spots on thin layer chromatograms were detected by fluorography. Thin layer plates were sprayed with En^{3}Hance (New England Nuclear) and exposed to a Kodak X-OMAT S film.

Radioactivity was determined by liquid scintillation counting with Packard Emulsifier Scint 299 in a Packard Tri-Carb 4530 counter. Quench corrections were performed by the spectral index of external standard.

Analytical methods

Chlorophyll was determined according the Whatley and Arnon [24]. The activity of NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13) was determined as described [13]. Experiments were performed at least 3 times. Standard errors of the mean are indicated.

Results

Course of synthesis in green and achlorophyllous leaves

Normal green or non-green etiolated rye leaves were grown at 22 °C in light or in darkness. Alternatively, seedlings were grown at 32 °C in light in

order to eliminate chloroplast ribosome formation in the leaves. Except for the tip parts, the 32 °C-grown leaves were 70S ribosome-deficient and bleached [10, 13]. The contents of prenylquinones in achlorophyllous tissues of etiolated 22 °C-grown or bleached 32 °C-grown leaves were below 20–30% of those found in green leaves [8]. Also carotenoid contents were low, although carotenoids were the predominating pigments of heat-bleached leaves [10]. The content of β-carotene (per g fresh weight) in bleached 32 °C-grown leaf sections was only about 2% of that in normal green leaves. In order to investigate differences and potential controls occurring in the course of development of isoprenoid compounds in green and non-green leaves we followed the same protocol that was previously described for investigations of glycolipid synthesis [15]. The lower halves of the leaves of 6-day-old seedlings were dissected into three parts of equal length (I–III, Fig. 1) and separately labeled with [^{14}C]mevalonate. Because cereal leaves are growing from a basal meristem the leaf segments reflect consecutive stages of progressive leaf age and allow a comparison of synthetic activities along the developmental gradient of the leaves.

The total [^{14}C]mevalonate uptake (radioactivity in crude total acetone extract) was under all growing conditions slightly higher in the younger than in the older leaf segments. Uptake in segment III was in green leaves about 40%, in the non-green leaves up to about 20% lower than in segment I. Uptake of [^{14}C]mevalonate into segments of etiolated leaves was up to 50%, and uptake into segments of heat-bleached leaves 50–100%, higher than into segments from green leaves (not shown). In accordance with the differences in total [^{14}C]mevalonate uptake also total incorporation into the *n*-hexane-extractable fraction of non-polar lipids was slightly decreasing with the age of the leaf tissue in green and heat-bleached, but not in etiolated leaves (Fig. 1). The most striking difference between the different types of leaf differentiation was that, at all stages, incorporation into total non-polar lipids was much higher in heat-bleached than in 22 °C-grown green or etiolated leaves. In heat-bleached 32 °C-grown leaf segments, on the average, about 50% of total [^{14}C]mevalonate uptake were incorporated into total non-polar lipids, while in 22 °C-grown leaves

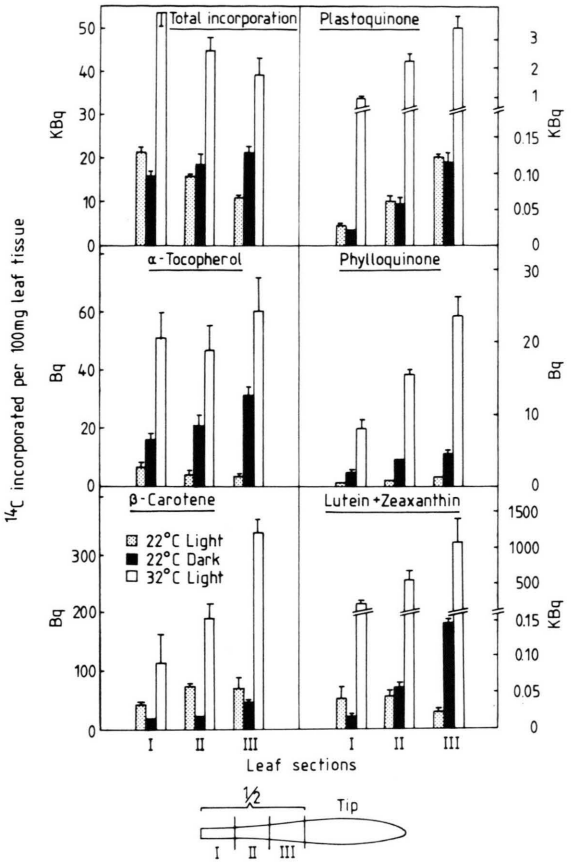

Fig. 1. Incorporation of radioactivity during a 6 h incubation with [^{14}C]mevalonate into total *n*-hexane-soluble non-polar lipids and into major prenylquinones and carotenoids of three consecutive segments (I–III) cut from the lower halves of primary leaves of 6-day-old rye seedlings, as illustrated by the scheme. Seedlings were grown at 22 °C in light (green leaves), at 22 °C in darkness (etiolated leaves), or at 32 °C in light (70S ribosome-deficient bleached leaves). Labeling was performed under the same light and temperature conditions under which the plants were raised.

only about 30% were incorporated. In heat-bleached leaves up to 12% of the radioactivity (in segment III) incorporated into non-polar lipids were contained in the prenylquinones (PQ-9, α-T, phylloquinone) and carotenoids (β-carotene, lutein) analysed by us, while these accounted for only up to 2% in 22 °C-grown leaves. Correspondingly, in heat-bleached 32 °C-grown leaves incorporation into individual prenylquinones and carotenoids was several-fold higher than in 22 °C-

grown leaves and, except for α-T, increased continuously with leaf age (Fig. 1). Since the amounts of the isoprenoids were lower in the 32 °C-grown than in green 22 °C-grown leaves, specific radioactivities were even more increased. The specific radioactivity of the β-carotene of the complete lower halves (segments I–III) was 49 Bq μg^{-1} for green 22 °C-grown and 7358 Bq μg^{-1} for bleached 32 °C-grown leaves. Among the 22 °C-grown leaves incorporation into PQ-9 was equal in green and etiolated leaves. Incorporation into α-T, phylloquinone and lutein was higher in etiolated than in green leaves. Only for β-carotene [^{14}C]mevalonate incorporation was higher in green than in etiolated leaves and leveled off with leaf age, similar to the previously described behavior of acyl lipids [15]. In green leaves (22 °C light) also labeling of lutein declined with leaf age.

Since the comparison of radioactive labeling in green and non-green leaves did not provide evidence for the existence of any correlation of the apparent synthesis of prenylquinones or carotenoids with chlorophyll formation, we examined, in addition, by inhibitor application, whether any immediate coupling between chlorophyll synthesis and the formation of isoprenoid compounds could be demonstrated in green leaf segments. Labeling experiments with [^{14}C]mevalonate were performed with segment III of green leaves in the presence of gabaculin, an inhibitor of δ-aminolevulinic acid synthesis [25, 26] and 4,6-dioxoheptanoic acid, an inhibitor of the aminolevulinic acid dehydratase [27], at concentrations which efficiently block chlorophyll synthesis. Dioxoheptanoic acid almost completely suppressed the synthesis of isoprenoid compounds, except for α-T, for which labeling was even increased (Fig. 2). The suppression of isoprenoid synthesis through dioxoheptanoic acid cannot be regarded as a mere consequence of the inhibition of chlorophyll synthesis, but must reflect more severe direct effects on this pathway because the action of the alternative and equally effective chlorophyll synthesis inhibitor gabaculin did not confirm the results obtained for dioxoheptanoic acid. In the presence of gabaculin incorporation into the total non-polar lipids and into PQ-9 was markedly diminished, incorporation into α-T and phylloquinone was not affected and labeling of the carotenoids was slightly increased (Fig. 2).

Comparison of the turnover in green and achlorophyllous leaves

Since the rates of [^{14}]mevalonate incorporation were not in accordance with the much lower steady-state contents of prenylquinones and carotenoids in non-green, relative to green, leaves we have examined by chase experiments whether these compounds might be less stable in the absence of chlorophyll and normal thylakoid differentiation. After termination of [^{14}C]mevalonate application the radioactivity in total non-polar lipids and in most individual compounds analysed by us increased for the next 3–6 h of a subsequent chase (Fig. 3). The majority of the isoprenoid compounds was fairly stable during the next 24 h. Except for β-carotene, degradation rates were not markedly higher in heat-bleached and 22 °C-grown etiolated, relative to normal green leaves, and did not provide a sufficient explanation for the differences in the prenylquinone and carotenoid contents. Only the turnover of β-carotene in 32 °C-grown heat-bleached leaves was much higher than in normal green leaves which might reflect increased photodegradation. Its half time in 32 °C-grown leaves was about 12 h, while it was about 58 or 41 h in green or etiolated 22 °C-grown leaves (Fig. 3). During the total chase period of 24 h radioactivity continued to accumulate in α-T, particularly in heat-bleached leaves, and apparent degradation could not be observed.

Uptake and incorporation by isolated plastids

To examine whether differences of the capacity of precursor transport across the envelope membranes or of enzymatic activities were responsible for the greatly higher rates of apparent synthesis of isoprenoid compounds in heat-bleached than in green leaves, uptake of substrates and capacities for *in vitro* incorporation were compared in Percoll-gradient-purified isolated plastids. Microscopic examinations indicated that 90% of the organelles were still intact at the end of the incubation experiments. Uptake of [^{14}C]mevalonate and of [^{14}C]isopentenyl pyrophosphate was compared by the silicon-layer filtering centrifugation method. Uptake into chloroplasts or bleached plastids from 32 °C-grown leaves differed markedly. Both mevalonate and isopentenyl pyrophosphate uptake was faster in heat-bleached plastids than in

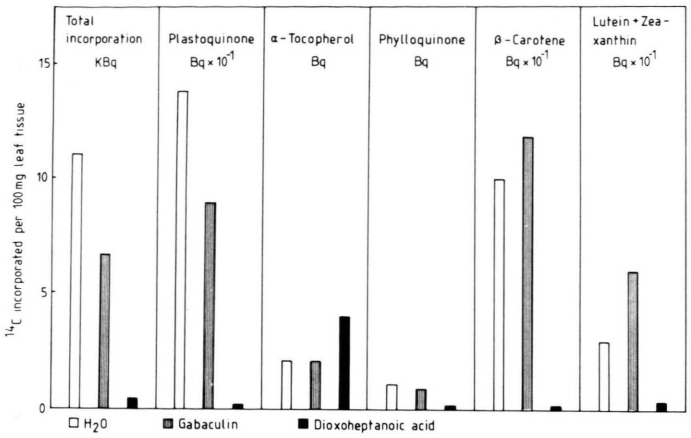

Fig. 2. Effect of 1 mM gabaculin and of 10 mM 4,6-dioxoheptanoic acid on incorporation of [^{14}C]mevalonate (6 h incubation) into total *n*-hexane-soluble non-polar lipids and into major prenylquinones and carotenoids of segment III (see scheme of Fig. 1; upper one third of lower halves of leaves) from green leaves of 6-day-old rye seedlings grown at 22 °C in light.

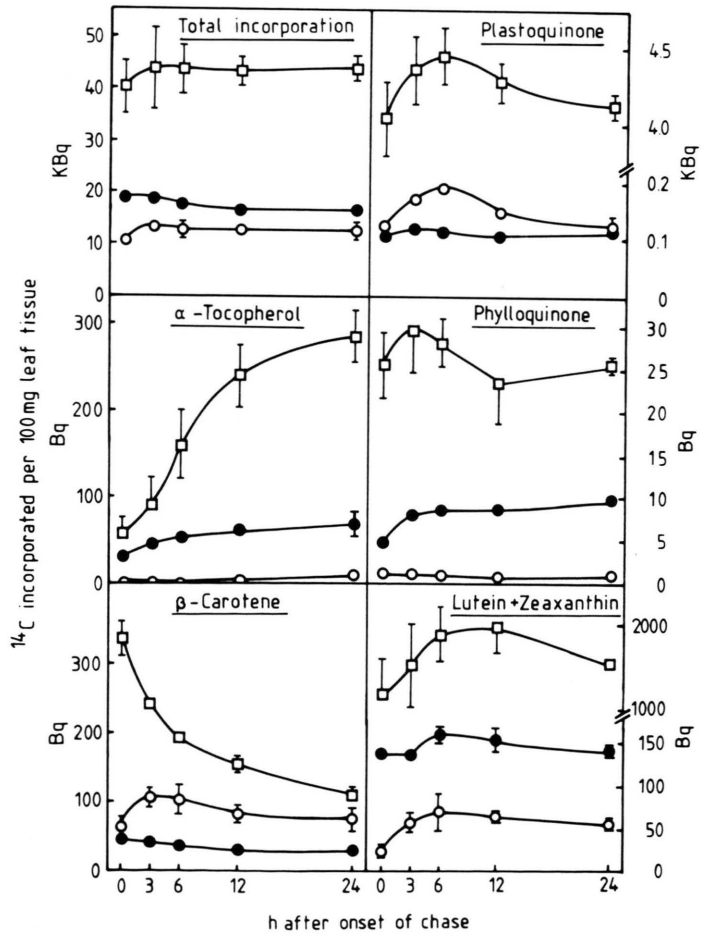

Fig. 3. Chase of the radioactivity incorporated in total *n*-hexane-soluble non-polar lipids or in major prenylquinones or carotenoids after a 6 h labeling with [^{14}C]mevalonate. For labeling and chase, only the segment no. III (see Scheme of Fig. 1; upper one third of lower halves of leaves) from primary leaves of 6-day-old rye seedlings was used. ○, green leaves grown at 22 °C in light; ●, etiolated leaves grown at 22 °C in darkness; □, 70S ribosome-deficient bleached leaves grown at 32 °C in light.

Table I. Comparison of the incorporation of [^{14}C]isopentenyl pyrophosphate into total *n*-hexane-soluble non-polar lipids and into plastoquinone (PQ-9) by Percoll-gradient-purified chloroplasts from 22 °C-grown leaves or by ribosome-deficient bleached plastids from 32 °C-grown leaves of 6-day-old rye seedlings. The 4 h incubations were performed at the same temperature, 22 °C or 32 °C, at which the corresponding leaves had been grown. The data are referred to either equal numbers of plastids or equal amounts of leaf tissue. Incorporation per amount of fresh weight of original leaf tissue used for organelle isolation was calculated from estimations of the yields of intact plastids. 100 mg (fresh weight) tissue from lower halves of green leaves contain about 81 ± 3 µg chlorophyll.

Measurement	Chloroplasts (22 °C)	Bleached plastids (32 °C)
[^{14}C] incorporation into total non-polar lipids		
Bq per 10^8 plastids	2655	2972
Bq per 100 mg leaf tissue	2393	1491
[^{14}C]incorporation into PQ-9		
Bq per 10^8 plastids	5.5	9.4
Bq per 100 mg leaf tissue	4.9	4.7

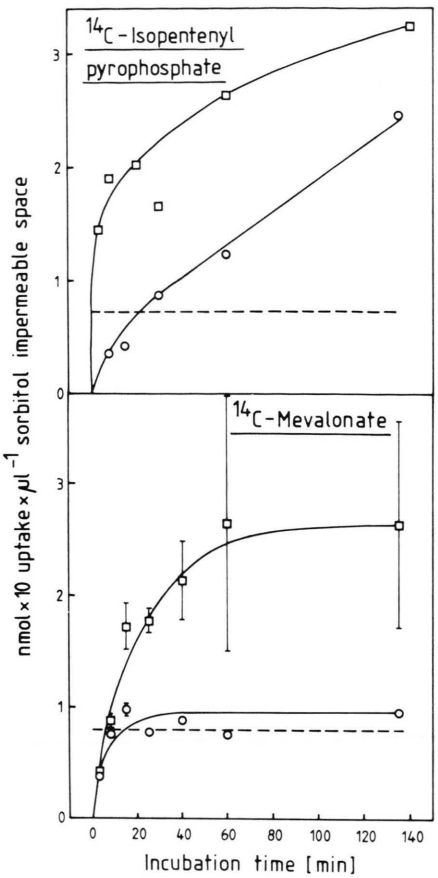

Fig. 4. Time course of the uptake of [^{14}C]isopentenyl pyrophosphate and [^{14}C]mevalonate into the sorbitol-impermeable space of isolated chloroplasts (○) from green leaves (grown at 22 °C in light), or of ribosome-deficient plastids (□) from heat-bleached leaves (grown at 32 °C in light). – – –, external mevalonate concentration of the medium.

chloroplasts (Fig. 4). While uptake of mevalonate into chloroplasts occurred only to the level of equilibration to the concentration of the external medium, it was greatly enriched in heat-bleached plastids above its external concentration. Also the accumulation of isopentenyl pyrophosphate in heat-bleached plastids was markedly higher than in chloroplasts.

Analysis of the products formed during incubation of isolated organelles showed that [^{14}C]mevalonate was not to any detectable extent incorporated into lipids. Isopentenyl pyrophosphate was, however, incorporated into numerous distinct lipids. Under our assay conditions formation of PQ-9 was clearly identified and fluorographs of TCL separations indicated a faint labeling of β-carotene in heat-bleached plastids. The capacities of isolated chloroplasts and heat-bleached plastids for synthesis of total non-polar lipids and PQ-9 appeared to be of similar order. If incorporation was referred to plastid number, heat-bleached plastids appeared to be more active than chloroplasts (Table I). Incorporation into the total non-polar lipids in chloroplasts was about 15%, in bleached plastids about 7% of the [^{14}C]mevalonate incorporation found in equivalent quantities of intact leaf tissue. The labeling of PQ-9 was 3% in chloroplasts or 0.2% in bleached plastids of the [^{14}C]mevalonate incorporation into PQ-9 in equivalent amounts of intact leaf tissue.

Discussion

Previous investigations comparing the rates of acetate incorporation along the developmental gradient above the basal meristem zone of cereal leaves, such as rye, revealed a clear correlation of plastidic glycolipid synthesis with greening and

chloroplast differentiation [15]. Glycolipids were formed in low amounts also in achlorophyllous, etiolated or heat-bleached, leaves but the activity of new synthesis leveled off at an early developmental stage while their stability was not markedly different from that observed in green leaves. Thus, glycolipid accumulation was mainly regulated by control of its biosynthesis. While the total contents of prenylquinones and carotenoids remained also much lower in etiolated and heat-bleached, than in normal green, leaves [8], these differences were not at all reflected by the rates of incorporation of external mevalonate by intact leaves since these were far highest in 32 °C-grown leaves and for phylloquinone, α-T and lutein also higher in etiolated than in green leaves. In 32 °C-grown bleached leaves up to 19- or 36-fold more radioactivity than in green leaves was incorporated *e.g.* into phylloquinone or PQ-9.

Ratios of apparent mevalonate incorporation will diverge from true rates of isoprenoid synthesis to the extent that internal metabolite pool sizes, their compartmentation or the rates of turnover of the compounds would differ in different tissues. Nevertheless, apparent incorporation rates could illustrate relative changes during the course of development. In this respect it was, however, surprising that rates of mevalonate incorporation into the isoprenoids of etiolated and heat-bleached leaves, analysed by us, increased continuously with increasing age of the tissue and only incorporation into the total non-polar lipids declined, as expected, in 32 °C-grown leaves. Some immediate correlation with greening and thylakoid differentiation was only indicated by the reduction of total lipid and PQ-9 labeling after application of the chlorophyll synthesis inhibitor gabaculin.

According to previous work [1] exposure of green leaves to darkness induced an apparent degradation of prenylquinones. Also in achlorophyllous leaves an increased turnover of prenylipids might conceivably be accompanied by higher apparent incorporation rates but, nevertheless, result in lower steady state levels than in green leaves. However, except for β-carotene, the other compounds were under all conditions quite stable and the lower isoprenoid quantities of achlorophyllous leaves cannot be explained by increased degradation, relative to green leaves. Within our 24 h chase periods half times could not be determined very precisely, can, however, be estimated to range mostly between 2–3 days. These values are fairly similar to those estimated from apparent degradation rates after dark incubation of leaves [1], however, much longer than those observed in the unicellular alga *Chlorella* [28, 29]. For β-carotene, degradation was greatly increased in heat-bleached leaves, with a half time of only 12 h instead of about 60 h in green leaves, and could thus sufficiently explain the lower β-carotene accumulation, as compared to green leaves, while in etiolated leaves β-carotene synthesis appeared to be low. It is conceivable that under the conditions of disturbed plastid differentiation in 32 °C-grown leaves β-carotene suffered more from photooxidative degradation than in normal chloroplasts. In green rye leaves photooxidation of β-carotene was, in contrast to observations with *Chlorella* [28], not much higher, relative to lutein. Similar to *Chlorella,* xanthophylls and α-T had lowest turnover also in rye leaves [29]. For α-T no degradation at all was observed during the 24 h chase periods. Its initial labeling was always very low and radioactivity was slowly but continuously accumulating in α-T, particularly in 32 °C-grown leaves. This behavior might either result from the slowness of the cyclization of the prenylquinol [30] or from its gradual accumulation in plastoglobuli which have a high α-T content [31, 32]. However, the extended high radioactive incorporation into α-T in non-green leaves cannot represent true higher accumulation of substance because the final contents of α-T were much lower than in green leaves [8]. For PQ-9 which is the major prenylquinone constituent of plastoglobuli the course of labeling differed greatly from that of α-T and degradation became apparent already after 6 h of chase.

Since for the majority of the isoprenoids differences of turnover cannot account for the strong labeling in achlorophyllous, particularly heat-bleached, leaves external mevalonate incorporation must be greatly affected and modified by thorough ontogenetic changes of uptake, intracellular compartmentation, transport or pool sizes, or by varying routes of metabolic channelling of isoprenoid metabolites which appear to accompany, in particular, patterns of leaf development with greatly diverging types of plastid differentiation. Several of the potential mechanisms appear to contribute to the strikingly high mevalonate incor-

poration in heat-bleached leaves, which were most thoroughly analysed, as the most remarkable example. The uptake of external mevalonate and the total incorporation capacity were about two times higher than in green leaves. In addition, the uptake and accumulation of the isoprenoid precursors mevalonate and isopentenyl pyrophosphate into bleached plastids was greatly enhanced, as compared to the uptake by chloroplasts. While mevalonate was not incorporated into lipids in isolated plastids from rye, in accordance with results of other publications, some authors believe that chloroplasts can be fully autonomous and capable to both synthesize and use mevalonate for prenylipid synthesis, but the mevalonate pyrophosphate decarboxylase seems to be labile *in vitro* [6, 33]. However, also during different stages of normal chloroplast development the capacity for uptake and incorporation of isoprenoid precursors from the cytoplasm appears to change quite markedly. Heintze *et al.* [34] discriminate between an autonomous stage in young leaf tissue where CO_2 is the preferential source of isoprenoid synthesis in chloroplasts, and a "division-of-labor" stage in mature tissue where the precursor flow from photosynthesis declines and, instead, isopentenyl pyrophosphate is imported from the cytoplasm. Leaves with non-green plastids and, in particular the rudimentary, proplastid-like, heat-bleached plastids, would necessarily be in a "division-of-labor" situation and totally dependent on precursor supply from the cytoplasm. Nevertheless, also ribosome-deficient heat-bleached plastids were still able to synthesize a full complement of prenylipids, thus emphasizing that the enzymes involved were nuclear-encoded and synthesized in the cytoplasm.

External mevalonate is to a major proportion incorporated into sterols in cereal leaves [34, 35]. Since sterol formation was greatly increased in 32 °C-grown leaves [10] the internal isoprenoid precursor pools might be much more depleted than in normal leaves. When internal pools are smaller, they will obtain a higher specific radioactivity from [^{14}C]mevalonate uptake and this will also increase apparent incorporation rates. The observation that *in vitro,* in the presence of an isoprenoid precursor supply of equal specific radioactivity, the incorporation into lipids or PQ-9 in bleached plastids was similar as in chloroplasts but, relative to the capacities of equivalent amounts of intact leaf tissues, much more diminished than for chloroplasts, provides strong arguments, that in the intact tissue of heat-bleached leaves plastids must be supplied with precursors of higher specific radioactivity than in green leaves, to explain the higher incorporation from external mevalonate. In addition to such general effects, as enhanced organellar uptake and higher specific radioactivity of the precursors, also more specific actions of metabolic channelling must be responsible for the peculiar differences which exist between the labeling patterns of different plastidic isoprenoids in heat-bleached leaves, *e.g.* between PQ-9 and β-carotene. From the overall comparison of our results it has to be anticipated that prenyllipid synthesis is lower in achlorophyllous, than in green, leaves and that the differences of mevalonate incorporation do not so much reflect true differences of synthetic rates but rather illustrate very fundamental developmental changes in the metabolic flow of isoprenoid intermediates which are not observed during glyolipid formation from acetate.

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