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Barry J. Pogson and Heather M. Rissler

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 Genetic manipulation of carotenoid
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biosynthesis and photoprotection
biosynthesis and photoprotection

Barry J. Pogson* **and Heather M. Rissler**

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Division of Biochemistry and Molecular Biology, The Australian National University, Canberra, ACT 0200, Australia
There are multiple complementary and redundant mechanisms to provide protection against photo-
oxidative dam There are multiple complementary and redundant mechanisms to provide protection against photo-
oxidative damage, including non-photochemical quenching (NPQ). NPQ dissipates excess excitation
energy as heat by using xanthop There are multiple complementary and redundant mechanisms to provide protection against photo-
oxidative damage, including non-photochemical quenching (NPQ). NPQ dissipates excess excitation
energy as heat by using xanthop oxidative damage, including non-photochemical quenching (NPQ). NPQ dissipates excess excitation
energy as heat by using xanthophylls in combination with changes to the light-harvesting complex
(LHC) antenna. The xanthophyl energy as heat by using xanthophylls in combination with changes to the light-harvesting complex (LHC) antenna. The xanthophylls are oxygenated carotenoids that in addition to contributing to NPQ can quench singlet or trip (LHC) antenna. The xanthophylls are oxygenated carotenoids that in addition to contributing to NPQ
can quench singlet or triplet chlorophyll and are necessary for the assembly and stability of the antenna.
We have genetic can quench singlet or triplet chlorophyll and are necessary for the assembly and stability of the antenna. We have genetically manipulated the expression of the ε -cyclase and β -carotene hydroxylase carotenoid biosy We have genetically manipulated the expression of the ε -cyclase and β -carotene hydroxylase carotenoid
biosynthetic enzymes in *Arabidopsis thaliana*. The ε -cyclase overexpression confirmed that *lut2* (lutein d biosynthetic enzymes in *Arabidopsis thaliana*. The ε -cyclase overexpression confirmed that $lut2$ (lutein deficient) is a mutation in the ε -cyclase gene and demonstrated that lutein content can be altered at the l cient) is a mutation in the *ε*-cyclase gene and demonstrated that lutein content can be altered at the level
of mRNA abundance with levels ranging from 0 to 180% of wild-type. Also, it is clear that lutein affects
the ind *Chelamydomonas* are additive, no matter what the genetic background, whether *npq1* (zeaxanthin deficient), *Chlamydomonas* are additive, no matter what the genetic background, whether *npq1* (zeaxanthin deficient), the induction and extent of NPQ. The deleterious effects of lutein deficiency on NPQ in *Arabidopsis* and *Chlamydomonas* are additive, no matter what the genetic background, whether *mpql* (zeaxanthin deficient), *abal* Chlamydomonas are additive, no matter what the genetic background, whether $npql$ (zeaxanthin deficient), abal or antisense β -hydroxylase (xanthophyll cycle pool decreased). Additionally, increasing lutein content causes $abal$ or antisense β -hydroxyla
causes a marginal, but signif
xanthophyll cycle pool size. we significant, increase in the rate or induction or NTQ despite a
bool size.
Keywords: carotenoid; lutein; xanthophyll cycle; photoprotection;
non-photochemical quenching (NPO)

nrotenoid; lutein; xanthophyll cycle; phot
non-photochemical quenching (NPQ)

1. INTRODUCTION

(a) *Carotenoid biosynthesis and function*

Carotenes and their oxygenated derivatives, the xantho-(a) *Carotenoid biosynthesis and function*
Carotenes and their oxygenated derivatives, the xantho-
phylls, are integral components of the photosynthetic
annarative and consequently are essential to plant viabi-Carotenes and their oxygenated derivatives, the xantho-
phylls, are integral components of the photosynthetic
apparatus and consequently are essential to plant viabi-
lity contributing to numerous processes—including phylls, are integral components of the photosynthetic
apparatus and consequently are essential to plant viabi-
lity, contributing to numerous processes—including
assembly and stability of nigment-protein complexes apparatus and consequently are essential to plant viability, contributing to numerous processes—including
assembly and stability of pigment-protein complexes,
light harvesting and photoprotection. Four carotenoids lity, contributing to numerous processes—including
assembly and stability of pigment–protein complexes,
light harvesting and photoprotection. Four carotenoids,
lutein. B-carotene, violaxanthin and neoxanthin, are assembly and stability of pigment–protein complexes,
light harvesting and photoprotection. Four carotenoids,
lutein, β -carotene, violaxanthin and neoxanthin, are
nearly ubiquitous among bigher plants (figure 1) (Young light harvesting and photoprotection. Four carotenoids,
lutein, β -carotene, violaxanthin and neoxanthin, are
nearly ubiquitous among higher plants (figure 1) (Young
1993*a b*) with one of the few exceptions being the lutein, β -carotene, violaxanthin and neoxanthin, are
nearly ubiquitous among higher plants (figure 1) (Young
1993*a*,*b*) with one of the few exceptions being the para-
sitic angiosperm *Custuta releva* where neoxanthi nearly ubiquitous among higher plants (figure 1) (Young 1993*a*,*b*) with one of the few exceptions being the parasitic angiosperm *Cuscuta reflexa* where neoxanthin is replaced by lutein-5.6-epoxide (Bungard *et al.* 1999 1993*a*,*b*) with one of the few exceptions being the parasitic angiosperm *Cuscuta reflexa* where neoxanthin is replaced by lutein-5,6-epoxide (Bungard *et al.* 1999).

The critical roles of carotenoids are reflected in the replaced by lutein-5,6-epoxide (Bungard *et al.* 1999).
The critical roles of carotenoids are reflected in the
tight regulation of carotenoid biosynthesis during plant
development and adaptation to environmental stress The critical roles of carotenoids are reflected in the
tight regulation of carotenoid biosynthesis during plant
development and adaptation to environmental stress.
Their biosynthesis in plants has been reviewed elsewhere tight regulation of carotenoid biosynthesis during plant
development and adaptation to environmental stress.
Their biosynthesis in plants has been reviewed elsewhere
(Sandmann 1994: Hirschberg et al. 1997: Cunningham & development and adaptation to environmental stress. for
Their biosynthesis in plants has been reviewed elsewhere in
(Sandmann 1994; Hirschberg *et al.* 1997; Cunningham & cy
Gantt 1998). In brief lyconene is either cyclize Their biosynthesis in plants has been reviewed elsewhere
(Sandmann 1994; Hirschberg *et al.* 1997; Cunningham &
Gantt 1998). In brief, lycopene is either cyclized twice by
the B-cyclase to produce B-carotene or once each b (Sandmann 1994; Hirschberg *et al.* 1997; Cunningham & Gantt 1998). In brief, lycopene is either cyclized twice by the β -cyclase to produce β -carotene or once each by the Gantt 1998). In brief, lycopene is either cyclized twice by
the β -cyclase to produce β -carotene or once each by the
 β -cyclase and ε -cyclase (*lut2*) to produce α -carotene
(figure 1) The α -carotene rings the β -cyclase to produce β -carotene or once each by the β -cyclase and ε -cyclase (*lut2*) to produce α -carotene (figure 1). The α -carotene rings are hydroxylated by the ε -hydroxylase (*lut1*) and β β -cyclase and ε -cyclase (*lut2*) to produce α -carotene (figure 1). The α -carotene rings are hydroxylated by the ε -hydroxylase to make lutein. β -carotene is hydroxylated twice by the β -hydroxylase to ϵ -hydroxylase (*lutl*) and β-hydroxylase to make lutein. β-
carotene is hydroxylated twice by the β-hydroxylase to
make zeaxanthin. The hydroxyl groups are added to the
ends of each ring resulting in the xanthonbylls carotene is hydroxylated twice by the β -hydroxylase to make zeaxanthin. The hydroxyl groups are added to the ends of each ring resulting in the xanthophylls being more polar than β -carotene Zeaxanthin is enoxidated make zeaxanthin. The hydroxyl groups are added to the table 1).

ends of each ring resulting in the xanthophylls being The ε -cyclase is believed to be a key rate-limiting step,

more polar than β -carotene. Zeaxanth twice to make violaxanthin, which is subsequently

modified to make neoxanthin. Mutations have been iden-
tified in a number of enzymes in the pathway and in a modified to make neoxanthin. Mutations have been identified in a number of enzymes in the pathway and in a range of species: those that disrupt xanthonbull biomodified to make neoxanthin. Mutations have been identified in a number of enzymes in the pathway and in a range of species; those that disrupt xanthophyll bio-synthesis are listed in table 1 tified in a number of enzymes in the pathway and in a
range of species; those that disrupt xanthophyll biosynthesis are listed in table 1.
(i) *Cyclization reactions*

Cyclization reactions
The cyclization reactions occur at the branch point in
a pathway resulting in B B-carotenoids such as violax-(i) Cyclization reactions
The cyclization reactions occur at the branch point in
the pathway resulting in β, β -carotenoids such as violax-
anthin or β s-carotenoids such as lutein. Insight into the The cyclization reactions occur at the branch point in
the pathway resulting in β , β -carotenoids such as violax-
anthin or β , ϵ -carotenoids such as lutein. Insight into the
enzymes has come from isotone labellin the pathway resulting in β , β -carotenoids such as violax-
anthin or β , ϵ -carotenoids such as lutein. Insight into the
enzymes has come from isotope labelling, the use of anthin or β , ε -carotenoids such as lutein. Insight into the enzymes has come from isotope labelling, the use of herbicides that inhibit cyclization and genetic studies (Chunaey *et al* 1991: Bramley 1993: Bishon 199 enzymes has come from isotope labelling, the use of
herbicides that inhibit cyclization and genetic studies
(Chunaev *et al.* 1991; Bramley 1993; Bishop 1996; Pogson
et al. 1996). Lesions in the B-cyclase are lethal resu herbicides that inhibit cyclization and genetic studies (Chunaev *et al.* 1996; Bramley 1993; Bishop 1996; Pogson *et al.* 1996). Lesions in the β -cyclase are lethal, resulting in the accumulation of the monocyclic s (Chunaev et al. 1991; Bramley 1993; Bishop 1996; Pogson *et al.* 1996). Lesions in the β -cyclase are lethal, resulting in the accumulation of the monocyclic ε, Ψ -carotene in *Arabidopsis* (B. J. Pogson & D. DellaPenna, unpublished data), indicating a requirement for bic in the accumulation of the monocyclic ε, Ψ -carotene in
Arabidopsis (B. J. Pogson & D. DellaPenna, unpublished
data), indicating a requirement for bicyclic carotenoids
for plant viability. The accumulation of ε, Ψ Arabidopsis (B. J. Pogson & D. Della Penna, unpublished data), indicating a requirement for bicyclic carotenoids
for plant viability. The accumulation of ε, Ψ -carotene
indicates that the *Arabidopsis* ε -cyclase is incapable of
cyclizing both ends of the linear lyconene for plant viability. The accumulation of ε, Ψ -carotene indicates that the *Arabidopsis* ε -cyclase is incapable of cyclizing both ends of the linear lycopene in contrast to all *B*-cyclases and the lettuce ε -cy indicates that the *Arabidopsis* ε -cyclase is incapable of cyclizing both ends of the linear lycopene in contrast to all β -cyclases and the lettuce ε -cyclase (Phillip & Young 1995) which is in agreement with th cyclizing both ends of the linear lycopene in contrast to
all β -cyclases and the lettuce ε -cyclase (Phillip & Young
1995), which is in agreement with the activity of the
cyclases expressed in *Escherichia coli* (Cu all β -cyclases and the lettuce ε -cyclase (Phillip & Young 1995), which is in agreement with the activity of the cyclases expressed in *Escherichia coli* (Cunningham *et al.* 1996). Mutations have been identified th cyclases expressed in *Escherichia coli* (Cunningham *et al.* 1996). Mutations have been identified that apparently disrupt the activity of the ε -cyclase enzyme in *Arabidopsis* (*lut2*), *Scenedesmus* (C-2A-34) and *C* 1996). Mutations have been identified that apparently disrupt the activity of the ε -cyclase enzyme in *Arabidopsis* t2), *Scenedesmus* (C-2A-34) and *Chlamydomonas* (lor1) (see
ple 1).
The ε -cyclase is believed to be a key rate-limiting step,
sed on in viva and in vitra studies (Cunningham et al. 1996;

table 1).
The ε-cyclase is believed to be a key rate-limiting step,
based on *in vivo* and *in vitro* studies (Cunningham *et al.* 1996;
Pogson *et al.* 1996) In vivo the *lut*? mutation results in lutein The *ε*-cyclase is believed to be a key rate-limiting step,
based on *in vivo* and *in vitro* studies (Cunningham *et al.* 1996;
Pogson *et al.* 1996). *In vivo*, the *lut2* mutation results in lutein
being replaced by an based on *in vivo* and *in vitro* studies (Cunningham *et al.* 1996;
Pogson *et al.* 1996). *In vivo*, the *lut2* mutation results in lutein
being replaced by an equimolar increase in β, β xantho-
phylls specifically v Pogson *et al.* 1996). *In vivo*, the *lut2* mutation results in lutein
being replaced by an equimolar increase in β, β xantho-
phylls, specifically violaxanthin and antheraxanthin.

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violaxanthin de-epoxidase; NS, neoxanthin synthase.
Heterozygotes are not completely recessive, that is lutein Figure 1. Carotenoid biosynthetic pathway in higher plants. Biosynthetic mutations, *lut1*, *lut2*, *aba1* and *npq1* are shown. Figure 1. Carotenoid biosynthetic pathway in higher plants. Biosynthetic mutations, *lut1, lut2, aba1* and mpq1 are shown.
βLCY, β-cyclase; εLCY, ε-cyclase; βOHase, β-hydroxylase; εOHase, ε-hydroxylase; ZE, zeaxanthin epox Figure 1. Carotenoid biosynthetic pathway in higher p
βLCY, β-cyclase; εLCY, ε-cyclase; βOHase, β-hydroxy
violaxanthin de-epoxidase; NS, neoxanthin synthase.

Heterozygotes are not completely recessive, that is lutein
is slightly reduced, which is consistent with limiting levels
of the s-cyclase (Pogson et al. 1996) Sustained changes in Heterozygotes are not completely recessive, that is lutein
is slightly reduced, which is consistent with limiting levels
of the *ε*-cyclase (Pogson *et al.* 1996). Sustained changes in
light intensity can alter flux down t is slightly reduced, which is consistent with limiting levels
of the ε -cyclase (Pogson *et al.* 1996). Sustained changes in
light intensity can alter flux down the two branches,
which reflects both changes in photosys of the ε -cyclase (Pogson *et al.* 1996). Sustained changes in xanthophylls, neoxanthin and violaxanthin (Rissler & light intensity can alter flux down the two branches, Pogson 2000). However, this strategy did not eli light intensity can alter flux down the two branches,
which reflects both changes in photosystem composition
(Anderson & Osmond 1987; Senger *et al.* 1993; Young
1993*a*: Sonoike 1998) and possibly regulation of secyclase which reflects both changes in photosystem composition
(Anderson & Osmond 1987; Senger *et al.* 1993; Young
1993*a*; Sonoike 1998) and possibly regulation of ε -cyclase
enzyme mRNA (Hirschberg *et al.* 1997) If the lat (Anderson & Osmond 1987; Senger *et al.* 1993; Young 1993*a*; Sonoike 1998) and possibly regulation of ε -cyclase enzyme mRNA (Hirschberg *et al.* 1997). If the latter is the case then genetic manipulation of ε -cyc 1993a; Sonoike 1998) and possibly regulation of ε -cyclase This is being further investigated by using the *lut1* and enzyme mRNA (Hirschberg *et al.* 1997). If the latter is *lut2* mutations in combination with the an the case, then genetic manipulation of ε -cyclase mRNA branches.

(ii) *Hydroxylation reactions* an ε-hydroxylase and a β-hydroxylase based on the *lut1* It is presumed that there are two hydroxylase enzymes,
an ε -hydroxylase and a β -hydroxylase based on the *lutl*
mutation and isotope labelling studies, although only the
 β -hydroxylase has been cloned (Milborrow an ε -hydroxylase and a β -hydroxylase based on the *lutl*
mutation and isotope labelling studies, although only the
 β -hydroxylase has been cloned (Milborrow *et al.* 1982;
Porson *et al.* 1996; Sun *et al.* 1996) mutation and isotope labelling studies, although only the β -hydroxylase has been cloned (Milborrow *et al.* 1982; Pogson *et al.* 1996; Sun *et al.* 1996). There have been no renorts of herbicides specific for the hydro \Box β -hydroxylase has been cloned (Milborrow *et al.* 1982;
 \Box Pogson *et al.* 1996; Sun *et al.* 1996). There have been no

— reports of herbicides specific for the hydroxylation reac-Pogson *et al.* 1996; Sun *et al.* 1996). There have been no reports of herbicides specific for the hydroxylation reactions and only one mutation has been reported that clearly discuss one of the hydroxylation reactions—t reports of herbicides specific for the hydroxylation reactions and only one mutation has been reported that
clearly disrupts one of the hydroxylation reactions—the
lutl mutation of *Arabidobsis*, which blocks s-ring hydrox tions and only one mutation has been reported that
clearly disrupts one of the hydroxylation reactions—the
lut1 mutation of *Arabidopsis*, which blocks ε-ring hydroxy-
lation (Pogson *et al.* 1996) Celearly disrupts one of the hydroxylation reactions—the *C lut1* mutation of *Arabidopsis*, which blocks ε -ring hydroxylation (Pogson *et al.* 1996).

One possible explanation for the lack of β -hydroxylase lation (Pogson *et al.* 1996).
One possible explanation for the lack of β-hydroxylase mutations is that there are multiple β-hydroxylase genes, which appears to be the case in *Arabidotsis* and *Cohsicum* One possible explanation for the lack of β -hydroxylase mutations is that there are multiple β -hydroxylase genes, the value of the case in *Arabidopsis* and *Capsicum* cannum (Bouvier et al. 1996; Sun et al. 1996; Ti mutations is that there are multiple β-hydroxylase genes,
which appears to be the case in *Arabidopsis* and *Capsicum*
annum (Bouvier *et al.* 1996; Sun *et al.* 1996; Tian *et al.* 1999).
The alternative explanation is which appears to be the case in *Arabidopsis* and *Capsicum* annum (Bouvier et al. 1996; Sun et al. 1996; Tian et al. 1999). The alternative explanation is that the ε -hydroxylase retains some β -hydroxylase activity $\frac{1}{2}$ annum (Bouvier *et al.* 1996; Sun *et al.* 1996; Tian *et al.* 1999).
The alternative explanation is that the ε-hydroxylase retains some β-hydroxylase activity providing functional redundancy. A number of the c The alternative explanation is that the ε -hydroxylase retains some β -hydroxylase activity providing functional
redundancy. A number of the carotenoid enzymes theo-
retically arose from gene duplications and divergence and
some still retain the enzymatic activity of the an redundancy. A number of the carotenoid enzymes theoretically arose from gene duplications and divergence and
some still retain the enzymatic activity of the ancestral
protein (Bouvier et al. 1994). We expressed an antisen retically arose from gene duplications and divergence and
some still retain the enzymatic activity of the ancestral
protein (Bouvier *et al.* 1994). We expressed an antisense protein (Bouvier *et al.* 1994). We expressed an antisense *Phil. Trans. R. Soc. Lond.* B (2000)

^b-hydroxylase transgene in *A. thaliana* with the result of β -hydroxylase transgene in *A. thaliana* with the result of effectively reducing the levels of β -carotene-derived
xanthophylls, neoxanthin and violaxanthin (Rissler & β -hydroxylase transgene in *A. thaliana* with the result of effectively reducing the levels of β -carotene-derived xanthophylls, neoxanthin and violaxanthin (Rissler & Porson 2000). However, this strategy did not eli effectively reducing the levels of β-carotene-derived
xanthophylls, neoxanthin and violaxanthin (Rissler &
Pogson 2000). However, this strategy did not eliminate
the β-bydroxylation reactions which lends support to the xanthophylls, neoxanthin and violaxanthin (Rissler & Pogson 2000). However, this strategy did not eliminate
the β-hydroxylation reactions, which lends support to the
thesis of functional redundancy by the s-hydroxylase Pogson 2000). However, this strategy did not eliminate
the β -hydroxylation reactions, which lends support to the
thesis of functional redundancy by the ε -hydroxylase.
This is being further investigated by using the the β -hydroxylation reactions, which lends support to the thesis of functional redundancy by the ϵ -hydroxylase.
This is being further investigated by using the *lut1* and *lut2* mutations in combination with the ant thesis of functional redundancy by the ε -hydroxylase.
This is being further investigated by using the *lutl* and *lut2* mutations in combination with the antisense- β -hydroxylase.

(b) *Photosystem assembly and photoprotection*

Photosystem II consists of a core complex including (b) *Photosystem assembly and photoprotection*
Photosystem II consists of a core complex including
CP43, CP47, Dl/D2 encircled by the antenna, comprising
an inner ring of minor light-harvesting complexes Photosystem II consists of a core complex including
CP43, CP47, Dl/D2 encircled by the antenna, comprising
an inner ring of minor light-harvesting complexes
(LHCs) surrounded by trimers of the major LHC IIb CP43, CP47, Dl/D2 encircled by the antenna, comprising
an inner ring of minor light-harvesting complexes
(LHCs) surrounded by trimers of the major LHC IIb
(Lhch^{1/9)} (Dreyfuss & Thornber 1994: Jansson 1994). In an inner ring of minor light-harvesting complexes (LHCs) surrounded by trimers of the major LHC IIb (Lhcbl/2) (Dreyfuss & Thornber 1994; Jansson 1994). In (LHCs) surrounded by trimers of the major LHC IIb (Lhcbl/2) (Dreyfuss & Thornber 1994; Jansson 1994). In brief, β -carotene is bound to Dl/D2 and the xanthophylls to the antenna proteins. The lethal photobleaching in th (Lhcbl/2) (Dreyfuss & Thornber 1994; Jansson 1994). In
brief, β -carotene is bound to Dl/D2 and the xanthophylls
to the antenna proteins. The lethal photobleaching in the
absence of cyclic carotenoids in plants was thou brief, β -carotene is bound to Dl/D2 and the xanthophylls
to the antenna proteins. The lethal photobleaching in the
absence of cyclic carotenoids in plants was thought to reflect
the critical role of β -carotene, in t to the antenna proteins. The lethal photobleaching in the absence of cyclic carotenoids in plants was thought to reflect the critical role of β -carotene in the reaction centre. This is based on the fundamental photopro absence of cyclic carotenoids in plants was thought to reflect
the critical role of β -carotene in the reaction centre.
This is based on the fundamental photoprotective role of
 β -carotene in triplet quenching in bact the critical role of β -carotene in the reaction centre.
This is based on the fundamental photoprotective role of β -carotene in triplet quenching in bacteria, which
requires the carotenoid to be in Van der Waals cont This is based on the fundamental photoprotective role of β -carotene in triplet quenching in bacteria, which requires the carotenoid to be in Van der Waals contact β -carotene in triplet quenching in bacteria, which
requires the carotenoid to be in Van der Waals contact
(*ca.* 0.4 Å) to enable the electron exchange mechanism of
triplet quenching (Kripsky 1971) However in plants th requires the carotenoid to be in Van der Waals contact $(a, 0.4 \text{ Å})$ to enable the electron exchange mechanism of triplet quenching (Krinsky 1971). However, in plants the oxidative potential of the chlorophyll special pai $(ca. 0.4 \text{ Å})$ to enable the electron exchange mechanism of
triplet quenching (Krinsky 1971). However, in plants the
oxidative potential of the chlorophyll special pair, P_{680} is
such that it would oxidize any caroteno triplet quenching (Krinsky 1971). However, in plants the oxidative potential of the chlorophyll special pair, P_{680} is such that it would oxidize any carotenoids in close proximity. In fact, instead of triplet quenchin oxidative potential of the chlorophyll special pair, P_{680} is
such that it would oxidize any carotenoids in close
proximity. In fact, instead of triplet quenching, a cationic
 β -carotene may donate an electron to the such that it would oxidize any carotenoids in close proximity. In fact, instead of triplet quenching, a cationic β -carotene may donate an electron to the oxidized special pair, P_{680}^+ , and thereby provide a limited form of photoprotection perhaps via electron transp β-carotene may donate an electron to the oxidized special
pair, P_{680}^+ , and thereby provide a limited form of
photoprotection perhaps via electron transport involving
chl and Cyt b (Hanley et al. 1999: Vectros et al. pair, P_{680}^+ , and thereby provide a limited form of photoprotection perhaps via electron transport involving chl_z and Cyt b_{559} (Hanley *et al.* 1999; Vrettos *et al.* 1999).

Table 1. *Xanthophyll and NPQ mutations*

(References are Koornneef *et al*. (1982); Bishop (1996), Marin *et al*. (1996), Pogson *et al*. (1996), Niyogi *et al*. (1997, 1998), Li **Fable 1. Additionally**

(References are *et al.* (2000).)

aIndicates mutations whose genetic identifications are not confirmed. Alternative names are given in parentheses.

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If β -carotene is not involved in quenching of singlet or triplet excited chlorophyll, there is the prospect of a more If β -carotene is not involved in quenching of singlet or
triplet excited chlorophyll, there is the prospect of a more
fundamental role for the xanthophylls in these processes.
However, there are no xanthophylls proxima triplet excited chlorophyll, there is the prospect of a more
fundamental role for the xanthophylls in these processes.
However, there are no xanthophylls proximal to P_{680} .
Instead, they are located in the LHCs and the However, there are no xanthophylls proximal to P_{680} .
Instead, they are located in the LHCs and the inner
antenna proteins, CP43 and CP47, where they could
provide protection by quenching singlet and triplet Instead, they are located in the LHCs and the inner
antenna proteins, CP43 and CP47, where they could
provide protection by quenching singlet and triplet
excited states of chlorophyll. As yet, no one has had antenna proteins, CP43 and CP47, where they could
provide protection by quenching singlet and triplet
excited states of chlorophyll. As yet, no one has had
access to an oxygenic photosynthetic organism lacking all provide protection by quenching singlet and triplet excited states of chlorophyll. As yet, no one has had access to an oxygenic photosynthetic organism lacking all vanthophylls so it has not been possible to define the excited states of chlorophyll. As yet, no one has had
access to an oxygenic photosynthetic organism lacking all
xanthophylls so it has not been possible to define the
respective functions of xanthophylls and B-carotene in access to an oxygenic photosynthetic organism lacking all
xanthophylls so it has not been possible to define the
respective functions of xanthophylls and β -carotene in
plants plants. respective functions of xanthophylls and β -carotene in plants.
In vitro reconstitution studies have shown that xantho-

plants.
 In vitro reconstitution studies have shown that xantho-

phylls are required for folding and stability of the LHCs

including I hcbl/2. I hcb4. (CP29) and I hcb5. (CP26) In vitro reconstitution studies have shown that xantho-
phylls are required for folding and stability of the LHCs
including Lhcb1/2, Lhcb4 (CP29) and Lhcb5 (CP26)
(Plumley & Schmidt 1987: Giuffra et al. 1996: Ros et al. phylls are required for folding and stability of the LHCs
including Lhcbl/2, Lhcb4 (CP29) and Lhcb5 (CP26)
(Plumley & Schmidt 1987; Giuffra *et al.* 1996; Ros *et al.*
1998). The binding site for lutein that is essential f (Plumley & Schmidt 1987; Giuffra *et al.* 1996; Ros *et al.* 1998). The binding site for lutein that is essential for folding and a second site that can bind either violaxanthin or neovanthin have recently been identified 1998). The binding site for lutein that is essential for folding and a second site that can bind either violaxanthin or neoxanthin have recently been identified (Bassi *et al.* 1999). In the green alga *Scenedesmus*, the or neoxanthin have recently been identified (Bassi *et al.* induces the xanthophyll cycle and the protonation of two 1999). In the green alga *Scenedesmus*, the absence of lutein glutamate residues of Lhcbl/2 (Walters *et* 1999). In the green alga *Scenedesmus*, the absence of lutein gresults in reduced LHCs and, as a consequence, an C increase in the chlorophyll a/b ratio (Heinze *et al.* 1997). A N similar but less propounced trend is ob results in reduced LHCs and, as a consequence, an increase in the chlorophyll a/b ratio (Heinze *et al.* 1997). A similar, but less pronounced, trend is observed in the *Chlamydomanas* orthologue *lorl* supporting the vi increase in the chlorophyll a/b ratio (Heinze *et al.* 1997). A similar, but less pronounced, trend is observed in the *Chlamydomonas* orthologue, *lor1*, supporting the view that lutein has been evolutionarily conserved similar, but less pronounced, trend is observed in the *Chlamydomonas* orthologue, *lor1*, supporting the view that lutein has been evolutionarily conserved in all green plants due to a fundamental role in LHC assembly *Chlamydomonas* orthologue, *lorl*, supporting the view that lutein has been evolutionarily conserved in all green plants due to a fundamental role in LHC assembly (Chunaey et al. 1991). However in higher plants eliminalutein has been evolutionarily conserved in all green
plants due to a fundamental role in LHC assembly
(Chunaev *et al.* 1991). However, in higher plants, elimination of lutein has little, if any, effect on growth and develop-(Chunaev *et al.* 1991). However, in higher plants, elimination of lutein has little, if any, effect on growth and development (Pogson *et al.* 1996, 1998). Thus, despite evidence that lutein is more effective than other v tion of lutein has little, if any, effect on growth and development (Pogson *et al.* 1996, 1998). Thus, despite evidence that lutein is more effective than other xanthophylls in reconstitution studies its implied identifi ment (Pogson *et al.* 1996, 1998). Thus, despite evidence that lutein is more effective than other xanthophylls in reconstitution studies, its implied identification in the crystal structure of the LHC and its evolutionar In that lutein is more effective than other xanthophylls in \bigcup reconstitution studies, its implied identification in the \bigcap crystal structure of the LHC and its evolutionary conserreconstitution studies, its implied identification in the crystal structure of the LHC and its evolutionary conservation (Plumley & Schmidt 1987; Kuhlbrandt *et al.* 1994), it is not essential *in nine* (Pogson *et al.* 19 crystal structure of the LHC and its evolutionary conservation (Plumley & Schmidt 1987; Kuhlbrandt *et al.* 1994), it is not essential *in vivo* (Pogson *et al.* 1996). This does not show that lutein is dispensable but rat vation (Plumley & Schmidt 1987; Kuhlbrandt *et al.* 1994), it is not essential *in vivo* (Pogson *et al.* 1996). This does not show that lutein is dispensable but rather that it is replaceable by vanthophylls with similar it is not essential *in vivo* (Pogson *et al.* 1996). This does not show that lutein is dispensable but rather that it is replaceable by xanthophylls with similar chemical composition and structure. The replacement of the show that lutein is dispensable but rather that it is
replaceable by xanthophylls with similar chemical
composition and structure. The replacement of the wild-
type xanthophylls (lutein violaxanthin and neoxanthin) replaceable by xanthophylls with similar chemical
composition and structure. The replacement of the wild-
type xanthophylls (lutein, violaxanthin and neoxanthin)
by zeaxanthin in the double mutant lwt^2 abal caused composition and structure. The replacement of the wild-
type xanthophylls (lutein, violaxanthin and neoxanthin)
by zeaxanthin in the double mutant, *lut2 abal*, caused
delayed greening with high (about one-third) seedling type xanthophylls (lutein, violaxanthin and neoxanthin)
by zeaxanthin in the double mutant, *lut2 abal*, caused
delayed greening with high (about one-third) seedling
mortality and reduced NPO (Porson et al. 1998) Yet, the by zeaxanthin in the double mutant, *lut2 abal*, caused delayed greening with high (about one-third) seedling mortality and reduced NPQ (Pogson *et al.* 1998). Yet, the plants are still viable and the photosynthetic rate a delayed greening with high (about one-third) seedling
mortality and reduced NPQ (Pogson *et al.* 1998). Yet, the
plants are still viable and the photosynthetic rate and
chlorophyll *a*th ratio approach wild-type levels i mortality and reduced NPQ (Pogson *et al.* 1998). Yet, the plants are still viable and the photosynthetic rate and chlorophyll a/b ratio approach wild-type levels in mature leaves. Apparently, there is a substantial degr plants are still viable and the photosynthetic rate and
chlorophyll a/b ratio approach wild-type levels in mature
leaves. Apparently, there is a substantial degree of func-*Phil. Trans. R. Soc. Lond.* B (2000)
Phil. Trans. R. Soc. Lond. B (2000)

tional complementarity between structurally similar tional complementarity between structurally similar
xanthophylls (Pogson *et al.* 1998). The question remains as
to what are the limits of this canacity for carotenoids to tional complementarity between structurally similar
xanthophylls (Pogson *et al.* 1998). The question remains as
to what are the limits of this capacity for carotenoids to
substitute for each other? xanthophylls (Pogson *et al.* 1
to what are the limits of th
substitute for each other? **(c)** *Non-photochemical quenching*

In addition to their roles in assembly, some xantho-(c) **Non-photochemical quenching**
In addition to their roles in assembly, some xantho-
phylls are involved in photoprotection by quenching
chlorophyll fluorescence via non-radiative dissination in a In addition to their roles in assembly, some xantho-
phylls are involved in photoprotection by quenching
chlorophyll fluorescence via non-radiative dissipation in a
process referred to as non-photochemical quenching chlorophyll fluorescence via non-radiative dissipation in a process referred to as non-photochemical quenching (NPQ) (Demmig-Adams & Adams 1992; Frank *et al.* 1994; Gruszecki *et al.* 1999). In fact, the distinct roles of individual vanthonbulls are evidenced in the high light-(NPQ) (Demmig-Adams & Adams 1992; Frank *et al.* 1994; Gruszecki *et al.* 1999). In fact, the distinct roles of individual xanthophylls are evidenced in the high light-
induced accumulation of 'quenching' canotenoids zeav 1994; Gruszecki *et al.* 1999). In fact, the distinct roles of individual xanthophylls are evidenced in the high light-
induced accumulation of 'quenching' carotenoids, zeax-
anthin and anther vanishing by the de-enoxidat individual xanthophylls are evidenced in the high light-
induced accumulation of 'quenching' carotenoids, zeax-
anthin and antheraxanthin, by the de-epoxidation of
violaxanthin via the xanthophyll cycle (Yamamoto 1962induced accumulation of 'quenching' carotenoids, zeax-
anthin and antheraxanthin, by the de-epoxidation of
violaxanthin via the xanthophyll cycle (Yamamoto 1962;
Nivori 1999). Exposure to high photon flux densities anthin and antheraxanthin, by the de-epoxidation of
violaxanthin via the xanthophyll cycle (Yamamoto 1962;
Niyogi 1999). Exposure to high photon flux densities
results in an acidification of the thylakoid lumen, which violaxanthin via the xanthophyll cycle (Yamamoto 1962;
Niyogi 1999). Exposure to high photon flux densities
results in an acidification of the thylakoid lumen, which
induces the xanthophyll cycle and the protonation of two Niyogi 1999). Exposure to high photon flux densities
results in an acidification of the thylakoid lumen, which
induces the xanthophyll cycle and the protonation of two
glutamate residues of Lhchl/2 (Walters *et al.* 1996; results in an acidification of the thylakoid lumen, which
induces the xanthophyll cycle and the protonation of two
glutamate residues of Lhcbl/2 (Walters *et al.* 1996;
Gilmore *et al.* 1998; Kramer *et al.* 1999) Collecti induces the xanthophyll cycle and the protonation of two
glutamate residues of Lhcbl/2 (Walters *et al.* 1996;
Gilmore *et al.* 1998; Kramer *et al.* 1999). Collectively,
NPO leads to non-radiative quenching of singlet chl glutamate residues of Lhcbl/2 (Walters *et al.* 1996;
Gilmore *et al.* 1998; Kramer *et al.* 1999). Collectively,
NPQ leads to non-radiative quenching of singlet chloro--
phyll fluorescence thereby protecting against the Gilmore *et al.* 1998; Kramer *et al.* 1999). Collectively, NPQ leads to non-radiative quenching of singlet chloro-
phyll fluorescence, thereby protecting against the generation of chlorophyll triplets and thus oxidative damage phyll fluorescence, thereby protecting against the generation of chlorophyll triplets and thus oxidative damage
from singlet oxygen and other reactive oxygen species
(Nivogi 1999) tion of chlorop
from singlet ox
(Niyogi 1999).
The function Im singlet oxygen and other reactive oxygen species
Iiyogi 1999).
The function of the xanthophyll cycle is consistent with
the predicted excited singlet, state energy levels (S.)

(Niyogi 1999).
The function of the xanthophyll cycle is consistent with
the predicted excited singlet state energy levels (S_1) derived from the energy gap law for each of the carote-
noids. Neoxanthin and violaxanthin have predicted S_1 the predicted excited singlet state energy levels (S_1)
derived from the energy gap law for each of the carote-
noids. Neoxanthin and violaxanthin have predicted S_1
energy levels higher than that for chlorophyll $(Q_X$ noids. Neoxanthin and violaxanthin have predicted S_1
energy levels higher than that for chlorophyll $(Q_X \text{ and } Q_Y)$ and, accordingly, these molecules can effectively
transfer excitation energy to chlorophyll (Gruszecki *e* energy levels higher than that for chlorophyll $(Q_X$ and $Q_Y)$ and, accordingly, these molecules can effectively transfer excitation energy to chlorophyll (Gruszecki *et al.* 1999). Recently, the S₁ fluorescence spectra transfer excitation energy to chlorophyll (Gruszecki *et al.* 1999). Recently, the S₁ fluorescence spectra for violax-
anthin and zeaxanthin were determined and the S₁ energy 1999). Recently, the S_1 fluorescence spectra for violax-
anthin and zeaxanthin were determined and the S_1 energy
levels calculated by this method support the role of
violaxanthin in resonance energy transfer to chlo anthin and zeaxanthin were determined and the S_1 energy
levels calculated by this method support the role of
violaxanthin in resonance energy transfer to chlorophyll
(Frank et al. 2000). Conversely, the predicted S, st levels calculated by this method support the role of
violaxanthin in resonance energy transfer to chlorophyll
(Frank *et al.* 2000). Conversely, the predicted S₁ state of
zeavanthin is lower than that of chlorophyll theo violaxanthin in resonance energy transfer to chlorophyll
(Frank *et al.* 2000). Conversely, the predicted S_1 state of
zeaxanthin is lower than that of chlorophyll, theoretically
promoting fluorescence, quenching (Frank (Frank *et al.* 2000). Conversely, the predicted S_1 state of zeaxanthin is lower than that of chlorophyll, theoretically promoting fluorescence quenching (Frank *et al.* 1994, 2000). The photophysical properties of lut zeaxanthin is lower than that of chlorophyll, theoretically
promoting fluorescence quenching (Frank *et al.* 1994,
2000). The photophysical properties of lutein and anther-
axanthin would theoretically enable either light promoting fluorescence quenching (Frank *et al.* 1994, 2000). The photophysical properties of lutein and anther-
axanthin would theoretically enable either light harvesting
or quenching (Frank *et al.* 1994). In fact, the 2000). The photophysical properties of lutein and anther-
axanthin would theoretically enable either light harvesting
or quenching (Frank *et al.* 1994). In fact, the recent direct
measurement of the xanthophyll S, energy axanthin would theoretically enable either light harvesting
or quenching (Frank *et al.* 1994). In fact, the recent direct
measurement of the xanthophyll S_1 energy states and their

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corresponding spectral overlap with the excited singlet state of chlorophyll would suggest that either violaxanthin corresponding spectral overlap with the excited singlet
state of chlorophyll would suggest that either violaxanthin
or zeaxanthin can harvest or quench (Frank *et al.* 2000).
However, this is clearly not the case *in nine* state of chlorophyll would suggest that either violaxanthin
or zeaxanthin can harvest or quench (Frank *et al.* 2000).
However, this is clearly not the case *in vivo*, where violax-
anthin and zeaxanthin have defined roles or zeaxanthin can harvest or quench (Frank *et al.* 2000).
However, this is clearly not the case *in vivo*, where violax-
anthin and zeaxanthin have defined roles in impeding
and facilitating quenching respectively. Factor However, this is clearly not the case *in vivo*, where violax-
anthin and zeaxanthin have defined roles in impeding
and facilitating quenching, respectively. Factors other
than just the photophysical properties of the caro anthin and zeaxanthin have defined roles in impeding
and facilitating quenching, respectively. Factors other
than just the photophysical properties of the carotenoids
must be involved (Horton *et al.* 1996). The roles of and facilitating quenching, respectively. Factors other
than just the photophysical properties of the carotenoids
must be involved (Horton *et al.* 1996). The roles of lutein
and antheraxanthin *in nino* are still being cl than just the photophysical properties of the carotenoids
must be involved (Horton *et al.* 1996). The roles of lutein
and antheraxanthin *in vivo* are still being clarified
(Gilmore 2000: Gilmore *et al.* 1998: Pogson *e* must be involved (Horton *et al.* 1996). The roles of lutei
and antheraxanthin *in vivo* are still being clarifie
(Gilmore 2000; Gilmore *et al.* 1998; Pogson *et al.* 1998).
One of the ways that the mechanisms of NPO is b d antheraxanthin *in vivo* are still being clarified

illmore 2000; Gilmore *et al.* 1998; Pogson *et al.* 1998).

One of the ways that the mechanisms of NPQ is being

usidated is by analysing quenching in carotenoid bios

(Gilmore 2000; Gilmore *et al.* 1998; Pogson *et al.* 1998).
One of the ways that the mechanisms of NPQ is being
elucidated is by analysing quenching in carotenoid biosyn-
thetic and NPO mutants of *Chlamydomonas* and *Ar* One of the ways that the mechanisms of *NPQ* is being
elucidated is by analysing quenching in carotenoid biosyn-
thetic and *NPQ* mutants of *Chlamydomonas* and *Arabidopsis*
(table 1) *rhal* is a lesion in the violaxanthi elucidated is by analysing quenching in carotenoid biosynthetic and NPQ mutants of *Chlamydomonas* and *Arabidopsis*
(table 1). $npql$ is a lesion in the violaxanthin de-epoxidase thetic and NPQ mutants of *Chlamydomonas* and *Arabidopsis*
(table 1). $npql$ is a lesion in the violaxanthin de-epoxidase
and exhibits markedly reduced NPQ, presumably due to
the lack of antheraxanthin and zeavanthin where (table 1). $npqI$ is a lesion in the violaxanthin de-epoxidase
and exhibits markedly reduced NPQ, presumably due to
the lack of antheraxanthin and zeaxanthin, whereas $npq4$,
which recently has been identified as a lesion i the lack of antheraxanthin and zeaxanthin, whereas $npq4$, which recently has been identified as a lesion in PsbS (CP22), has almost no NPQ yet still accumulates antherwhich recently has been identified as a lesion in PsbS (CP22), has almost no NPQ yet still accumulates anther-
axanthin and zeaxanthin (Niyogi *et al.* 1998; Li *et al.* 2000). The *npq* mutants are discussed in detail els axanthin and zeaxanthin (Niyogi *et al.* 1998; Li *et al.* 2000). The mpq mutants are discussed in detail elsewhere (Baroli & Niyogi, this issue). In brief, insight from such studies supports a major role for zeaxanthin 2000). The npq mutants are discussed in detail elsewhere (Baroli & Niyogi, this issue). In brief, insight from such studies supports a major role for zeaxanthin and PsbS in the quenching process (Baroli & Niyogi, this is
studies supports a major
the quenching process.
Significantly, that mu redies supports a major role for zeaxanthin and PsbS in

e quenching process.

Significantly, *mpq1* mutants still had some reversible

PO, which could be eliminated by preventing lutein

the quenching process.

Significantly, $npql$ mutants still had some reversible

NPQ, which could be eliminated by preventing lutein Significantly, *npql* mutants still had some reversible NPQ, which could be eliminated by preventing lutein biosynthesis (Niyogi *et al.* 2000). This supports our earlier observations that the lutein-deficient lines of *A* NPQ, which could be eliminated by preventing lutein
biosynthesis (Niyogi *et al.* 2000). This supports our earlier
observations that the lutein-deficient lines of *A. thaliana,*
lutl and *lut*? exhibit a delayed inductio biosynthesis (Niyogi *et al.* 2000). This supports our earlier
observations that the lutein-deficient lines of *A. thaliana*,
lut1 and *lut2*, exhibit a delayed induction of NPQ kinetics
as well as a lower maximal NPO (P observations that the lutein-deficient lines of *A. thaliana,*
lutl and *lut2*, exhibit a delayed induction of NPQ kinetics
as well as a lower maximal NPQ (Pogson *et al.* 1998). The
induction kinetics of NPO can be acce *lutl* and *lut2*, exhibit a delayed induction of NPQ kinetics
as well as a lower maximal NPQ (Pogson *et al.* 1998). The
induction kinetics of NPQ can be accelerated in constitu-
tive zeavanthin-accumulating mutants, abo as well as a lower maximal NPQ (Pogson *et al.* 1998). The induction kinetics of NPQ can be accelerated in constitu-
tive zeaxanthin-accumulating mutants, *abal* and *lut2 abal*, despite a reduced level of maximum NPQ and tive zeaxanthin-accumulating mutants, *abal* and *lut2 abal*, ve
despite a reduced level of maximum NPQ and a loss of clum
LHC IIb trimers (Tardy & Havaux 1996; Hurry *et al.* 1997; Pogson *et al.* 1998). Analyses of carot LHC IIb trimers (Tardy & Havaux 1996; Hurry *et al.* number U58919) in the antisense orientation in the same vector 1997; Pogson *et al.* 1998). Analyses of carotenoid and NPQ using the same promoter (Rissler & Pogson 200 1997; Pogson *et al.* 1998). Analyses of carotenoid and NPQ using the same promoter (Rissler & Pogson 2000). An *npt* II kanamutants, and the effects of LHC IIb protonation on mycin resistance gene provided for antibiotic mutants, and the effects of LHC IIb protonation on
NPQ, suggest that antenna structure, a functional PsbS
protein and xanthophyll molecules all influence
quenching kinetics. The mechanisms for these inter-NPQ, suggest that antenna structure, a functional PsbS
protein and xanthophyll molecules all influence
quenching kinetics. The mechanisms for these inter-
actions however remain unclear Is there a direct involprotein and xanthophyll molecules all influence
quenching kinetics. The mechanisms for these inter-
actions, however, remain unclear. Is there a direct invol-
yement of 'quenching' carotenoids with PshS and does quenching kinetics. The mechanisms for these inter-
actions, however, remain unclear. Is there a direct involvement of 'quenching' carotenoids with PsbS and does
this include zeavanthin, anthersyanthin and lutein? actions, however, remain unclear. Is there a direct involvement of 'quenching' carotenoids with PsbS and does
this include zeaxanthin, antheraxanthin and lutein?
Alternatively does lutein facilitate changes in antenna vement of 'quenching' carotenoids with PsbS and does
this include zeaxanthin, antheraxanthin and lutein?
Alternatively, does lutein facilitate changes in antenna this include zeaxanthin, antheraxanthin and lutein?
Alternatively, does lutein facilitate changes in antenna
structure that promote quenching and if so how do
antenna proteins and PsbS interact? Alternatively, does lutein facilitate clare
structure that promote quenching a
antenna proteins and PsbS interact?
The carotenoids aid in the cantur ucture that promote quenching and if so how do
tenna proteins and PsbS interact?
The carotenoids aid in the capture of light, provide
otoprotection against excess light and promote antenna

antenna proteins and PsbS interact?
The carotenoids aid in the capture of light, provide
photoprotection against excess light and promote antenna
assembly. Recent work has helped to define how the most The carotenoids aid in the capture of light, provide
photoprotection against excess light and promote antenna
assembly. Recent work has helped to define how the most
abundant carotenoid, lutein, might contribute to these photoprotection against excess light and promote antenna
assembly. Recent work has helped to define how the most
abundant carotenoid, lutein, might contribute to these
processes. However, much remains to be learned. The \Box assembly. Recent work has helped to define how the most \Box abundant carotenoid, lutein, might contribute to these \Box processes. However, much remains to be learned. The abundant carotenoid, lutein, might contribute to these
processes. However, much remains to be learned. The
dual role of xanthophylls in LHC assembly and photo-
protection makes it difficult to design definitive experiprocesses. However, much remains to be learned. The
dual role of xanthophylls in LHC assembly and photo-
protection makes it difficult to design definitive experi-
ments because changes in LHC structure may alter dual role of xanthophylls in LHC assembly and photo-
protection makes it difficult to design definitive experi-
ments because changes in LHC structure may alter
photoprotection. Therefore, we are taking a new **Oprotection makes it difficult to design definitive experi-**

ments because changes in LHC structure may alter

photoprotection. Therefore, we are taking a new coefficients (Porra *et al.* 1989). Chlorophyll fluorescence photoprotection. Therefore, we are taking a new photoprotection. Therefore, we are taking a new
approach to further elucidate the roles of lutein and the
xanthophyll cycle pigments by under- and overexpressing
key genes in the carotenoid biosynthesis pathway Our in approach to further elucidate the roles of lutein and the
xanthophyll cycle pigments by under- and overexpressing
key genes in the carotenoid biosynthesis pathway. Our *in*
i'in approach, which replaces one or more xanth *xanthophyll cycle pigments by under- and overexpressing*
key genes in the carotenoid biosynthesis pathway. Our <i>in
vivo approach, which replaces one or more xanthophylls
with their physiological homologye minimizes pr key genes in the carotenoid biosynthesis pathway. Our *in*
vivo approach, which replaces one or more xanthophylls
with their physiological homologue, minimizes problems
with perturbations to the antenna. These transcenic p *vivo* approach, which replaces one or more xanthophylls
with their physiological homologue, minimizes problems
with perturbations to the antenna. These transgenic plants
are being used to study the regulation of the s-cyc with their physiological homologue, minimizes problems
with perturbations to the antenna. These transgenic plants
are being used to study the regulation of the ε -cyclase with perturbations to the antenna. These transgenic plants
are being used to study the regulation of the ε -cyclase
and β -hydroxylase *in vivo* and to understand the effects of
altered xanthonbyll content on Γ HC are being used to study the regulation of the ε -cyclase
and β -hydroxylase *in vivo* and to understand the effects of
altered xanthophyll content on LHC structure and photo-
protection protection.

Figure 2. Lutein levels in wild-type (col) , $lut2$ and 24 T_2
plants overexpressing the ε -cyclase transgene in a wild-type
background. Pigments were extracted from leaves of two- to
five-week-old plants and quantif plants overexpressing the ε-cyclase transgene in a wild-type
background. Pigments were extracted from leaves of two- to
five-week-old plants and quantified by HPLC. Values are
presented as per cent of wild-type levels for background. Pigments were extracted from leaves of two- to
five-week-old plants and quantified by HPLC. Values are
presented as per cent of wild-type levels for which lutein
content was $168 \text{ mmol mol}^{-1}$ chl a five-week-old plants and quantified by HPLC. Values are presented as per cent of wild-type levels for which lutein content was $168 \text{ mmol mol}^{-1}$ chl *a*.

2. METHODS

(a) *Transformation of* **A. thaliana** *with the sense* ^e*-cyclase and the antisense* ^b*-hydroxylase transgene aation of A. thaliana with*
e antisense β-hydroxyla
and growth of plants
P⁹⁴⁴ wes septimated to sont ε -cyclase and the antisense β -hydroxylase transgene
and growth of plants
The transgene pBP244 was constructed to contain the ε -cyclase

and growth of plants
The transgene pBP244 was constructed to contain the ϵ -cyclase
cDNA in the sense orientation under the control of the cauli-
flavor massic vinus (CoMV) promotes in the port^{7/97} binory The transgene pBP244 was constructed to contain the ε -cyclase
cDNA in the sense orientation under the control of the cauli-
flower mosaic virus (CaMV) promoter in the part7/27 binary
wester (Clasus 1993). The plasmid flower mosaic virus ($CaMV$) promoter in the part7/27 binary
vector (Gleave 1992). The plasmid pBP254 was constructed by cloning the *Arabidopsis* ^b-hydroxylase cDNA (GenBank accession vector (Gleave 1992). The plasmid pBP254 was constructed by
cloning the *Arabidopsis* β -hydroxylase cDNA (GenBank accession
number U58919) in the antisense orientation in the same vector
weing the same promotor (Bissla cloning the *Arabidopsis* β-hydroxylase cDNA (GenBank accession
number U58919) in the antisense orientation in the same vector
using the same promoter (Rissler & Pogson 2000). An *npt* II kana-
music resistance gene provi using the same promoter (Rissler & Pogson 2000). An npt II kanausing the same promoter (Rissler & Pogson 2000). An *npt* II kana-
mycin resistance gene provided for antibiotic selection of the trans-
genic plants (Gleave 1992). Both constructs were independently
transformed into drab mycin resistance gene provided for antibiotic selection of the trans-
genic plants (Gleave 1992). Both constructs were independently
transformed into *Arabidopsis thaliana*, Columbia ecotype and *lut2*,
using the floral di transformed into *Arabidopsis thaliana*, Columbia ecotype and *lut2*,
using the floral dip method followed by vacuum infiltration
(Clough & Bent 1998). Seeds were harvested and transgenic
generation and (T) and lines wer using the floral dip method followed by vacuum infiltration (Clough & Bent 1998). Seeds were harvested and transgenic generation one (T_1) seedlings were selected for kanamycin resis-
tange on Munching Shage media contai generation one (T_1) seedlings were selected for kanamycin resistance on Murashige-Skoog media containing 2% sucrose and generation one (T₁) seedlings were selected for kanamycin resis-
tance on Murashige–Skoog media containing 2% sucrose and
 $30 \,\mu g \text{ ml}^{-1}$ kanamycin. Plants were grown at 21 °C under continu-
sur-light (20 una-lm⁻² -⁻ tance on Murashige-Skoog media containing 2% sucrose and
30 μ g ml⁻¹ kanamycin. Plants were grown at 21 °C under continuous light (80 μ mol m⁻² s⁻¹). T₂ seeds were collected from the ous light $(80 \,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1})$. T₂ seeds were collected from the kanamycin resistant plants and grown in soil under an 18 h lightdark cycle (150 μ mol m⁻²s⁻¹) at 21 °C.

(150 μ mol m⁻²s⁻¹) at 21 °C.
 (b) *Pigment analysis and measuring*
 ablong pull fluorescence chient analysis and measur
chlorophyll fluorescence
extracted from leaves of two-to

 $chlorophyll$ fluorescence
Pigments were extracted from leaves of two- to five-week-old **chlorophyll fluorescence**
Pigments were extracted from leaves of two- to five-week-old
plants and analysed by HPLC chromatography (Hewlett-Packard
conject 1100) as described proviously (Berson at al. 1009). Chloro Pigments were extracted from leaves of two- to five-week-old
plants and analysed by HPLC chromatography (Hewlett-Packard
series 1100) as described previously (Pogson *et al.* 1998). Chloro-
phylloweng quantified by HPLC an plants and analysed by HPLC chromatography (Hewlett-Packard
series 1100) as described previously (Pogson *et al.* 1998). Chloro-
phylls were quantified by HPLC and by using published extinction
coefficients (Dawn at al. 1 series 1100) as described previously (Pogson *et al.* 1998). Chloro-
phylls were quantified by HPLC and by using published extinction
coefficients (Porra *et al.* 1989). Chlorophyll fluorescence measure-
mante and NPO colo coefficients (Porra *et al.* 1989). Chlorophyll fluorescence measurements and NPQ calculations were performed on intact leaves as described previously (Niyogi *et al.* 1998; Pogson *et al.* 1998). ments and NPQ calculations were performed on intact leaves as

3. RESULTS AND DISCUSSION

(a) *Establishing transgenic plants with a range of carotenoid compositions* (i) *Increased lutein by overexpressing the* ^e*- cyclase*

of carotenoid compositions
Increased lutein by overexpressing the ε -cyclase
We screened wild-type and *lut2* transgenics for
sertion of the BP244 s-cyclase transgene by kanamycin (i) Increased lutein by overexpressing the ε -cyclase
We screened wild-type and $lut2$ transgenics for
insertion of the BP244 ε -cyclase transgene by kanamycin Table 2. *Carotenoid levels in* A. thaliana *leaves overexp ressing the* ^e*-cyclase transgene* (*sense* ^e*-cyclase BP244*)

Table 2. Carotenoid levels in A. thaliana leaves overexpressing the ε-cyclase transgene (sense ε-cyclase BP244)
(Values given are for carotenoid content expressed as mmol mol⁻¹ chlorophyll *a*; *wt*, wild type; *lut2*, Table 2. Carotenoid tevels in A. than and teader overlap ressing the e-typical change (sense
(Values given are for carotenoid content expressed as mmol mol⁻¹ chlorophyll a; wt,
neoxanthin; V, violaxanthin; A, antheraxan

Table 3. *Carotenoid levels in wild-type and* lut2 A. thaliana *leaves expressing the antisense* β -hydroxylase

Table 3. Carotenoid levels in wild-type and lut2 A. thaliana leaves expressing the antisense β -hydroxylase
(Values given are for carotenoid content expressed as mmol mol⁻¹ chlorophyll *a*; wt, wild type; *lut2*, lute Table 3. Carotenoid tevels in with-sight and fut2 A. thanana teaves expressing the antisense p-hydroxylase.
(Values given are for carotenoid content expressed as mmol mol⁻¹ chlorophyll a; wt, wild type; *lut2*, lutein d \Box N, neoxanthin; V, violaxanthin; A, antheraxanthin; Z, zeaxanthin; L, lutein; β , β -carotene; β OHase, β -hydroxylase.)

						∸		total
◠	\sim wt	62 ± 2	59 ± 2			$172 + 18$	91 ± 2	380
	V wt anti-βOHase (BP254-K1) lut2	25 ± 2 41 ± 4	21 ± 3 124 ± 2	25 ± 2	6 ± 2	164 ± 15	111 ± 16 136 ± 2	320 330
	$lut2$ anti- β OHase (BP254-B1)	34 ± 4	$72 + 2$	$22 + 2$	$6 + 2$		162 ± 3	300

resistance and increased lutein content. Significantly, resistance and increased lutein content. Significantly,
lutein accumulation was restored in all transformed *lut2*
lines. The *lut2* mutation has a biochemical phenotype resistance and increased lutein content. Significantly,
lutein accumulation was restored in all transformed *lut2*
lines. The *lut2* mutation has a biochemical phenotype
consistent with a disruption to the s-cyclase i.e. lutein accumulation was restored in all transformed *lut2*
lines. The *lut2* mutation has a biochemical phenotype
consistent with a disruption to the ε -cyclase, i.e. a
complete lack of carotenoids with an ensilon ring lines. The *lut2* mutation has a biochemical phenotype consistent with a disruption to the ε -cyclase, i.e. a complete lack of carotenoids with an epsilon ring (Pogson *et al.* 1996). Additionally, the *lut2* and ε complete lack of carotenoids with an epsilon ring (Pogson complete lack of carotenoids with an epsilon ring (Pogson *et al.* 1996). Additionally, the *lut2* and ε -cyclase loci map to the same region of the genome, that is the bottom of chromosome 5. Thus data from biochemical *et al.* 1996). Additionally, the *lut2* and *ε*-cyclase loci map
to the same region of the genome, that is the bottom of
chromosome 5. Thus, data from biochemical, genetic and
functional complementation experiments all su to the same region of the genome, that is the bottom of chromosome 5. Thus, data from biochemical, genetic and functional complementation experiments all support the conclusion that $h\nu t^2$ is a mutation in the s-cyclase chromosome 5. Thus, data from biochemical, genetic and functional complementation experiments all support the conclusion that $lut2$ is a mutation in the ε -cyclase gene.
Figure 2 expresses lutein content as a percentage functional complementation experiments all support the

conclusion that $lut2$ is a mutation in the ε -cyclase gene.
Figure 2 expresses lutein content as a percentage of wild-type for a range of wild-type plants transformed
with the BP244 s-cyclase transgene. This procedure Figure 2 expresses lutein content as a percentage of wild-type for a range of wild-type plants transformed with the BP244 ε -cyclase transgene. This procedure identified dozens of plants with altered lutein content with wild-type for a range of wild-type plants transformed
with the BP244 ϵ -cyclase transgene. This procedure iden-
tified dozens of plants with altered lutein content with
levels ranging from 10 to 150% of wild-type levels with the BP244 ε -cyclase transgene. This procedure identified dozens of plants with altered lutein content with levels ranging from 10 to 150% of wild-type levels in the T, generation indicating a combination of co-sup tified dozens of plants with altered lutein content with
levels ranging from 10 to 150% of wild-type levels in the
 T_1 generation, indicating a combination of co-suppression
and overexpression. In the T_2 generation, levels ranging from 10 to 150% of wild-type levels in the T_1 generation, indicating a combination of co-suppression
and overexpression. In the T_2 generation, most of the 25
transformed lines segregated 3:1 for kanam T_1 generation, indicating a combination of co-suppression
and overexpression. In the T_2 generation, most of the 25
transformed lines segregated 3:1 for kanamycin resistance, and overexpression. In the T_2 generation, most of the 25
transformed lines segregated 3:1 for kanamycin resistance,
indicating one insertion, some segregated 15:1, indicating
two and lutein content ranged from 5 to 180 transformed lines segregated 3:1 for kanamycin resistance,
indicating one insertion, some segregated 15:1, indicating
two, and lutein content ranged from 5 to 180% of wild-
type, which was the same or higher than the respe indicating one insertion, some segregated 15:1, indicating
two, and lutein content ranged from 5 to 180% of wild-
type, which was the same or higher than the respective
parental T. Interestingly in the T. generation the lu two, and lutein content ranged from 5 to 180% of wild-
type, which was the same or higher than the respective the state in content ranged from 5 to 180% of wild-
h was the same or higher than the respective
. Interestingly, in the T_3 generation the lutein
come high accumulating lines dropped to wildtype, which was the same or higher than the respective
parental T_1 . Interestingly, in the T_3 generation the lutein
content of some high accumulating lines dropped to wild-
type or lower levels, perhaps suggesting a parental T_1 . Interestingly, in the T_3 generation the lutein
content of some high accumulating lines dropped to wild-
type or lower levels, perhaps suggesting a subsequent
gene silencing although this has not been co content of some high accumulating lines dropped to wild-
type or lower levels, perhaps suggesting a subsequent
gene silencing, although this has not been confirmed.
The increase in lutein of up to 180% preferentially type or lower levels, perhaps suggesting a subsequent switched off (dark) at 170s. Open bar, actinic light on; closed

gene silencing, although this has not been confirmed.
The increase in lutein of up to 180% preferentially
resulted in a reduction in violaxanthin content and, in
some lines an increase in the total carotenoid pool The increase in lutein of up to 180% preferentially
resulted in a reduction in violaxanthin content and, in
some lines, an increase in the total carotenoid pool
(table 2) We did not detect a marked change in β -carotene resulted in a reduction in violaxanthin content and, in
some lines, an increase in the total caroteneid pool
(table 2). We did not detect a marked change in β -carotene
or neovanthin and neither α -carotene nor α -c some lines, an increase in the total carotenoid pool Pogson 2000). That is, whenever possible, the altera

(table 2). We did not detect a marked change in β -carotene in levels of one xanthophyll results in a converse c (table 2). We did not detect a marked change in β -carotene
or neoxanthin and neither α -carotene nor α -cryptoxanthin
were detected when flux down either branch of the
pathway was increased which indicates that the or neoxanthin and neither α -carotene nor α -cryptoxanthin
were detected when flux down either branch of the
pathway was increased, which indicates that the hydroxyl-
ation reactions are not limiting. The change in th were detected when flux down either branch of the pathway was increased, which indicates that the hydroxylation reactions are not limiting. The change in the total pool size requires more detailed study to determine if pathway was increased, which indicates that the hydroxylation reactions are not limiting. The change in the total
pool size requires more detailed study to determine if
there is a feedback effect due to the transgene. Many ation reactions are not limiting. The change in the total
pool size requires more detailed study to determine if
there is a feedback effect due to the transgene. Many
studies that have genetically altered vanthonbyll accum pool size requires more detailed study to determine if
there is a feedback effect due to the transgene. Many
studies that have genetically altered xanthophyll accumu-
lation have demonstrated that carotenoid accumulation i there is a feedback effect due to the transgene. Many
studies that have genetically altered xanthophyll accumulation have demonstrated that carotenoid accumulation is tightly regulated (Chunaev *et al*. 1991; Rock & Zeevaart

Figure 3. Non-photochemical quenching of chlorophyll
fluorescence (NPQ) in wild-type, *lut2* and plants expressing
the antisense ⁸ hydroxylage transgene (anti-8OHase) Figure 3. Non-photochemical quenching of chlorophyll
fluorescence (NPQ) in wild-type, $lut2$ and plants express
the antisense β -hydroxylase transgene (anti- β OHase).
I eaves from five-week-old plants were exposed to a fluorescence (NPQ) in wild-type, *lut2* and plants expressing
the antisense β -hydroxylase transgene (anti- β OHase).
Leaves from five-week-old plants were exposed to actinic
light (1000umolm⁻²s⁻¹ bigh light) from the antisense β -hydroxylase transgene (anti- β OHase).
Leaves from five-week-old plants were exposed to actinic
light (1000 µmol m⁻² s⁻¹, high light) from 0 to 170 s with
10.000 µmol m⁻² s⁻¹ pulses every 10 s-Leaves from five-week-old plants were exposed to actinic
light $(1000 \,\mu\text{mol m}^{-2} \text{s}^{-1})$, high light) from 0 to 170s with
 $10\,000 \,\mu\text{mol m}^{-2} \text{s}^{-1}$ pulses every 10s. Actinic light was
switched off $(dark)$ at 170s. Open light (1000µmol m⁻²s⁻¹, high light) from 0 to 170s with
10 000µmol m⁻²s⁻¹ pulses every 10s. Actinic light was
switched off (dark) at 170s. Open bar, actinic light on; closed
bar, actinic light off. NPO was calcula 10 000 µmol m⁻²s⁻¹ pulses every 10s. Actinic light was
switched off (dark) at 170s. Open bar, actinic light on; closed
bar, actinic light off. NPQ was calculated as $(F_m - F'_m)/F'_m$ at
10s intervals bar, actinic light off. NPQ was calculated as $(F_m - F'_m)/F'_m$ at

10s intervals.
Pogson 2000). That is, whenever possible, the alteration in levels of one xanthophyll results in a converse change Pogson 2000). That is, whenever possible, the alteration
in levels of one xanthophyll results in a converse change
in levels of a structurally or functionally similar carotenoid
in preference to a more global affect on all in levels of one xanthophyll results in a converse change
in levels of a structurally or functionally similar carotenoids.
in preference to a more global affect on all carotenoids.

in preference to a more global affect on all carotenoids.
(ii) *Antisense inhibition of the β-hydroxylase:biosynthesis and LHC*
secombly assembly Antisense inhibition of the β -hydroxylase:biosynthesis and LHC
embly
An antisense β -carotene hydroxylase transgene, BP254,
wild-type plants resulted in a maximum reduction in

tightly regulated (Chunaev *et al.* 1991; Rock & Zeevaart both T_1 and T_2 generations (Rissler & Pogson 2000). In 1991; Bishop 1996; Pogson *et al.* 1996, 1998; Rissler & addition, there was a 5% reduction in lutein, An antisense β -carotene hydroxylase transgene, BP254, in wild-type plants resulted in a maximum reduction in An antisense β -carotene hydroxylase transgene, BP254,
in wild-type plants resulted in a maximum reduction in
violaxanthin of *ca*. 64% and in neoxanthin of 41% for
both T and T generations (Rissler & Pogson 2000) In in wild-type plants resulted in a maximum reduction in
violaxanthin of *ca*. 64% and in neoxanthin of 41% for
both T_1 and T_2 generations (Rissler & Pogson 2000). In
addition, there was a 5% reduction in lutein a 22% violaxanthin of *ca*. 64% and in neoxanthin of 41% for
both T_1 and T_2 generations (Rissler & Pogson 2000). In
addition, there was a 5% reduction in lutein, a 22%

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Table 4. *Summary of xanthophyll and NPQ levels in carotenoid biosynthetic mutants and transgenics*

Table 4. Summary of xanthophyll and NPQ levels in carotenoid biosynthetic mutants and transgenics
(The measure for NPQ incorporates both the rapidly reversible $({}^tq_E)$) and slowly reversible (q_1) components. We have Table 4. Sammary of xammophyticana NPQ tevers in carolenoid biosymmetric matants and transgenits

(The measure for NPQ incorporates both the rapidly reversible (iq_E) and slowly reversible (q_1) components. We have es (The measure for NPQ incorporates both the rapidly reversible $({}^4F_E)$ and slowly reversible (q_I) components. We have estimated q_E as maximal NPQ minus NPQ after 30s of dark relaxation $({}^4F_E)$. This illustrates th nearly eliminated in the double mutants, $npq1 lut2$ and $npq1 lort. V + A + Z$ (violaxanthin+antheroxanthin+zeaxanthin); β OHase, β -hydroxylase.)

^a Data derived from Niyogi *et al.* (1997, 2000), Pogson *et al.* (1998) and Rissler & Pogson (2000).

 $\frac{b}{b}$ Not calculated.
increase in β -carotene and a 16% reduction in total increase in β -carotene and a 16% reduction in total carotenoid levels (table 3). The disruption of one step in the pathway resulting in a reduction in the total pool of increase in β -carotene and a 16% reduction in total carotenoid levels (table 3). The disruption of one step in the pathway resulting in a reduction in the total pool of carotenoids is something not observed for *lutl* carotenoid levels (table 3). The disruption of one step in
the pathway resulting in a reduction in the total pool of
carotenoids is something not observed for $lult$, $lult$ or the
abal mutations. In $lult$ there was a marked i the pathway resulting in a reduction in the total pool of explanation for the lower ratio is that there may be an carotenoids is something not observed for *lutl*, *lut2* or the increase in Lhcbl/2 trimers (Rissler & Pogs carotenoids is something not observed for *lutl, lut2* or the *abal* mutations. In *lutl* there was a marked increase in violaxanthin and antheraxanthin, despite the fact that the discussion was after the branch point in t *abal* mutations. In *lutl* there was a marked increase in violaxanthin and antheraxanthin, despite the fact that the disruption was after the branch point in the pathway, implying feedback or sink-driven regulation (Pogso violaxanthin and antheraxanthin, despite the fact that the disruption was after the branch point in the pathway, implying feedback or sink-driven regulation (Pogson *et al.* 1996). However, the antisense hydroxylase plants implying feedback or sink-driven regulation (Pogson *et al.* 1996). However, the antisense hydroxylase plants cannot have a corresponding increase in lutein to compensate for lost violaxanthin and neovanthin as lutein als 1996). However, the antisense hydroxylase plants cannot
have a corresponding increase in lutein to compensate for
lost violaxanthin and neoxanthin as lutein also requires
the B-hydroxylase for its synthesis—instead the onl have a corresponding increase in lutein to compensate for
lost violaxanthin and neoxanthin as lutein also requires
the β -hydroxylase for its synthesis—instead the only
ontion is for an increase in β -carotene. Given lost violaxanthin and neoxanthin as lutein also requires
the β -hydroxylase for its synthesis—instead the only
option is for an increase in β -carotene. Given that caro-
tenes are rarely, if ever, found in LHC complex the β -hydroxylase for its synthesis—instead the only tenes are rarely, if ever, found in LHC complexes, turn-
over of unincorporated β -carotene could be reflected in and a functional PsbS protein (Walters *et al.* 1996; Niyogi the reduction in the total carotenoid pool. er of unincorporated β -carotene could be reflected in
e reduction in the total carotenoid pool.
The limit of 60% reduction in violaxanthin in one-third
the lines could either he the result of post-transcriptional

the reduction in the total carotenoid pool.
The limit of 60% reduction in violaxanthin in one-third
of the lines could either be the result of post-transcriptional
regulation inefficient inhibition of the second putati The limit of 60% reduction in violaxanthin in one-third
of the lines could either be the result of post-transcriptional
regulation, inefficient inhibition of the second putative β -
hydroxylase gene product, or a degree regulation, inefficient inhibition of the second putative β -
hydroxylase gene product, or a degree of functional
redundancy due to the ε -hydroxylase (Rissler & Pogson
2000) Considering the latter the *Arabidotesi* hydroxylase gene product, or a degree of functional
redundancy due to the ε-hydroxylase (Rissler & Pogson
2000). Considering the latter, the *Arabidopsis* β-hydroxylase
enzyme does exhibit some s-hydroxylase activity in redundancy due to the *ε*-hydroxylase (Rissler & Pogson 2000). Considering the latter, the *Arabidopsis* β-hydroxylase
enzyme does exhibit some *ε*-hydroxylase activity in *E. coli*
and *lutl* accumulates some lutein (Pogs 2000). Considering the latter, the *Arabidopsis* β-hydroxylase enzyme does exhibit some ε-hydroxylase activity in *E. coli* enzyme does exhibit some ε -hydroxylase activity in *E. coli*
and *lutl* accumulates some lutein (Pogson *et al.* 1996; Sun
et al. 1996). If the converse occurs, then the ε -hydroxylase
could be hydroxylating 8-ca and *lutl* accumulates some lutein (Pogson *et al.* 1996; Sun *et al.* 1996). If the converse occurs, then the ε -hydroxylase could be hydroxylating β -carotene in the antisense plants.
Currently we are testing the t \Box *et al.* 1996). If the converse occurs, then the ε -hydroxylase \Box could be hydroxylating β -carotene in the antisense plants. \Box Currently, we are testing the three hypotheses by crossing the most severe antisense lines into the *lut1* background to Currently, we are testing the three hypotheses by crossing
the most severe antisense lines into the *lutl* background to
determine whether or not a concomitant decrease in the
 β - and s-bydroxylases will result in a furt the most severe antisense lines into the *lutl* background to
determine whether or not a concomitant decrease in the
 β - and ϵ -hydroxylases will result in a further reduction in
violaxanthin and neovanthin Clearly, m determine whether or not a concomitant decrease in the β - and ϵ -hydroxylases will result in a further reduction in violaxanthin and neoxanthin. Clearly, many questions remain about the regulation of the pathway in v β - and ϵ -hydroxylases will result in a further reduction in violaxanthin and neoxanthin. Clearly, many questions remain about the regulation of the pathway *in vivo*.
Chlorophyll content, Western blots of major LHCs

remain about the regulation of the pathway *in vivo*.
Chlorophyll content, Western blots of major LHCs and
non-denaturing 'green gels' all consistently indicated that
little alteration in antenna composition had occurred i Chlorophyll content, Western blots of major LHCs and
non-denaturing 'green gels' all consistently indicated that
little alteration in antenna composition had occurred in
the antisense B-hydroxylase plants (Rissler & Pogson non-denaturing 'green gels' all consistently indicated that de-epoxidase mutation, reversible quenching was reduced
little alteration in antenna composition had occurred in but never eliminated (Gilmore & Yamamoto 1991; N $\frac{1}{2}$ little alteration in antenna composition had occurred in the antisense β-hydroxylase plants (Rissler & Pogson 2000). This preservation of LHCs is in agreement with *in vitro* reconstitution data that show vio 2000). This preservation of LHCs is in agreement with *in* 2000). This preservation of LHCs is in agreement with *in*
vitro reconstitution data that show violaxanthin is
preferred for optimal stability, but is not essential for
folding except for I hcb5 (Ros et al. 1998). Interes *vitro* reconstitution data that show violaxanthin is
preferred for optimal stability, but is not essential for
folding except for Lhcb5 (Ros *et al.* 1998). Interestingly, in
the most severe antisense B-bydroxylase plants preferred for optimal stability, but is not essential for folding except for Lhcb5 (Ros *et al.* 1998). Interestingly, in the most severe antisense β -hydroxylase plants the the most severe antisense β-hydroxylase plants the *Phil. Trans. R. Soc. Lond.* B (2000)

chlorophyll a/b ratio was 2.33 ± 0.1 , which was significhlorophyll a/b ratio was 2.33 ± 0.1 , which was significantly lower than wild-type (2.85 ± 0.28) . A possible explanation for the lower ratio is that there may be an chlorophyll a/b ratio was 2.33 ± 0.1 , which was significantly lower than wild-type (2.85 ± 0.28) . A possible explanation for the lower ratio is that there may be an increase in I hchl/2 trimers (Rissler & Pogson 2000 cantly lower than wild-type (2.85 ± 0.28) . A possible explanation for the lower ratio is that there may be an increase in Lhcbl/2 trimers (Rissler & Pogson 2000), which have a chlorophyll a/h ratio of 13 (Peter & explanation for the lower ratio is that there may be an increase in Lhcbl/2 trimers (Rissler & Pogson 2000), which have a chlorophyll a/b ratio of 1.3 (Peter & Thornber 1991: Bassi *et al.* 1993). Additionally exposure o increase in Lhcbl/2 trimers (Rissler & Pogson 2000), which have a chlorophyll a/b ratio of 1.3 (Peter & Thornber 1991; Bassi *et al.* 1993). Additionally, exposure of wild-type and antisense β -bydroxylase plants to bi which have a chlorophyll a/b ratio of 1.3 (Peter & Thornber 1991; Bassi *et al.* 1993). Additionally, exposure of wild-type and antisense β -hydroxylase plants to high light showed that the pool of violaxanthin is tigh Thornber 1991; Bassi *et al.* 1993). Additionally, exposure of wild-type and antisense β -hydroxylase plants to high light
showed that the pool of violaxanthin is tightly controlled
to optimize I HC structure at the exp wild-type and antisense β -hydroxylase plants to high light showed that the pool of violaxanthin is tightly controlled to optimize LHC structure at the expense of availability of violaxanthin for de-epoxidation.
(iii) *Photop rotection*

The *Photoprotection*
The xanthophyll cycle has been proposed to play a role
photoprotection in conjunction with LHC protonation (iii) *Photoprotection*
The xanthophyll cycle has been proposed to play a role
in photoprotection in conjunction with LHC protonation
and a functional PebS protein (Walters *et al.* 1996; Nivori and a functional PsbS protein (Walters *et al*. 1996; Niyogi in photoprotection in conjunction with LHC protonation
and a functional PsbS protein (Walters *et al.* 1996; Niyogi
1999; Li *et al.* 2000). In the absence of zeaxanthin,
quenching is greatly reduced (Niyogi *et al.* 1998) and a functional PsbS protein (Walters *et al.* 1996; Niyogi 1999; Li *et al.* 2000). In the absence of zeaxanthin, quenching is greatly reduced (Niyogi *et al.* 1998). However when zeaxanthin was reduced by 75% there 1999; Li *et al.* 2000). In the absence of zeaxanthin, quenching is greatly reduced (Niyogi *et al.* 1998). However, when zeaxanthin was reduced by 75% there was only a slight 16% reduction in the extent of NPO quenching is greatly reduced (Niyogi *et al.* 1998).
However, when zeaxanthin was reduced by 75% there was only a slight 16% reduction in the extent of NPQ However, when zeaxanthin was reduced by 75% there
was only a slight 16% reduction in the extent of NPQ
(Rissler & Pogson 2000) (figure 3). These results demon-
strate that only a limited fraction of the total zeaxanthi was only a slight 16% reduction in the extent of NPQ
(Rissler & Pogson 2000) (figure 3). These results demonstrate that only a limited fraction of the total zeaxanthin
in wild-type plants is required for optimal chlorophyl (Rissler & Pogson 2000) (figure 3). These results demonstrate that only a limited fraction of the total zeaxanthin
in wild-type plants is required for optimal chlorophyll
fluorescence quenching in viva and are in agreemen Eurate that only a limited fraction of the total zeaxanthin
in wild-type plants is required for optimal chlorophyll
fluorescence quenching *in vivo* and are in agreement with
studies on isolated thylakoids that suggested o in wild-type plants is required for optimal chlorophyll
fluorescence quenching *in vivo* and are in agreement with
studies on isolated thylakoids that suggested only a few
zeavanthin molecules are required (Gilmore *et al* fluorescence quenching *in vivo* and are in agreement with studies on isolated thylakoids that suggested only a few zeaxanthin molecules are required (Gilmore *et al.* 1998). This would imply that in wild-type plants, more studies on isolated thylakoids that suggested only a few zeaxanthin molecules are required (Gilmore *et al.* 1998). This would imply that in wild-type plants, more zeaxanthin is synthesized than is actually required for zeaxanthin molecules are required (Gilmore et al. 1998). This would imply that in wild-type plants, more
zeaxanthin is synthesized than is actually required for
achieving maximal NPQ. The 'excess' zeaxanthin that is
not directly involved in chlorophyll fluorescence quenching zeaxanthin is synthesized than is actually required for
achieving maximal NPQ. The 'excess' zeaxanthin that is
not directly involved in chlorophyll fluorescence quenching
may, contribute, to other processes, such as protec achieving maximal NPQ. The 'excess' zeaxanthin that is
not directly involved in chlorophyll fluorescence quenching
may contribute to other processes such as protection
against linid peroxidation (Hayaux & Nivogi 1999) not directly involved in chlorophyll fluorescence quenching
may contribute to other processes such as protection
against lipid peroxidation (Havaux & Niyogi 1999).
Whenever zeaxanthin accumulation has been may contribute to other processes such as protection

against lipid peroxidation (Havaux & Niyogi 1999).
Whenever zeaxanthin accumulation has been
prevented, either by dithiothreitol treatment or a
de-enoxidase mutation reversible quenching was reduced Whenever zeaxanthin accumulation has been
prevented, either by dithiothreitol treatment or a
de-epoxidase mutation, reversible quenching was reduced
but never eliminated (Gilmore & Yamamoto 1991: Nivogi prevented, either by dithiothreitol treatment or a
de-epoxidase mutation, reversible quenching was reduced
but never eliminated (Gilmore & Yamamoto 1991; Niyogi
et al. 1997–1998) This residual component of the rapidly de-epoxidase mutation, reversible quenching was reduced
but never eliminated (Gilmore & Yamamoto 1991; Niyogi
et al. 1997, 1998). This residual component of the rapidly
reversible quenching (a_c) is ca. 60% in Chlamydom but never eliminated (Gilmore & Yamamoto 1991; Niyogi
et al. 1997, 1998). This residual component of the rapidly
reversible quenching (q_E) is *ca.* 60% in *Chlamydomonas* and
14% in *Arabidotesis* (table 4). The two lute *et al.* 1997, 1998). This residual component of the rapidly
reversible quenching (q_E) is $ca. 60\%$ in *Chlamydomonas* and
14% in *Arabidopsis* (table 4). The two lutein deficient
mutations of *Arabidopsis* and *Chlamydo* mutations of *Arabidopsis* and *Chlamydomonas*, *lut2* and *lor1*, respectively, have delayed and reduced NPQ (Niyogi *et al*. 1997; Pogson *et al*. 1998). When they are crossed into the

See figure 3 for details.
 npq1 background, the remaining rapidly reversible NPQ is time (s)
Figure 4. Non-photochemical quenching of chlorophyll
fluorescence (NPO) in wild-type (wt) and a plant Figure 4. Non-photochemical quenching of chlorop
fluorescence (NPQ) in wild-type (wt) and a plant
overgyntessipe the S cyclose transcene (wt BP944, T Figure 4. Non-photochemical quenching of chlorophyll
fluorescence (NPQ) in wild-type (wt) and a plant
overexpressing the ε-cyclase transgene (wt-BP244, T₃
generation), in which lutein levels were 130% of wild-type.
See overexpressing the ε-cycl
generation), in which lut
See figure 3 for details.

essentially eliminated (table 4) (Niyogi *et al*. 1997, 2000). Additionally, when the antisense β -hydroxylase line that essentially eliminated (table 4) (Niyogi *et al.* 1997, 2000).
Additionally, when the antisense β -hydroxylase line that
has a 16% reduction in NPQ is crossed into the *lut2*
hackground there is a further 34% reducti Additionally, when the antisense β -hydroxylase line that
has a 16% reduction in NPQ is crossed into the *lut2* in
background, there is a further 34% reduction in NPQ 30 has a 16% reduction in NPQ is crossed into the $lut2$
background, there is a further 34% reduction in NPQ
(figure 3). Conversely, constitutively increasing the
proportion of lutein in RP244 (figure 4) and zeavanthin background, there is a further 34% reduction in NPQ
(figure 3). Conversely, constitutively increasing the
proportion of lutein in BP244 (figure 4) and zeaxanthin
in *ahal* (Porson *et al.* 1998) increases the rate of i (figure 3). Conversely, constitutively increasing the proportion of lutein in BP244 (figure 4) and zeaxanthin
in *abal* (Pogson *et al.* 1998) increases the rate of induction
of NPO I ikewise, the delayed quench in lvt^2 proportion of lutein in BP244 (figure 4) and zeaxanthin
in *abal* (Pogson *et al.* 1998) increases the rate of induction of NPQ. Likewise, the delayed quench in lut2 can be zeaxanthin. restored by constitutively accumulating either lutein or zeaxanthin.
Overexpressing the ε -cyclase resulted in a 31% reduc-

zeaxanthin.
Overexpressing the ε -cyclase resulted in a 31% reduction in the xanthophyll pool size but a more rapid induc-
tion of NPO (table 4, figure 4) which is contrary to the Overexpressing the ε -cyclase resulted in a 31% reduction in the xanthophyll pool size but a more rapid induction of NPQ (table 4, figure 4), which is contrary to the antisense. B-bydroxylase, data, where, decreasing t tion of NPQ (table 4, figure 4), which is contrary to the antisense β -hydroxylase data where decreasing the tion of NPQ (table 4, figure 4), which is contrary to the
antisense β -hydroxylase data where decreasing the
xanthophyll cycle pool delayed induction (table 4,
figure 3). Reducing the xanthophyll cycle pool therefore antisense β -hydroxylase data where decreasing the xanthophyll cycle pool delayed induction (table 4, figure 3). Reducing the xanthophyll cycle pool therefore reduces quenching if lutein content is unaltered but if the xanthophyll cycle pool delayed induction (table 4, figure 3). Reducing the xanthophyll cycle pool therefore reduces quenching if lutein content is unaltered, but if the reduced xanthophyll cycle pool is replaced by a corre figure 3). Reducing the xanthophyll cycle pool therefore reduces quenching if lutein content is unaltered, but if the reduced xanthophyll cycle pool is replaced by a corresponding increase in lutein, the rate of induction is reduced xanthophyll cycle pool is replaced by a corre-
sponding increase in lutein, the rate of induction is
slightly and significantly accelerated (figure 4). This
annarent paradox may be explained by the bypothesis sponding increase in lutein, the rate of induction is
slightly and significantly accelerated (figure 4). This
apparent paradox may be explained by the hypothesis
that increasing lutein resulted in more 'free' lutein which slightly and significantly accelerated (figure 4). This
apparent paradox may be explained by the hypothesis
that increasing lutein resulted in more 'free' lutein, which
could substitute for zeavanthin and participate in th apparent paradox may be explained by the hypothesis
that increasing lutein resulted in more 'free' lutein, which
could substitute for zeaxanthin and participate in the that increasing lutein resulted in more 'free' lutein, which
could substitute for zeaxanthin and participate in the
initial phase of NPQ since most of the lutein would be
tightly bound within the LHC (Kuhlbrandt et al. 19 could substitute for zeaxanthin and participate in the initial phase of NPQ since most of the lutein would be tightly bound within the LHC (Kuhlbrandt *et al.* 1994) and probably not participate in NPO initial phase of NPQ since most of th
tightly bound within the LHC (Kuhll
and probably not participate in NPQ. and probably not participate in NPQ.
4. CONCLUSIONS

Genetic manipulation of the ε -cyclase and β -hydroxylase $\frac{4}{5}$ CONCLUSIONS
has shown that it is possible to alter flux through the
pathway and the carotenoid pool size by manipulating Genetic manipulation of the ε -cyclase and β -hydroxylase
has shown that it is possible to alter flux through the
pathway and the carotenoid pool size by manipulating
mRNA abundance of one gene. However, there appear has shown that it is possible to alter flux through the pathway and the carotenoid pool size by manipulating mRNA abundance of one gene. However, there appear to be limits to the extent that manipulating gene expression pathway and the carotenoid pool size by manipulating Study of Early Events in Photosynthesis at ASU.

mRNA abundance of one gene. However, there appear to

be limits to the extent that manipulating gene expression

results mRNA abundance of one gene. However, there appear to be limits to the extent that manipulating gene expression results in altered carotenoid accumulation. That is, there is a maximal increase of ca . 180% for lutein in th be limits to the extent that manipulating gene expression ^e-cyclase lines and a maximal decrease of *ca.* 60% for is a maximal increase of *ca*. 180% for lutein in the sense
E-cyclase lines and a maximal decrease of *ca*. 60% for
violaxanthin in the antisense β -hydroxylase lines. How
transcription is regulated and to what extent p ϵ -cyclase lines and a maximal decrease of *ca*. 60% for violaxanthin in the antisense β -hydroxylase lines. How transcription is regulated and to what extent post-transcriptional processes—such as chaperone-mediated violaxanthin in the antisense β -hydroxylase lines. How
transcription is regulated and to what extent post-
transcriptional processes—such as chaperone-mediated transcriptional processes—such as chaperone-mediated *Phil. Trans. R. Soc. Lond.* B (2000)

sis and photop rotection B. J. Pogson and H. M. Rissler 1401
targeting, the proposed multi-enzyme complexes and
incorporation into LHCs (Giuffra *et al.* 1996; Bonk *et al.* targeting, the proposed multi-enzyme complexes and
incorporation into LHCs (Giuffra *et al.* 1996; Bonk *et al.*
1997; Cunningham & Gantt 1998)—affect accumulation
remains to be determined incorporation into LHCs (C
1997; Cunningham & Gant
remains to be determined.
The physiological relevan 97; Cunningham & Gantt 1998)—affect accumulation
mains to be determined.
The physiological relevance of xanthophylls and NPQ
exemplified by the bleaching phenotype that is

remains to be determined.
The physiological relevance of xanthophylls and NPQ
is exemplified by the bleaching phenotype that is The physiological relevance of xanthophylls and NPQ
is exemplified by the bleaching phenotype that is
observed in several carotenoid biosynthetic and NPQ
mutants. In the absence of both zeavanthin and lutein is exemplified by the bleaching phenotype that is
observed in several carotenoid biosynthetic and NPQ
mutants. In the absence of both zeaxanthin and lutein,
Chlamydomonas, cultures, photobleach, and mature observed in several carotenoid biosynthetic and NPQ
mutants. In the absence of both zeaxanthin and lutein,
Chlamydomonas cultures photobleach and mature
Arabidoteis leaves senesce and photobleach under birh mutants. In the absence of both zeaxanthin and lutein,
 Chlamydomonas cultures photobleach and mature
 Arabidopsis leaves senesce and photobleach under high

light (Nivogi et al. 1997–2000). There is a much less Chlamydomonas cultures photobleach and mature

Arabidopsis leaves senesce and photobleach under high

light (Niyogi *et al.* 1997, 2000). There is a much less

bleaching if only zeavanthin is eliminated and there is *Arabidopsis* leaves senesce and photobleach under high
light (Niyogi *et al.* 1997, 2000). There is a much less
bleaching if only zeaxanthin is eliminated and there is
residual NPO Also photobleaching is not as extensive light (Niyogi *et al.* 1997, 2000). There is a much less bleaching if only zeaxanthin is eliminated and there is residual NPQ. Also, photobleaching is not as extensive in *npq4* mutants that exhibit a loss of NPQ without a change residual NPQ. Also, photobleaching is not as extensive in $mpq4$ mutants that exhibit a loss of NPQ without a change
in xanthophyll biosynthesis (Baroli & Niyogi, this issue).
These differences may reflect the role of xant $mpq4$ mutants that exhibit a loss of NPQ without a change
in xanthophyll biosynthesis (Baroli & Niyogi, this issue).
These differences may reflect the role of xanthophylls in
other processes in addition to NPO including p in xanthophyll biosynthesis (Baroli & Niyogi, this issue).
These differences may reflect the role of xanthophylls in
other processes in addition to NPQ, including photo-
system stability quenching of chlorophyll singlets a These differences may reflect the role of xanthophylls in
other processes in addition to NPQ, including photo-
system stability, quenching of chlorophyll singlets and
triplets and protection against linid peroxidation other processes in addition to NPQ, including postern stability, quenching of chlorophyll singlet triplets, and protection against lipid peroxidation.
It is possible and photophysically feasible that system stability, quenching of chlorophyll singlets and
triplets, and protection against lipid peroxidation.
It is possible and photophysically feasible that lutein

triplets, and protection against lipid peroxidation.
It is possible and photophysically feasible that lutein
might participate in the quenching process, particularly
during the first 30 s of high light prior to substantial It is possible and photophysically feasible that lutein
might participate in the quenching process, particularly
during the first 30 s of high light prior to substantial zeax-
anthin synthesis. Chlorophyll singlets are for might participate in the quenching process, particularly
during the first 30s of high light prior to substantial zeax-
anthin synthesis. Chlorophyll singlets are formed within
seconds of high light exposure and intersystem during the first 30s of high light prior to substantial zeax-
anthin synthesis. Chlorophyll singlets are formed within
seconds of high light exposure and intersystem crossing to anthin synthesis. Chlorophyll singlets are formed within
seconds of high light exposure and intersystem crossing to
form potentially damaging triplets occurs rapidly. An
increase in lutein did increase NPO levels during th seconds of high light exposure and intersystem crossing to
form potentially damaging triplets occurs rapidly. An
increase in lutein did increase NPQ levels during the first
30s and maximal NPO was unaffected (figure 4) Thi form potentially damaging triplets occurs rapidly. An increase in lutein did increase NPQ levels during the first 30s and maximal NPQ was unaffected (figure 4). This increase in the early phase of NPQ was despite a decreas increase in lutein did increase NPQ levels during the first
30s and maximal NPQ was unaffected (figure 4). This
increase in the early phase of NPQ was despite a decrease
in the xanthonbull cycle pool size X_{eff} a reduc 30s and maximal NPQ was unaffected (figure 4). This
increase in the early phase of NPQ was despite a decrease
in the xanthophyll cycle pool size. Yet, a reduction in the
xanthophyll cycle pool in the antiense hydroxylase l increase in the early phase of NPQ was despite a decrease
in the xanthophyll cycle pool size. Yet, a reduction in the
xanthophyll cycle pool in the antisense hydroxylase lines caused a 16% reduction in maximal NPQ if lutein xanthophyll cycle pool in the antisense hydroxylase lines
caused a 16% reduction in maximal NPQ if lutein
content was unaltered, and this decrease could be
enhanced by another 34% if lutein was eliminated caused a 16% reduction in maximal NPQ if lutein
content was unaltered, and this decrease could be
enhanced by another 34% if lutein was eliminated
(figure 3) In fact lutein deficiency has a deleteriously content was unaltered, and this decrease could be
enhanced by another 34% if lutein was eliminated
(figure 3). In fact, lutein deficiency has a deleteriously
additive effect on NPO in a range of *Arabidotesis* and enhanced by another 34% if lutein was eliminated (figure 3). In fact, lutein deficiency has a deleteriously additive effect on NPQ in a range of *Arabidopsis* and *Chlamydomonas* genotypes, including wild-type, *npq1* (zea *Chalmydomonasignamy in the finite monastypers, in the signal (figure 3).* In fact, lutter definitions a deleteriously additive effect on NPQ in a range of *Arabidopsis* and *Chlamydomonas* genotypes, including wild-type, $npql$ (zeax-
anthin deficient), antisense β -hydroxylase (xanthophyll
cycle pool decreased) or *abal* (table 4). Ques $Chlamydomonas$ genotypes, including wild-type, $npql$ (zeax-
anthin deficient), antisense β -hydroxylase (xanthophyll
cycle pool decreased) or *abal* (table 4). Questions remain
concerning the number of lutein molecules contribu anthin deficient), antisense β -hydroxylase (xanthophyll cycle pool decreased) or *abal* (table 4). Questions remain concerning the number of lutein molecules contributing to NPO (Gilmore 2000) and whether lutein direct cycle pool decreased) or *abal* (table 4). Questions remain concerning the number of lutein molecules contributing to NPQ (Gilmore 2000) and whether lutein directly or indirectly affects chlorophyll singlet quenching and concerning the number of lutein molecules contributing acts with PsbS. We still do not understand the mechanism indirectly affects chlorophyll singlet quenching and inter-
acts with PsbS. We still do not understand the mechanism
of NPQ, but recent progress has enabled more specific
questions to be addressed and has shown that it is acts with PsbS. We still do not understand the mechanism
of NPQ, but recent progress has enabled more specific
questions to be addressed and has shown that it is indeed
a physiologically relevant process of NPQ, but recent progress has
questions to be addressed and has s
a physiologically relevant process. a physiologically relevant process.
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insight gained from *Arabidopsis thaliana* L. mutants that lack
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violaxanthin deepoxidase activity and or lutein. *Photosyn. Res.*
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H₀11 Hall.

Discussion

Discussion
E. Hideg (*Institute of Plant Biology, Biological Research Centre,*
Szeged Hungary) In order to 25t 25 efficient triplet **Discussion**

E. Hideg (*Institute of Plant Biology, Biological Research Centre,*
 Szeged, Hungary). In order to act as efficient triplet

quenchers or singlet oxygen quenchers carotenoids have E. Hideg (*Institute of Plant Biology, Biological Research Centre, Szeged, Hungary*). In order to act as efficient triplet quenchers or singlet oxygen quenchers, carotenoids have to be close enough to chlorophylls or other to be close enough to chlorophylls or other potential sites quenchers or singlet oxygen quenchers, carotenoids have
to be close enough to chlorophylls or other potential sites
of O_2 production. When the carotenoid composition is
altered do pigments have the same localization as to be close enough to chlorophylls or other potential sites
of O_2 production. When the carotenoid composition is
altered, do pigments have the same localization as in the
wild-type? of O₂ produce
altered, do pie
wild-type?

wild-type?
B. J. Pogson. That is a good question to which the answers
are still being sought. It would appear that there are not B. J. Pogson. That is a good question to which the answers
are still being sought. It would appear that there are not
dramatic changes to chlorophyll content and antenna B. J. Pogson. That is a good question to which the answers
are still being sought. It would appear that there are not
dramatic changes to chlorophyll content and antenna
function in the single mutants $lutl$, $lut2$ and $abal$ are still being sought. It would appear that there are not
dramatic changes to chlorophyll content and antenna
function in the single mutants *lut1*, *lut2* and *abal*. Also, the
antisense 8-hydroxylase plants have a simil dramatic changes to chlorophyll content and antenna
function in the single mutants *lutl*, *lut2* and *abal*. Also, the
antisense β -hydroxylase plants have a similar pigment
content and I han content as wild-type. Base function in the single mutants *lutl, lut2* and *abal*. Also, the antisense β -hydroxylase plants have a similar pigment content and Lhcp content as wild-type. Based on those results I suspect there are no dramatic chan antisense β -hydroxylase plants have a similar pigment content and Lhcp content as wild-type. Based on those results, I suspect there are no dramatic changes to the capacity to quench triplet chlorophylls in the mutants content and Lhep content as wild-type. Based on those
results, I suspect there are no dramatic changes to the
capacity to quench triplet chlorophylls in the mutants or
transgenic lines that contain substantial levels of at results, I suspect there are no dramatic changes to the capacity to quench triplet chlorophylls in the mutants or transgenic lines that contain substantial levels of at least one wild-type vanthophyll. However, we require capacity to quench triplet chlorophylls in the mutants or
transgenic lines that contain substantial levels of at least
one wild-type xanthophyll. However, we require more
detailed analyses to confirm this I think the story transgenic lines that contain substantial levels of at least
one wild-type xanthophyll. However, we require more
detailed analyses to confirm this. I think the story will be
more complex for xanthophyll-reduced and xanthop one wild-type xanthophyll. However, we require more
detailed analyses to confirm this. I think the story will be
more complex for xanthophyll-reduced and xanthophyll-
deficient lines detailed analyses to confirm this. I think the story will be
more complex for xanthophyll-reduced and xanthophyll-
deficient lines.

BIOLOGICAL
SCIENCES

THE ROYAL **PHILOSOPHICAL**
TRANSACTIONS