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ROLE OF CAROTENOIDS IN PROTECTING CHLOROPHYLL FROM PHOTODESTRUCTION—II. STUDIES ON THE EFFECT OF FOUR MODIFIERS OF THE ALBINO cl_1 MUTANT OF MAIZE*

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Abstract—By combining independent, dominant, modifier genes of the albino cl_1 mutant it is possible to produce a spectrum of phenotypes ranging from normal green to albino. Analysis of plastid pigments reveals that the albino possesses the ability to produce as much or more chlorophyll than normal siblings and that this ability is not impaired by the presence of the modifier genes. The modifiers do influence, however, the amount of carotene and xanthophyll the plants produced. The level of the three plastid pigments (chlorophyll, carotene and xanthophyll) vary simultaneously and, in most modified phenotypes, occur in approximately the same concentrations relative to their normal siblings. Since chlorophyll production appears to be normal in these mutants, it is suggested that the modifier genes do not directly influence the concentration of this pigment. Rather, the ultimate amount of chlorophyll will not rise above that which can be protected from photodestruction by the carotenoid levels determined by the various modifier genotypes.

INTRODUCTION

STUDIES with carotenoid deficient photosynthetic bacteria[1,2] have shown that colored carotenoids are required for protecting bacteriochlorophyll from photodestruction, and in 1959 Stanier[3] proposed that colored carotenoids might play a role in protecting chlorophyll from autophotodestruction in all photoautotrophs. The work of Anderson and Robertson[4] on the maize mutant, w_3, supported this hypothesis. This mutant, which is devoid of colored carotenoids, retains the ability to synthesize chlorophyll. Exposure of leaves to light under aerobic conditions results in rapid destruction of chlorophyll, while under anaerobic conditions, the pigment is stable in strong light. Catalase in this mutant also proved to be photosensitive under aerobic conditions,[5,6] suggesting that colored carotenoids may have a role in protecting porphyrins other than chlorophyll from photo-destruction.

Further evidence on the role of carotenoids in protecting chlorophyll has been obtained through the study of plastid pigment development in a series of mutant phenotypes resulting from the modification of the albino phenotype of the cl_1 mutant of maize by a series of modifier genes.

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GENETIC MATERIAL

The cl_1 mutant of maize was first described by Everett.[7] It is one of many white endosperm-albino seedling mutants that have been found in corn,[8] However, Everett[7] found that, unlike the others, the albinism of this mutant could be completely or partially suppressed if it occurred in a plant in which one or the other of two independent, dominant modifier genes were present ($Cl^2{}_M$ or $Cl^3{}_M$).

Since Everett's original work, two other alleles of cl_1 have been found which have phenotypes indistinguishable from cl_1 and also two additional dominant modifier genes. A thorough description of these mutants and the genetic tests which establish that the modifiers are allelic are given by Robertson.[9] Table 1 summarizes the list of the cl_1

TABLE 1. ALLELES AT THE cl_1 LOCUS, THEIR MODIFIERS AND PHENOTYPES

		Phenotype as affected by modifier					
		Endosperm		Seedlings		Mature plant	
Allele	Modifier	hetero-zygous	homo-zygous	hetero-zygous	homo-zygous	hetero-zygous	homozygous
cl_1	$Cl^2{}_M$	white*	white*	pale green†	pale green†	lethal	lethal
	$Cl^3{}_M$	white*	white*	green	green	pale green white sheaths zebra leaves	green—about $\frac{2}{3}$–$\frac{3}{4}$ height of normal and 2–3 days later maturing
cl_p	$Cl^4{}_M$	white*	white*	green	green	lethal	green—about $\frac{1}{2}$–$\frac{2}{3}$ height of normals and 2–3 days later maturing
w_{7716}	$Cl^5{}_M$	white*	white*	pale green†	green	lethal	green—less vigorous and later maturing than $Cl^4{}_M$

*In certain genetic backgrounds the endosperm will be pale yellow instead of white but this affect seems to be independent of the Cl_M genes.

†The three pale green seedling phenotypes can be arranged in the following order on the basis of the amount of pigment present: homozygous $Cl^2{}_M$> heterozygous $Cl^5{}_M$> heterozygous $Cl^2{}_M$.

alleles, their modifiers and the resulting phenotypes. It will be noted that the modifiers alter only the phenotype of the seedling and/or mature plant but not that of the endosperm. Robertson[9] also reported on the phenotypes for plants that were heterozygous for all of the possible combinations of the four modifier alleles. These observations are summarized in Table 2.

The phenotypes recorded in Tables 1 and 2 were determined on the basis of visual comparisons of normal and mutant plants from the same segregating ear. Criteria used were plant height at maturity, date of flowering and differences in plant pigmentation obvious to the eye. Observations such as those recorded in Tables 1 and 2 demonstrate that the modifier genes are responsible for partial or nearly complete (if not complete) suppression of the albino phenotype of cl_1.

TABLE 2. PHENOTYPE OF PLANTS HETEROZYGOUS FOR THE VARIOUS MODIFIERS OF THE cl_1 LOCUS*

Genotype	Phenotypes	
	as seedlings	at tasseling
$cl_p\ Cl^4{}_M$ / $cl_1\ Cl^3{}_M$	equal to normals	Average 1 day later. Main stalk as vigorous as normals but produces fewer tillers. Date of maturity same as normals.
$cl_1\ Cl^3{}_M$ / $w_{7716}\ Cl^5{}_M$	equal to normals	Vigor equals normals, average 1 day later.
$cl_1\ Cl^3{}_M$ / $cl_1\ Cl^2{}_M$	equal to normals	Vigor equals normals, average 1–2 days later.
$cl_1\ Cl^2{}_M$ / $cl_p\ Cl^4{}_M$	equal to normals	$\frac{2}{3}$ height of normals, average 1 week later.
$w_{7716}\ Cl^5{}_M$ / $cl_p\ Cl^4{}_M$	equal to normals	$\frac{2}{3}$ height of and paler green than normals, average 1 week later.
$cl_1\ Cl^2{}_M$ / $w_{7716}\ Cl^5{}_M$	pale green	Died as seedlings.

*From Robertson.[9]

METHODS

To determine the chlorophyll, carotene and xanthophyll levels of the phenotypes reported in Table 1 and 2, seedlings were grown in the light in a Percival plant growth chamber Model PGC–78, on a cycle of 12 hr light (2000 ft-c.), 12 hr dark, at 22°, for 12 days, at which time they were harvested. In all genotypes, normal and mutant seedlings from the same ear were planted in adjacent rows. Where possible 3 g of material were harvested for analysis.

The leaves were ground in a blender for 2½ min in 50 ml acetone and 40 ml hexane (b.p. 65–67°) and the pigments extracted by using a method modified from Zscheile and Porter.[10] The chlorophyll concentration was determined by reading the optical density of the extract on a Spectronic 505 or Beckman-DU at 667 mμ. After the chlorophyll determination, the xanthophyll was determined by reading the first methanol wash at 47 mμ. Following the extraction of the chlorophyll, the hexane was then read at 450 mμ for the carotene determination.

The concentrations of the three pigments were determined by using the following formulae: chlorophyll (O.D./34·5) × vol./wt. = mg/g;[11] xanthophyll (O.D./240) × vol./wt. = mg/g and carotene (O.D./240) × vol./wt. = mg/g.[10]

To determine the ability of the plants to produce protochlorophyllide and to convert this into chlorophyll, normal and mutant seedlings were grown in the growth chamber in the dark at 22° for 14 days after which time a 5-g sample was taken for protochlorophyllide determinations. The samples were ground in the dark in a volume of 80 per cent acetone equal to 2 ml/g of tissue using a mortar and pestle with sand. The coarse debris was removed by filtering each sample through four layers of cheese cloth, the liquid centrifuged and its optical density determined at 630 mμ.

To determine the ability of the plants to produce chlorophyll, the remaining plants of each genotype, from which the samples for protochlorophyllide were taken, were illuminated with 1000 ft-c. of light for 1 min and then returned to the dark. After an hour of

darkness, they were harvested and extracted as above. The optical density was then read at 667 mμ. The concentration of the two pigments were calculated from their O.D. values, using the following formula for protochlorophyllide: (O.D./35·6) × vol./wt. = mg/g.[12]

RESULTS

The results of the tests to determine the ability of albino and suppressed plants to make chlorophyll are given in Table 3.

The chlorophyll, carotene and xanthophyll determinations for the light grown seedlings are given in Table 4. These results are plotted graphically in Fig. 1. The values in Table 4

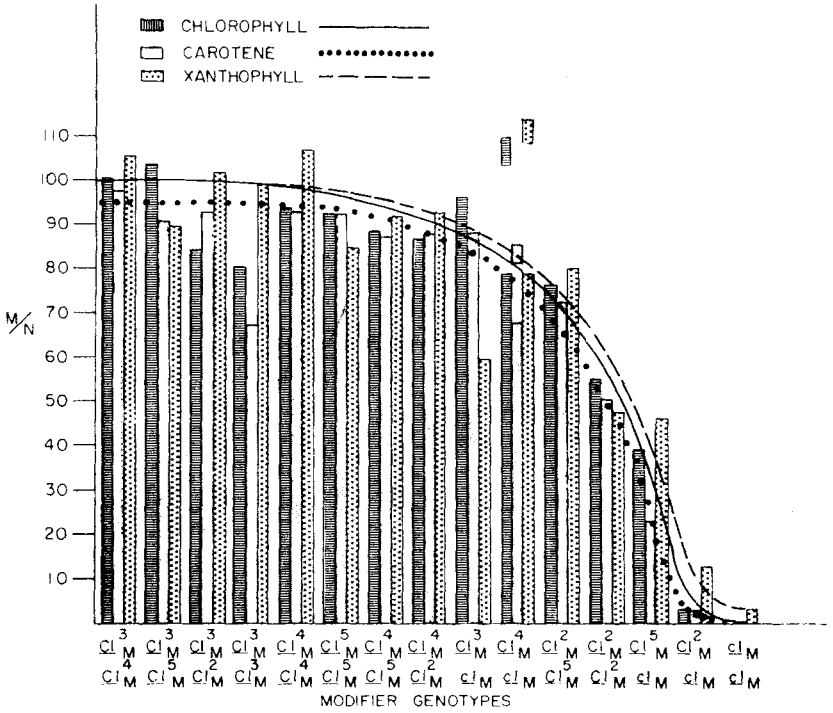

FIG. 1. Chlorophyll, carotene and xanthophyll produced by seedlings of the various modifier genotypes expressed in percentage the mutant concentration is of the normal (mutant/normal).

are selected from determinations made on several samples from the same and different ears. The total number of samples tested varied from three to eight, depending upon the amount of material available for testing and the consistency of the values determined. Three samples were used for each genotype to determine the average chlorophyll and carotene values in Table 4. For these two pigments, the values reported were from the same samples. These were selected because their percentage determinations were consistent with the majority of those found for *both* chlorophyll and carotene. The xanthophyll values are sometimes based on less than three samples, which did not necessarily include those used for the chlorophyll and carotene determination; although where possible this was done.

The pigment levels for the various phenotypes are expressed as a percentage that the mutant is of the normal. This is done to eliminate the affect of the considerable background variation between samples of the same genotype but taken from different ears

TABLE 3. THE FORMATION OF PROTOCHLOROPHYLLIDE AND CHLOROPHYLL IN NORMAL AND MUTANT SEEDLINGS FROM SELF-POLLINATED EARS OF PLANTS CARRYING cl_1 ALLELES WITH AND WITHOUT MODIFIERS

Genotype of self-pollinated plant	Seedlings tested	Dark grown seedlings: protochlorophyllide (mg/g)	Dark grown seedlings: protochlorophyllide (mutant/normal)	Dark grown seedlings: chlorophyll (mg/g)	Dark grown seedlings: chlorophyll (mutant/normal)
$Cl_1\ cl_1\ cl_M\ cl_M$	N*	0·00354	1·6	0·0	—
	M*	0·00572			
$W_{7716}\ w_{7716}\ cl_M\ cl_M$	N	0·00208	1·5	0·0	—
	M	0·00308			
$Clp\ cl_P\ cl_M\ cl_M$	N	0·00298	1·5	0·0	—
	M	0·00444			
$Cl_1\ cl_1\ Cl^2{}_M\ Cl^2{}_M$	N	0·00348	1·3	0·0	—
	M	0·00438			
$W_{7716}\ w_{7716}\ Cl^5{}_M\ Cl^5{}_M$	N	0·00394	0·8	0·0	—
	M	0·00314			
$Cl_p\ cl_p\ Cl^4{}_M\ Cl^4{}_M$	N	0·00292	1·2	0·0	—
	M	0·00354			
$Cl_1\ cl_1\ Cl^3{}_M\ Cl^3{}_M$	N	0·00264	1·4	0·0	—
	M	0·00360			
		Seedlings exposed to 1 min of light at 1000 ft-c. and harvested after 1 hr of darkness			
$Cl_1\ cl_1\ cl_M\ cl_M$	N	0·00091	1·9	0·00208	4·1
	M	0·00170		0·00846	
$W_{7716}\ w_{7716}\ cl_M\ cl_M$	N	0·00080	2·4	0·00139	4·5
	M	0·00162		0·00624	
$Cl_p\ cl_p\ cl_M\ cl_M$	N	0·00185	0·9	0·00069	6·5
	M	0·00150		0·00452	
$Cl_1\ cl_1\ Cl^2{}_M\ Cl^2{}_M$	N	0·00139	0·8	0·00133	2·0
	M	0·00121		0·00272	
$W_{7716}\ w_{7716}\ Cl^5{}_M\ Cl^5{}_M$	N	0·00207	1·1	0·00064	1·5
	M	0·00237		0·00099	
$Cl_p\ cl_p\ Cl^4{}_M\ Cl^4{}_M$	N	0·00214	0·9	0·00128	1·7
	M	0·00203		0·00215	
$Cl_1\ cl_1\ Cl^3{}_M\ Cl^3{}_M$	N	0·00185	1·7	0·00058	4·0
	M	0·00314		0·00232	

*N=normal, M=mutant

TABLE 4. TOTAL CHLOROPHYLL, CAROTENE AND XANTHOPHYLL FOR NORMAL AND MUTANT SEEDLINGS

Mutant genotype		Pigments (mg/g*) Chloro-phyll	Caro-tene	Xantho-phyll	Mutant as a percentage of Normal Chloro-phyll	Caro-tene	Xantho-phyll
	N	1·47	0·0489	0·0683			
$cl_1\ cl_1\ cl_M\ cl_M$					0·7	0·2	3·1
	M	0·01	0·0001	0·0021			
	N	1·88	0·0691	0·0577†			
$cl_1\ cl_1\ Cl^2{}_M\ cl_M$					3·7	3·2	13·1
	M	0·07	0·0022	0·0073†			
	N	1·45	0·0481	0·0714†			
$cl_1\ cl_1\ Cl^2{}_M\ Cl^2{}_M$					55·2	51·1	47·5
	M	0·80	0·0246	0·0339†			
$cl_1\ cl_1\ Cl^3{}_M\ cl_M$	*N*	1·93	0·0634	0·0772†			
or					96·9	88·2	59·6
$cl_1\ W_{7716}\ Cl^3{}_M\ cl_M$	*M*	1·87	0·0559	0·0460†			
	N	1·38	0·0470	0·0605‡			
$cl_1\ cl_1\ Cl^3{}_M\ Cl^3{}_M$					80·4	67·4	99·7
	M	1·11	0·0317	0·0603‡			
	N	1·79	0·0660	0·0777			
$cl_1\ cl_p\ Cl^4{}_M\ cl_M$					79·3	68·0	78·5
	M	1·42	0·0449	0·0610			
	N	1·36	0·0726	0·0633			
$cl_1\ cl_p\ Cl^4{}_M\ cl_M$					111·8	86·5	115·2
	M	1·52	0·0628	0·0729			
	N	1·62	0·0872	0·1059			
$cl_p\ cl_p\ Cl^4{}_M\ Cl^4{}_M$					93·8	93·0	107·3
	M	1·52	0·0811	0·1136			
	N	1·71	0·0660	0·0725			
$cl_1\ cl_1\ Cl^3{}_M\ Cl^2{}_M$					84·8	92·9	102·2
	M	1·45	0·0613	0·0741			
	N	1·42	0·0735	0·0977			
$cl_1\ cl_p\ Cl^4{}_M\ Cl^2{}_M$					86·6	87·9	93·0
	M	1·23	0·0646	0·0909			
	N	1·63	0·0571	0·0606†			
$cl_1\ cl_p\ Cl^3{}_M\ Cl^4{}_M$					100·6	97·4	105·6
	M	1·64	0·0556	0·0640†			
	N	2·15	0·0868	0·1280			
$cl_p\ w_{7716}\ Cl^5{}_M\ cl_M$					39·5	22·9	46·9
	M	0·85	0·0199	0·0600			
	N	1·41	0·0533	0·0833			
$w_{7716}\ w_{7716}\ Cl^5{}_M\ Cl^5{}_M$					93·6	93·2	84·9
	M	1·32	0·0497	0·0707			
	N	1·45	0·0646	0·1388			
$cl_1\ w_{7716}\ Cl^2{}_M\ Cl^5{}_M$					77·2	72·6	80·8
	M	1·12	0·0469	0·1122			
	N	1·45	0·0792	0·0698			
$cl_1\ w_{7716}\ Cl^3{}_M\ Cl^5{}_M$					104·1	90·7	89·7
	M	1·51	0·0718	0·0626			
	N	1·86	0·0745	0·0771			
$cl_p\ w_{7716}\ Cl^4{}_M\ Cl^5{}_M$					88·7	87·4	92·5
	M	1·65	0·0651	0·0713			

*Mean value for three samples except where indicated otherwise. *N*=normal, *M*=mutant.
†Mean value for two samples.
‡Determination for one sample.

and the affect of background variation between the lines from which the different genotypes were derived. Comparing the average values for normals of the different genotypes will give some indication of the extent of this background variation. The curves are superimposed on the bar graphs of Fig. 1 to emphasize the general trend in pigment concentration of the three pigments over the range of modifier genotypes listed along the abscissa. These genotypes are arranged in descending order, with those giving the closest approximation to normal plants at tasseling (Tables 1 and 2) to the left, and those deviating most from normal to the right. Since a numerical value cannot be assigned the points along the abscissa, the curves were not mathematically fitted but were drawn in free hand.

DISCUSSION

The cl_1 mutant is one of thirteen or more white-albino mutants of maize which have been described.[8] These mutants all have white (or pale yellow) endosperms and usually produce albino seedlings when germinated in the light. There are a few variations of the seedling phenotype observed among some of the mutants, as in the case of cl_1 with its modifiers. Some white-albinos, such as w_3 and vp_9, have alleles (pas_{8686} and pas_{4889}, respectively) that give pale green seedlings. One, albescent (*al*), has white seedlings which become green under certain conditions (most readily at high temperatures). Another, pink scutellum (*ps*), has pink endosperm and gives rise to pink seedlings devoid of chlorophyll if grown in light. The pink pigment is the result of the accumulation of lycopene.[13] Six of these mutants also germinate prematurely (viviparous) before the seed dries completely on the ear.

All these mutants were tested for the accumulation of colorless carotene precursors that absorb in the ultraviolet, and six accumulated one or more of these precursors.[13] The cl_1 mutant is one that does not accumulate any of these precursors.

These mutants also all have the ability to make protochlorophyllide in the dark and convert it to chlorophyll when exposed to light.[13]

These general observations on the white-albino mutants strongly suggest that the primary biosynthetic block of this class of mutants is in the biosynthetic pathway of the carotenoid pigments and not chlorophyll.

The data in Table 3 demonstrate that the albino alleles at the cl_1 locus certainly do not impair the ability of the mutant seedlings to make chlorophyll, nor does the presence of the modifiers seem to have any affect on this ability. In most instances, the mutants make at least as much protochlorophyllide as normals, and in some instances more. Similar results have been observed for the w_3 albino mutant of maize.[4, 14] The consistently higher values for chlorophyll in the mutants are of such a magnitude that they probably have some consistent physiological basis. This increase could be due to the lower carotene concentration, particularly in the albinos, $cl_1\ cl_1\ cl_M\ cl_M$, $cl_p\ cl_p\ cl_M\ cl_M$, and $w_{7716}\ w_{7716}\ cl_M\ cl_M$. In normal seedlings, the carotene might absorb some light energy that may otherwise be used in chlorophyll formation. If this were so, lower concentrations would be expected in the mutants which possess modifiers. This is observed for three of them but not for $cl_1\ cl_1\ Cl^3{}_M\ Cl^3{}_M$, which has a relatively high carotene concentration. The fact that the other three modified genotypes consistently make about twice as much chlorophyll as their normal siblings suggests that the value for the $Cl_1\ cl_1\ Cl^3{}_M\ Cl^3{}_M$ stock is abnormally high. Since these tests were mainly to establish that the albino mutants and their suppressed counterparts retain the ability to make chlorophyll, neither this discrepancy nor the

relationship between the carotenoid level and the efficiency with which protochlorophyllide is converted to chlorophyll was followed up at this time.

Table 4 and Fig. 1 demonstrate that the levels of the three main plastid pigments are altered in all the suppressed phenotypes. With few exceptions, the levels of the three plastid pigments, expressed as percentages of the normal level for each genotype, are remarkably similar. Also, there is general agreement between the degree to which the mature plant for a given genotype approximates normal and the level of pigment achieved. The one outstanding exception to this is $Cl^3{}_M\ Cl^3{}_M$. This mutant also shows considerable variation between pigments and thus is an exception in this regard also. Unfortunately, there was very little of this material with which to work. Since $cl_1\ cl_1\ Cl^3{}_M\ Cl^3{}_M$ plants are so vigorous, this stock had been perpetuated mostly in the homozygous condition in our genetic lines, so there were only a few ears from weak inbred plants of the $Cl_1\ cl_1\ Cl^3{}_M\ Cl^3{}_M$ genotype available for testing. It is almost certain that the chlorophyll and carotene percentages are too low. Unless there is a marked discrepancy between mature plant vigor and pigment production in the seedling in this particular genotype, a discrepancy which certainly is lacking for all the others, these values for $Cl^3{}_M\ Cl^3{}_M$ probably are not representative of this genotype. More vigorous $Cl_1\ cl_1\ Cl^3{}_M\ Cl^3{}_M$ lines are being produced, but it will require a year or two to complete the necessary crosses.

The two sets of values given for the $Cl^4{}_M\ cl_M$ genotypes were selected from eight determinations made on this genotype. Two were discarded, one because a chlorophyll sample was lost, and the other because of an extremely low carotene determination. Of the remaining determinations, half gave high values and half low. These are represented by the two sets of values. The phenotype of the mature plant would indicate that $Cl^4{}_M\ cl_m$ should correspond to the lower values. Further tests will be needed on better stocks to explain these high readings. In spite of the varied background of the genetic material and the complicated extraction procedure necessary to make the determinations summarized in Table 4 and Fig. 1, there is recognized an obvious tendency for the three plastid pigments to vary simultaneously in most of the modified phenotypes and to occur in approximately the same concentrations relative to their normal siblings. The parallel behavior of carotene and xanthophyll is reasonable since they both are produced via a common biosynthetic pathway. Thus, it would appear that the modifier genes are suppressing the mutant cl_1 (w_{7716} or cl_p) that blocks normal carotenoid synthesis.

But why should chlorophyll also closely approximate the levels of the other pigments in these suppressed genotypes? Tests have shown that, in albino or suppressed phenotypes, there is normal chlorophyll production; yet, in albinos ($cl_M\ cl_M$) and lethal pale-green seedlings ($Cl^2{}_M\ Cl_M$, $Cl^5{}_M\ cl_M$, $Cl^2{}_M\ Cl^2{}_M$, and lethal green seedlings ($Cl^2{}_M\ Cl^5{}_M$ and $Cl^4{}_M\ cl_M$), chlorophyll remains roughly proportional to the carotenoid pigments. When near normal levels of carotenoids are produced, near normal chlorophyll levels are observed. These parallel levels of chlorophyll and carotenoids, especially in light of the known ability of these genotypes to make chlorophyll, are in agreement with the work of Anderson and Robertson[4,5] which indicates that one of the roles of colored carotenoids is to protect chlorophyll from photodestruction. Low chlorophyll levels in plants with low carotenoid levels could be the result of the destruction of all chlorophyll produced above the amount that the limited carotenoids of these genotypes will protect.

It has been suggested that a correlation between chlorophyll and carotenoid production might result from the degradation of carotenoids to supply the phytol group of chlorophyll or that phytol and carotene utilize a common precursor.[15] In these albino mutants, the

former cannot explain the relationship between these two pigments since no colored carotenoids are produced. Nor can the latter explanation suffice since it has been demonstrated that the albino mutants can produce chlorophyll (with phytol) in the absence of colored carotenoids. If a common precursor is involved in synthesis of carotenoids and phytol, it must come before the biosynthetic block in these mutants.

Another possible explanation for the simultaneous involvement of these three pigments might be the result of some structural change in the plastid that would permit the production of chlorophyll but which would in some way interfere with carotenoid formation. The suppressors might then act to correct this defect in plastid structure, which would permit a parallel increase in these pigments depending upon the degree of normal structure realized. The condition of the plastids in these mutants is not known. Electron microscopy studies of dark grown plants of other white-albino mutants have shown that they are quite similar to those of dark grown normals. Studies are planned on the plastid structure of the cl_1 albinos and various suppressed mutants of this locus in an attempt to see if a structural change might be involved.

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