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Communication

Synthesis of Two Chromoplast-Specific Proteins During Fruit Development in *Capsicum annuum*[1]

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ABSTRACT

The time-course of accumulation of two membrane proteins during fruit ripening was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blots in tissue extracts of *Capsicum annuum* L., vars Emerald Giant, Albino, and DNAP VS-12. The proteins, named ChrA and ChrB, were previously shown to occur specifically in chromoplasts. Fruit development was divided into five stages based on changes in color. ChrA was not detectable in the first three stages, but accumulated to a high level in the fully mature, red fruit. ChrB was not detectable in the first, mature-green stage of fruit maturation, but was found in the second stage, when carotenoid accumulation first appeared, and in all later stages. The patterns of accumulation in chromoplasts that develop from proplastids or leucoplasts are similar to those in chromoplasts that develop from chloroplasts. We conclude that ChrA and ChrB are probably synthesized *de novo* during chromoplast development.

In analyzing the molecular genetics of chromoplast development, it is useful to consider *chr*[2] and we have undertaken a program for the isolation of members of the set. For reasons cited earlier (2), we have chosen to work with the bell pepper *Capsicum annuum* L.

From the available data it appears unlikely that *chr* genes will be found in the plasmid genome (2, 3, 7, 8, 11, 12). Plant breeders, however, have identified a number of genes that are probably *chr*. *C. annuum* has four Mendelian loci that affect chromoplast development in mature fruit (5) and two other loci that affect chromoplast development in immature fruits (10). Our strategy has been to isolate chromoplast-specific proteins and, through these, to isolate *chr* genes.

We previously reported the specific accumulation of a 58-kD and a 35-kD protein in chromoplasts of *C. annuum* (4). The data could not, however, distinguish between *de novo* synthesis of the proteins and the posttranslational conversion of these proteins from precursors. Evidence that these proteins are indeed synthesized during chromoplast development would be crucial in identifying the structural genes for these proteins as chromoplast specific. We have sought to resolve this question by analyzing proteins from developing pepper fruits by western blots. We employed three varieties of pepper which differ in chromoplast ontogeny: var Emerald Giant shows the familiar pattern of chromoplasts developing from chloroplasts; vars Albino and DNAP VS-12 have the *gg* genotype (10), in which chromoplasts arise from proplastids or leucoplasts.

MATERIALS AND METHODS

Plant Material

Fruits of *Capsicum annuum* L., vars Emerald Giant, Albino, and DNAP VS-12 were grown in the greenhouse. Fruits were harvested and the proteins extracted on the same day.

Stages of Fruit Ripening

Unlike the usual pattern in tomato, pigmentation of pepper fruits develops unevenly in different portions of the fruit. There is, therefore, no commonly employed system for designating stages of fruit ripening. We arbitrarily identified five stages for the three varieties of pepper (Table I).

Table I. *A System for Identifying Stages in the Maturation of Pepper Fruits*

Color development was used as an indicator of fruit maturation. Because carotenoids accumulate while Chl are still present in var Emerald Giant, parts of the fruit in stages 2 through 4 appear brown. In Albino and DNAP-VS 12 Chl is absent or nearly so at all stages of fruit development, so that fruit color changes directly from white to red. Color development occurs more uniformly in these varieties.

Developmental stage	Color	
	var Emerald Giant	vars Albino and DNAP-VS 12
1	Mature green	White
2	Green with some brown areas	Yellowish orange
3	Mostly brown	Orange
4	Mostly brown, some red areas	Reddish orange
5	Red	Red

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[2] Abbreviations: *chr*, the set of genes that control chromoplast development or are uniquely expressed during chromoplast development; ChrA and ChrB, chromoplast-specific proteins of 58- and 35-kD, respectively; TBS, Tris-buffered saline.

Isolation and Analysis of Chromoplast Proteins

Proteins were purified from chromoplasts as described by Hadjeb *et al.* (4), subjected to electrophoresis (1), and stained with Coomassie blue.

Preparation of Antibodies

Purified proteins were isolated from preparative gels (Russell Durbin, personal communication) and injected subcutaneously and intramuscularly in rabbits. Production of antisera was carried out by Lampire Biological Laboratories.

Protein Extraction from Fruit Membranes

Total membrane protein was extracted from whole tissue substantially as described by Marder *et al.* (9).

Immuno Blots

The proteins were transferred by western blotting onto Immobilon blotting membrane (Millipore; Bedford, MA) substantially as described by Towbin *et al.* (13) with modifications (14, 15). Blots were treated according to Huynh *et al.* (6).

RESULTS AND DISCUSSION

Detection of ChrA and ChrB

The two chromoplast-specific proteins, designated ChrA and ChrB, were originally identified as chromoplast-specific proteins by the electrophoretic analysis of gradient-purified plastids from fruits of *C. annuum* L. (4). Because we could not be certain of the efficiency of recovery of plastids from different fruit tissues, we employed total membrane proteins for the analysis of the time-course of accumulation of ChrA and ChrB during fruit ripening. Using a procedure developed for the analysis of Q_B-binding protein (9), we recovered polypeptides with the same electrophoretic mobilities as ChrA and ChrB in the clarified detergent extract and negligible amounts elsewhere (data not shown).

Accumulation of ChrA and ChrB in Ripening Fruit

Total membrane proteins isolated from three varieties of pepper fruits taken throughout ripening were analyzed by SDS-PAGE (Fig. 1). Proteins similar in mobility to ChrA were detected in all five stages of development. We had previously detected a protein in green fruits that comigrates with ChrA in SDS gels, but is distinct from ChrA in urea and 2D gels

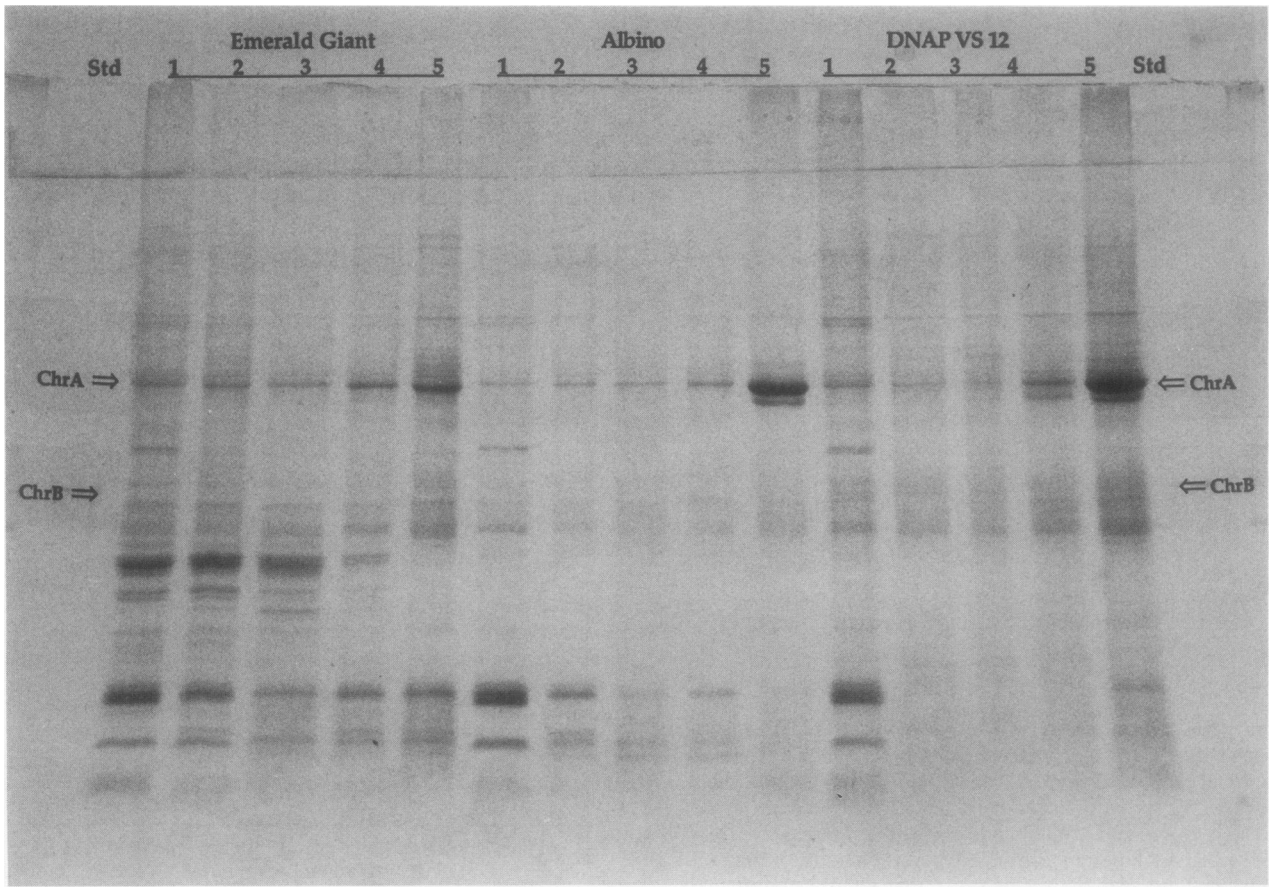

Figure 1. Electrophoresis of total membrane proteins of *Capsicum annuum* fruits collected at different stages of maturation. Membrane proteins were isolated from fruits and analyzed by SDS-PAGE and stained with Coomassie blue. Samples from vars Emerald Giant, Albino, and DNAP VS-12 were collected at stages of maturation described in Table I. The positions of authentic ChrA and ChrB are indicated at either side.

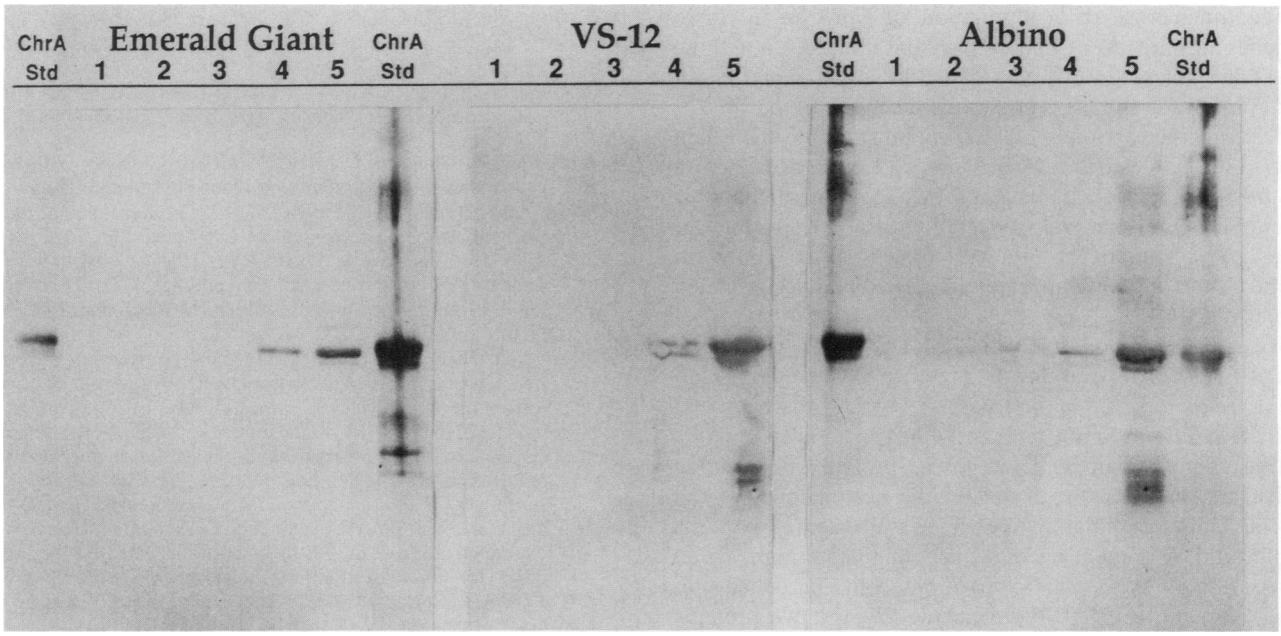

Figure 2. Western blot of ChrA during fruit maturation of *Capsicum annuum*. Membrane proteins from fruits of three varieties were collected at different stages of maturation and subjected to SDS-PAGE as in Figure 1. The gels were blotted and probed with antiserum to ChrA. Signals corresponding to ChrA are detected only in late stages of maturation.

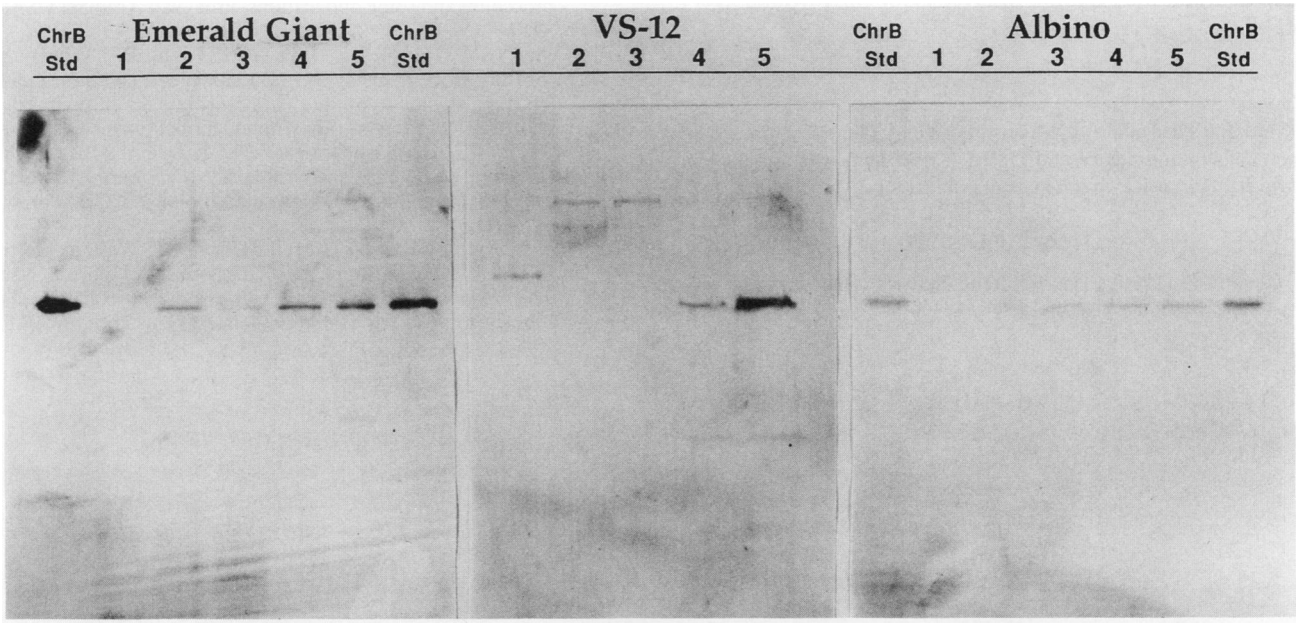

Figure 3. Western blot of ChrB during fruit maturation of *Capsicum annuum*. Membrane proteins from fruits of three varieties were collected at different stages of maturation and subjected to SDS-PAGE as in Figure 1. The gels were blotted and probed with antiserum to ChrB. In vars Emerald Giant and Albino, signals corresponding to ChrB are detected in stages 2 through 5. In var DNAP VS-12, signals of higher mol wt are visible in stages 1 through 3, whereas signals corresponding to ChrB itself accumulate only in stages 4 and 5.

(4). We did not detect ChrB in stained gels of total membrane proteins. Electrophoresis and protein staining alone, therefore, were insufficient for the analysis of these proteins in fruit development.

Blots of gels were challenged with antisera to ChrA and ChrB. Anti-ChrA (Fig. 2) detected no antigen in stages 1 through 3, small accumulations in stage 4, and large amounts in the mature, red fruit. Anti-ChrB (Fig. 3) detected the appearance of ChrB in stage 2 with increasing amounts in later stages. Several samples showed small amounts of cross-reacting material. ChrA and ChrB did not cross-react with one another.

The time-course of accumulation of ChrA in all three varieties appeared to be qualitatively the same. Although the time-course of accumulation of ChrB in vars Emerald Giant and Albino was similar, samples from stage 1 of var DNAP VS 12 contained a protein of lower mobility than ChrB which reacted strongly with anti-ChrB. This protein was not detected in subsequent stages. Also in var DNAP VS-12, small amounts of cross-reacting material of very high mol wt appeared in stages 2 and 3 and of low mol wt in stages 4 and 5. A protein of the correct mobility appeared in stage 4 and accumulated strongly in stage 5.

We also examined ripe fruits of tomato (*Lycopersicon esculentum* var Horizon) for the presence of ChrA and ChrB. We detected only the faintest bands in western blots probed with anti-ChrB and none with anti-ChrA (data not shown).

The absence of significant amounts of protein cross-reacting with ChrA in immature fruit and the near identity of the kinetics of its accumulation in varieties with disparate patterns of plastid development make it likely that ChrA arises by *de novo* synthesis. With some reservations, a similar conclusion can be drawn for ChrB. We conclude that the two proteins are synthesized as part of the program of chromoplast development and that they belong therefore to the proposed class of Chr proteins.

We shall report elsewhere (N Hadjeb, CA Price, unpublished data) on the isolation of cDNAs for *chrA* from a λgt11 library.

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LITERATURE CITED

1. **Chua N-H** (1980) Electrophoretic analysis of chromoplast proteins. Methods Enzymol **69:** 434–446
2. **Gounaris I, Michalowski CB, Bohnert HJ, Price CA** (1986) Restriction and gene maps of plastid DNA from *Capsicum annuum*. Curr Genet **11:** 7–16
3. **Gounaris I, Price CA** (1987) Plastid transcripts in chloroplast and chromoplasts of *Capsicum annuum*. Curr Genet **12:** 219–224
4. **Hadjeb N, Gounaris I, Price CA** (1988) Chromoplast-specific proteins in *Capsicum annuum*. Plant Physiol **88:** 42–45
5. **Hurtado-Hernández H, Smith PG** (1985) Inheritance of mature fruit color in *Capsicum annuum* L. J Hered **86:** 211–212
6. **Huynh TV, Young RA, Davis RW** (1984) Constructing and screening cDNA libraries in λgt10 and λgt11. *In* D Glover, ed, DNA Cloning Techniques: A Practical Approach. IRL Press, Oxford, pp 49–78
7. **Iwatsuki N, Hirai A, Asahi T** (1985) A comparison of tomato fruit chloroplast and chromoplast DNAs as analyzed with restriction endonucleases. Plant Cell Physiol **26:** 599–602
8. **Kuntz M, Evrard J-L, d'Harlingue A, Weil J-H, Camara B** (1989) Expression of plastid and nuclear genes during chromoplast differentiation in bell pepper (*Capsicum annuum*) and sunflower (*Helianthus annus*). Mol Gen Genet **216:** 156–163
9. **Marder JB, Mattoo AK, Edelman M** (1986) Identification and characterization of the *psbA* gene product: the 32-kDa chromoplast membrane protein. Methods Enzymol **118:** 384–396
10. **Odland ML, Porter AM** (1938) Inheritance of the immature fruit color of peppers. Proc Am Soc Hortic Sci **36:** 647–651
11. **Piechulla B, Chonoles Imlay KR, Gruissem W** (1985) Plastid gene expression during fruit ripening in tomato. Plant Mol Biol **5:** 373–384
12. **Thompson WW, Whatley JM** (1980) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA **76:** 4350–4354
14. **Tsang VCW, Hancock K, Wilson M, Palme DF, Whaley SD, McDougal JS, Kennedy S** (1987) Enzyme-linked immunoelectro transfer blot technique (western blot) for human T-lymphotropic virus type III/lymphodenopathy-associated virus (HTLV-III/LAV) antibodies. *In* K Jeysellan, MCM Chung, OL Kon, eds, Genes and Proteins: A Laboratory Manual of Selected Techniques in Molecular Biology, ISCU, Paris, pp 171–181
15. **Winston SE, Fuller SA, Hurrell JGR** (1987) Western blotting. *In* FM Ausubel, R Brent, RE Kingston, DD Moore, JG Seidman, JA Smith, K Struhl, eds, Current Protocols in Molecular Biology. Greene Publishing Associates, New York, pp 10.8.1–10.8.6