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Expression of Plant Chaperonin-60 Genes in *Escherichia coli**

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We have examined the expression in *Escherichia coli* of genes encoding a plant chloroplast molecular chaperone, chaperonin-60. Purified plant chaperonin-60 is distinct in that it contains two polypeptides, p60$^{cpn\text{-}60\alpha}$ and p60$^{cpn\text{-}60\beta}$, which have divergent amino acid sequences (Hemmingsen, S. M., and Ellis, R. J. (1986) *Plant Physiol.* 80, 269–276; Martel, R., Cloney, L. P., Pelcher, L. E., and Hemmingsen, S. M. (1990) *Gene (Amst.)* 94, 181–187). The precise polypeptide composition(s) of the active tetradecameric specie(s) (cpn60$_{14}$) has not been determined. Genes encoding the mature forms of the *Brassica napus* chaperonin polypeptides have been expressed separately and in combination in *E. coli* to produce three novel strains: α, β, and $\alpha\beta$. The plant cpn60 polypeptides accumulated in soluble forms and to similar high levels in each. There was no conclusive evidence that p60$^{cpn\text{-}60\alpha}$ assembled into cpn60$_{14}$ species in α cells. In β and $\alpha\beta$ cells, the plant gene products assembled efficiently into cpn60$_{14}$ species. Thus, the assembly of p60$^{cpn\text{-}60\alpha}$ required the presence of p60$^{cpn\text{-}60\beta}$, whereas the assembly of p60$^{cpn\text{-}60\beta}$ could occur in the absence of p60$^{cpn\text{-}60\alpha}$. Significant proportions of the endogenous groEL polypeptides were not assembled into tetradecameric groEL$_{14}$ in β and $\alpha\beta$ cells. Analysis of the tetradecameric species that did form indicated the presence of novel hybrid cpn60$_{14}$ species that contained both plant and bacterial cpn60 polypeptides.

Chaperonins (cpn),[1] are evolutionarily related molecular chaperones found in prokaryotes and eukaryotes (3–5). Chaperonin-60 has been implicated in the assembly of rubisco in higher plant plastids (6–9). Although the extent to which the functions of molecular chaperones are general or specific has not been defined, it has been suggested that in some cases of failed assembly of foreign proteins, co-expression of the appropriate molecular chaperone might overcome this problem (5, 10–12). This approach would require successful heterologous synthesis and assembly of functional molecular chaperones.

The *Escherichia coli* chaperonins, cpn60 (groEL) and cpn10 (groES), are encoded in the *groE* operon (3). groEL functions as a homotetradecamer (groEL$_{14}$) of 60-kDa polypeptides, and groES functions as a homoheptamer (groES$_7$) of 10-kDa polypeptides (4, 13). The cpn60 of higher plant plastids also functions as a tetradecamer (cpn60$_{14}$) (14). However, higher plant plastid cpn60$_{14}$ differs from groEL$_{14}$ in that the purified protein is composed of two distinct polypeptides, p60$^{cpn\text{-}60\alpha}$ and p60$^{cpn\text{-}60\beta}$ (1). These polypeptides are as divergent in sequence from one another as each is from groEL (2). The precise polypeptide composition of plastid cpn60$_{14}$ has not been determined. The compositions may be restricted, for example, α_{14}, β_{14}, or $\alpha_7\beta_7$, or unrestricted, for example, $\alpha_n\beta_{14-n}$. The functions of *cpn-60α* and *cpn-60β* may be independent or interdependent. Insight into these questions might be gained by expression of these genes in *E. coli*. Such a study would be simplified in an *E. coli* strain lacking groEL; however, *groE* functions are essential for viability (15), and complementation of *groEL* mutants by a closely related homolog has only recently been reported (16).

We have expressed the *Brassica napus cpn-60α* and *cpn-60β* genes separately and in combination in *E. coli* strains containing normal levels of the endogenous chaperonin proteins. We report the synthesis of *B. napus* chloroplast chaperonin polypeptides, their assembly into tetradecameric species, and their effects on the endogenous *E. coli* chaperonin groEL$_{14}$.

MATERIALS AND METHODS

Construction of Expression Plasmids—The *lacI*^{Q} gene, flanked by *Eco*RI (17), was adapted to *Bam*HI and ligated to pKK233-2 (18), which had been cut with *Bam*HI, to produce pKK. *cpn-60α* and *cpn-60β* cDNA clones (2) were modified by site-directed mutagenesis to introduce *Nco*I sites at the position of the mature N termini. The cDNA inserts were excised with *Eco*RI, repaired to blunt ends, and cut with *Nco*I, and each was ligated to pKK that had been cut with *Hin*dIII, repaired, and cut with *Nco*I. The resulting plasmids were pKKα and pKKβ. pKKβ was cut with *Sca*I, *Eco*RI linkers were added and cut with *Eco*RI, and the fragment containing *cpn-60β* was ligated to pKKα, which had been cut with *Eco*RI, to produce pKK$\alpha\beta$. The orientation shown (Fig. 1) was chosen for this study.

Bacterial Growth Conditions—*E. coli* DH5α cells (19) harboring the appropriate plasmid were innoculated from overnight cultures into 2 × YT medium containing 100 μg/ml ampicillin at 37 °C. Isopropylthiogalactoside was added to 0.5 mM final concentration when the apparent absorbance (590 nm) of the culture reached 0.5, typically after 2 to 3 h, and the culture was incubated for a further 15 h at 37 °C.

Extraction of Proteins from B. napus and E. coli—Crude chloroplast stromal protein extracts were prepared from 6-day-old *B. napus* cotyledons as described (1). *E. coli* cells were recovered by centrifugation and resuspended in either TBST (50 mM Tris-Cl, 150 mM NaCl, 0.05% v/v Tween-20, pH 7.9) or TBSB (50 mM Tris-Cl, 150 mM NaCl, pH 7.9, 20% v/v glycerol, 2 mM phenylmethylsulfonyl fluoride, 4 μg/ml chymostatin, 2 μg/ml leupeptin, 2 μg/ml pepstatin, 1 μg/ml aprotinin (all from Sigma)). Cell suspensions were passed through a French pressure cell at 6,000 p.s.i. Soluble protein extracts were defined as the supernatants recovered after centrifugation, 8000 × *g* for 10 min. Total protein extracts were supernatants recovered after cells were lysed at 95 °C for 5 min directly in SDS sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 2% SDS, 2% 2-mercaptoethanol, and 10% glycerol.

Polyacrylamide Gel Electrophoresis (PAGE) and Immunoblotting—

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[1] The abbreviations used are: cpn, chaperonin(s); rubisco, ribulose-P$_2$ carboxylase; PAGE, polyacrylamide gel electrophoresis.

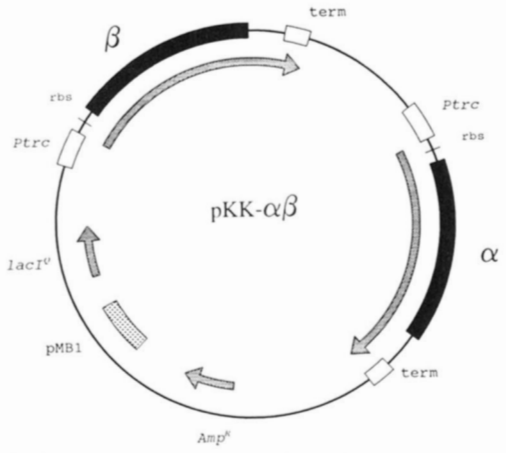

FIG. 1. **Schematic representation of vector for expression of plant *cpn60* genes in *E. coli*.** Plasmids pKKα, pKKβ, and pKK$\alpha\beta$ were constructed as described under "Materials and Methods." pKK$\alpha\beta$ is illustrated. The synthetic *trp-lac* promoter (*Ptrc*), *lac* repressor gene ($lacI^q$), ribosome binding sites (*rbs*), transcription terminators (*term*), high copy number origin of replication (*pMB1*), ampicillin resistance gene (Amp^R), and the orientations of the coding sequences for mature $p60^{cpn\text{-}60\alpha}$ (α), and $p60^{cpn\text{-}60\beta}$ (β), are indicated.

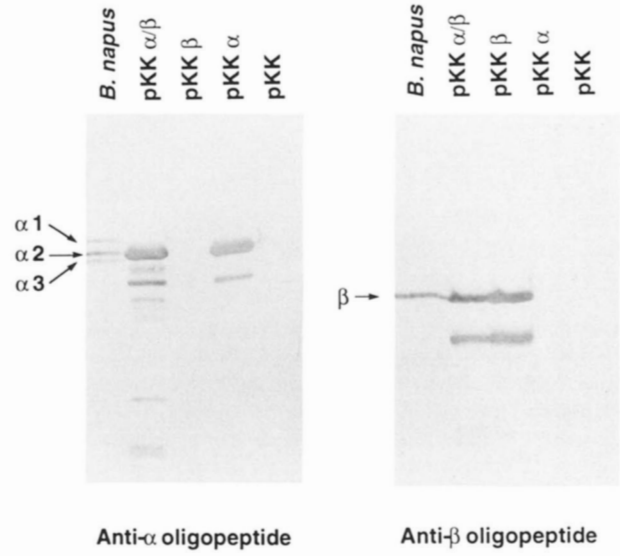

FIG. 2. **Identification of plastid *cpn60* gene products synthesized in *E. coli*.** Total extracts from *E. coli* cells harboring plasmids pKKα, pKKβ, or pKK$\alpha\beta$ were resolved by SDS-PAGE (15%), blotted onto nitrocellulose, and probed with the indicated oligopeptide-directed antisera. *B. napus* chloroplast stromal proteins were electrophoresed to provide references. *Arrows* indicate the positions of migration of the mature $p60^{cpn\text{-}60\alpha}$ polypeptides and the mature $p60^{cpn\text{-}60\beta}$ polypeptide. The mature α_2 and the mature β polypeptides have predicted relative molecular weights of 56,768 and 56,571, respectively (2). The resolution of these polypeptides requires the specific conditions described.

For SDS-PAGE analysis, protein preparations were treated with SDS sample buffer as described above and resolved on 15% gels (acrylamide:*N*,*N*′-methylenebisacrylamide, 30%:0.3%), or on 9% gels (acrylamide:*N*,*N*′-methylenebisacrylamide, 30%:0.8%) as indicated. For nondenaturing PAGE analysis, soluble protein preparations containing 10% glycerol were resolved on 5% gels as previously described (1). Immunoblotting was as described (1), except that the secondary antibody was an alkaline phosphatase conjugate from Promega Biotec. Color development was with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Antibodies—Two oligopeptides (LSIESSSSFETTVE and TLEEGKSAENAL) were synthesized chemically, conjugated to keyhole limpit hemocyanin (Multiple Peptide Systems, San Diego), and used to raise polyclonal antisera in rabbits. The first was derived from the predicted sequence of $p60^{cpn\text{-}60\alpha}$, the second from $p60^{cpn\text{-}60\beta}$. These antibodies were used to identify $p60^{cpn\text{-}60\alpha}$ and $p60^{cpn\text{-}60\beta}$ polypeptides. Anti-plant chaperonin-60 has been described (1). This antiserum, after depletion against an *E. coli* lysate, is specific for the plant gene products and does not recognize groEL on immunoblots. Anti-*E. coli* groEL was a gift from R. Hendrix (University of Pittsburgh). This antiserum is specific for groEL and does not recognize the plant cpn60 polypeptides on immunoblots.

Sucrose Density Gradient Centrifugation—Protein extracts made in TBST were layered onto 13-ml sucrose gradients (5–50% (w/v) sucrose in 10 mM Tris-Cl, 10 mM $MgSO_4$, 0.5 mM EDTA, pH 8.0) and centrifuged at 180,000 × *g* for 21.5 h at 4 °C (1).

Gel Permeation Chromatography—Soluble protein extracts made in TBSB were passed through 0.2-μ filters and chromatographed on a Superose 6 HR 10/30 (Pharmacia LKB Biotechnology Inc.) column pre-equilibrated and run with Buffer A (20 mM Tris-Cl, pH 7.3, 20% (v/v) glycerol, 100 mM KCl, 1 mM 2-mercaptoethanol). Molecular mass standards including carbonic anhydrase (29 kDa), albumin (66 kDa), alcohol dehydrogenase (150 kDa), α-amylase (200 kDa), apoferritin (443 kDa), and thyroglobulin (669 kDa) (Sigma) were used as references.

Ion Exchange Chromatography—Protein fractions recovered from gel permeation chromatography were dialyzed overnight against Buffer B (Buffer A minus KCl) and loaded onto a Mono Q HR 5/5 (Pharmacia LKB Biotechnology Inc.) column pre-equilibrated with Buffer B. The column was washed with 10 bed volumes of Buffer B, and bound proteins were eluted with a 0–500 mM linear NaCl gradient in Buffer B.

RESULTS AND DISCUSSION

Construction of Vectors for the Regulated Expression of Plant cpn60 Genes in E. coli—Plastid chaperonin polypeptides are synthesized in the cytoplasm as larger precursors (1, 2). To assess plastid chaperonin function in *E. coli*, synthesis of the mature forms of the polypeptides was directed by replacing the Asn and Gln codons corresponding to the mature N termini of $p60^{cpn\text{-}60\alpha}$ and $p60^{cpn\text{-}60\beta}$, respectively (2), with Met codons. These modified plant *cpn-60α* and *cpn-60β* genes were introduced separately and in combination into a modified version of vector pKK233-2 (18) under the control of P*trc* and a co-resident copy of $lacI^q$. Thus, three vectors were constructed: pKKα, pKKβ, and pKK$\alpha\beta$ (Fig. 1).

Identification of Plant cpn60 Gene Products Synthesized in E. coli—When purified *B. napus* chloroplast $cpn60_{14}$ is subjected to SDS-polyacrylamide gel electrophoresis, four stainable bands are observed. We have identified three of these as α-polypeptides and one as a β-polypeptide. The characterization of this protein will be reported elsewhere.[2]

E. coli cells harboring pKKα (α cells), accumulated a novel polypeptide, recognized by an α polypeptide-specific antiserum (Fig. 2). This band co-migrated with the middle of the three α bands present in *B. napus* plastid stromal protein extracts. Cells harboring pKKβ (β cells) accumulated a novel polypeptide, recognized by a β polypeptide-specific antiserum (Fig. 2). It co-migrated with the β band of *B. napus* plastid stromal protein. Cells harboring pKK$\alpha\beta$ ($\alpha\beta$ cells) accumulated both of these novel polypeptides. In total extracts from each of these strains, immunoreactive bands of lower apparent M_r were also observed. These may have resulted from proteolysis during isolation or from proteolysis within the cell or illegitimate translation.

Assembly of cpn-60α and cpn-60β Gene Products Synthesized in E. coli—To determine if the plant cpn60 polypeptides were assembled into $cpn60_{14}$ species in *E. coli*, soluble proteins from α, β, or $\alpha\beta$ cells were resolved by sucrose-density gradient centrifugation. Plant gene products present in each gradient fraction were identified by probing immunoblots with anti-plant chaperonin-60 serum (Fig. 3, *right*). A bimodal distri-

[2] L. P. Cloney and S. M. Hemmingsen, manuscript in preparation.

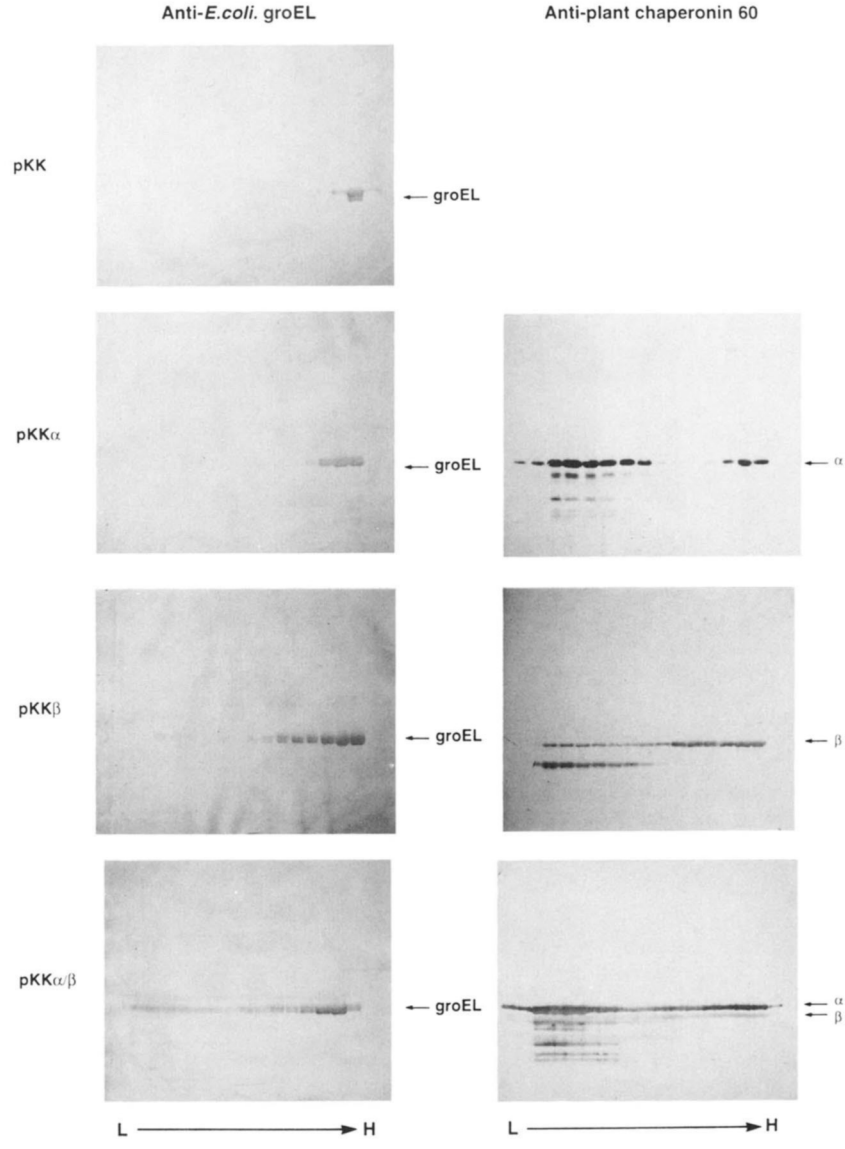

FIG. 3. **Size distribution of chaperonin gene products in extracts from *E. coli* cells expressing plant *cpn60* genes.** Proteins extracted from *E. coli* cells harboring pKK, pKKα, pKKβ, or pKK$\alpha\beta$ as indicated, were separated by sucrose density gradient centrifugation. Gradients were fractionated from high M_r (H) to low M_r (L), and proteins were resolved by SDS-PAGE (9%), blotted onto nitrocellulose, and probed with antibodies that recognized specifically either *E. coli* or plant chaperonin-60 polypeptides. *Arrows* indicate the positions of migration of groEL polypeptide, mature p60$^{cpn\text{-}60\alpha}$ (α), and mature p60$^{cpn\text{-}60\beta}$ (β).

bution of the plant gene products was observed when any of the three expression plasmids was present. The relative proportions of plant gene products found in the high M_r fractions (H) varied with the construct present. The greater proportion of mature p60$^{cpn\text{-}60\alpha}$ present in extracts from α cells appeared to migrate in the gradient as low M_r forms (L), whereas the greater proportion of mature p60$^{cpn\text{-}60\beta}$ in β cell extracts migrated as high M_r forms. Analysis of $\alpha\beta$ cell extracts revealed the presence of p60$^{cpn\text{-}60\alpha}$ and p60$^{cpn\text{-}60\beta}$ in both high and low M_r forms. In extracts from each of the three strains, the immunoreactive bands of smaller size were present only in the low M_r fractions. Only mature p60$^{cpn\text{-}60\alpha}$ and p60$^{cpn\text{-}60\beta}$ co-migrated with groEL$_{14}$.

The plant gene products that co-migrated with groEL$_{14}$ might have been present as (*a*) homotetradecamers, (*b*) α/β heterotetradecamers, (*c*) components with groEL of hybrid tetradecamers, or (*d*) polypeptides bound to the surface of the groEL tetradecamer in a groEL$_{14}$-polypeptide complex similar to the binary complexes that form between groEL$_{14}$ and other unfolded polypeptides (4, 20–23). In such complexes, it has been reported that groEL$_{14}$ binds a single unfolded polypeptide (24, 25). The expected ratio of bound plant p60$^{cpn\text{-}60}$ to groEL polypeptide in groEL$_{14}$-p60$^{cpn\text{-}60}$ complexes would therefore be low, probably less than 1:14.

Soluble extracts were prepared from α, β and $\alpha\beta$ strains (Fig. 4, *lanes S*). In each case, more plant cpn60 polypeptides were present than groEL polypeptide. Soluble proteins were resolved by gel permeation chromatography, and fractions corresponding to groEL$_{14}$ (H) and monomeric groEL (L) were isolated (Fig. 4). Plant cpn60 polypeptides were detectable in both of these fractions by immunoblot analysis (not shown). Quantities of plant polypeptides detectable by Coomassie Blue staining were present in fractions corresponding to monomeric groEL (L) from α and $\alpha\beta$ cell extracts. Quantities of plant polypeptides detectable by staining were present in the groEL$_{14}$ fractions (H) from α, β, and $\alpha\beta$ cell extracts. In agreement with the sucrose-density gradient analysis, the relative proportions of the plant polypeptides that co-migrated with groEL$_{14}$ varied depending on which plant genes were expressed.

In the groEL$_{14}$ fraction (H) from α cell extracts, considerably less plant polypeptide was present than groEL polypeptide. This low ratio of p60$^{cpn\text{-}60\alpha}$ to groEL may suggest that the plant polypeptide is bound unassembled to the surface of groEL$_{14}$ (groEL$_{14}$-p60$^{cpn\text{-}60\alpha}$) in α cells. Alternatively, p60$^{cpn\text{-}60\alpha}$ may be inefficiently assembled into a tetradecameric specie(s)

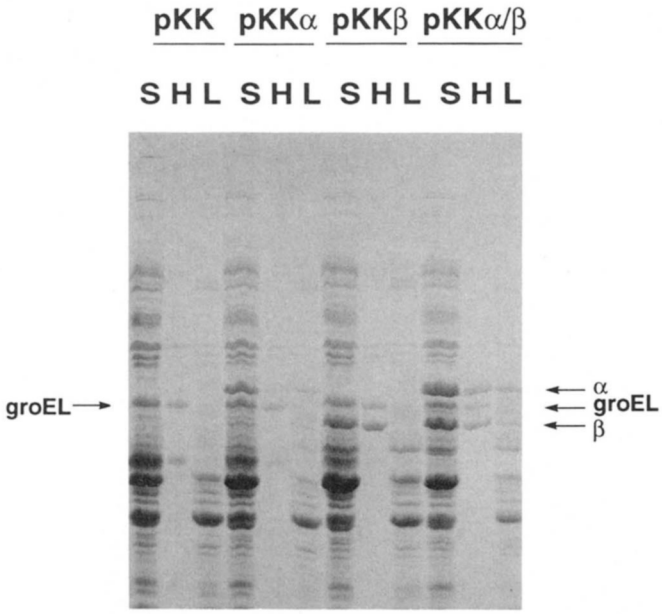

FIG. 4. **Levels of accumulation and assembly of bacterial and plant cpn60 proteins.** Soluble protein extracts were prepared from *E. coli* cells expressing the plant *cpn-60* genes indicated in the figure and resolved by gel permeation chromatography. Polypeptides from crude soluble protein (*S*), the fraction containing the peak quantity of oligomeric $cpn60_{14}$ (*H*) (approximately 840 kDa), and the fraction representing monomeric cpn60 (*L*) (approximately 60 kDa) were resolved by SDS-PAGE (15%) and visualized by Coomassie Blue R-250 staining. *Arrows* indicate the positions of migration of groEL polypeptide, mature $p60^{cpn\text{-}60\alpha}$ (α), and mature $p60^{cpn\text{-}60\beta}$ (β).

in α cells. In the high M_r fractions (*H*) from β and $\alpha\beta$ cell extracts, the levels of plant polypeptide present exceeded that of groEL polypeptide. This high ratio of plant $p60^{cpn60}$ to groEL polypeptide rules out, for these two strains, the possibility that the plant gene products are present only as unfolded polypeptides bound to the surface of $groEL_{14}$. Therefore, when $p60^{cpn\text{-}60\beta}$ is present alone, and when both $p60^{cpn\text{-}60\alpha}$ and $p60^{cpn\text{-}60\beta}$ are present together, they are assembled into tetradecameric species.

Effect of Expression of Plant cpn60 Genes on E. coli groEL Protein—The presence of high levels of foreign polypeptides might be expected to induce expression of the *groE* operon (26–29). To determine if the presence of plant cpn60 polypeptides resulted in higher levels of groEL polypeptide, total and soluble protein extracts were analyzed by SDS-PAGE and Coomassie Blue staining. No difference was seen between the levels of total and soluble groEL polypeptide (not shown), and the levels of soluble groEL polypeptide in each strain were very similar (Fig. 4, *S*). Thus, the presence of plant cpn60 polypeptides does not induce expression of the *groE* operon.

To determine if the synthesis of plant cpn60 polypeptides affected the assembly or stability of $groEL_{14}$, soluble proteins from α, β, and $\alpha\beta$ cells, resolved by sucrose density gradient centrifugation, were immunoblotted with a groEL-specific antiserum (Fig. 3, *left*). In control cell extracts, groEL polypeptide was detected as a single high M_r specie, $groEL_{14}$. The distribution of groEL polypeptide in the gradient in which α cell proteins were resolved was similar to the control. Analysis of extracts from β and $\alpha\beta$ cells revealed groEL polypeptide distributed throughout the gradients in what appeared to be bimodal distributions. Analysis of soluble proteins by gel permeation chromatography confirmed that in β and $\alpha\beta$ cell extracts, groEL polypeptide was found predominantly in two forms corresponding to monomer and tetradecamer (not shown). Thus, in β and $\alpha\beta$ cells, the presence of plant cpn60 polypeptides did affect the state of assembly of groEL polypeptides. We have shown that the plant polypeptides assemble into $cpn60_{14}$ species in these same two strains. It appears that the assembly of plant cpn60 polypeptides into $cpn60_{14}$ species rather than just their presence in the cell is required before groEL polypeptide accumulates in an unassembled form. This analysis could not distinguish whether the plant polypeptides disrupted preassembled $groEL_{14}$ oligomers or interfered with the assembly of $groEL_{14}$ from monomers. In either case, however, the result is a decrease in the abundance of $groEL_{14}$ in β and $\alpha\beta$ cells, since the total levels of groEL polypeptide are very similar.

Analysis of the Structure of $cpn60_{14}$ in Cells That Express Plant Chaperonin Genes—In the case of β and $\alpha\beta$ cells, we have shown that the plant polypeptides assemble into tetradecameric species, possibly (*a*) homotetradecamers, (*b*) α/β heterotetradecamers, or (*c*) hybrid tetradecamers. To further characterize these structures, the ion exchange characteristics of $cpn60_{14}$ from α, β, and $\alpha\beta$ cells were compared with those of authentic $groEL_{14}$ and authentic *B. napus* chloroplast $cpn60_{14}$ (Fig. 5).

Authentic $groEL_{14}$ and *B. napus* chloroplast $cpn60_{14}$ had distinctive ion exchange chromatographic characteristics. The peak elution of authentic $groEL_{14}$ was at 450 mM NaCl. Peak elution of both $p60^{cpn\text{-}60\alpha}$ and $p60^{cpn\text{-}60\beta}$ of authentic *B. napus* chloroplast $cpn60_{14}$ occurred at 405 mM NaCl. The ion exchange chromatographic characteristics of $cpn60_{14}$ protein from α, β, and $\alpha\beta$ cells were clearly different from one another. From this, we infer that different oligomeric species must have been present in each of these three strains. Closer inspection of the elution profiles confirmed this.

When *cpn-60α* was expressed alone in *E. coli*, the plant and bacterial cpn60 gene products present in $cpn60_{14}$ species coeluted with a peak at 450 mM NaCl. This peak and the appearance of the elution profile were characteristic of authentic $groEL_{14}$. Thus, in the case of α cells, this analysis could not determine whether the $p60^{cpn\text{-}60\alpha}$ polypeptides that co-migrated with $groEL_{14}$ did so as subunits of assembled $cpn60_{14}$ species or as polypeptides bound to the surface of $groEL_{14}$.

When *cpn-60β* was expressed alone in *E. coli*, the plant and bacterial cpn60 polypeptides eluted over a broadened range of salt concentration. Peak elution of $p60^{cpn\text{-}60\beta}$ and of groEL occurred at 375 and 405 mM NaCl, respectively. These elution profiles were not characteristic of either $groEL_{14}$ or plastid $cpn60_{14}$. When *cpn-60β* was co-expressed with *cpn-60α*, plant and bacterial cpn60 polypeptides eluted over an even broader range. Peak elution of groEL occurred at 435 mM NaCl. Fractions containing peak levels of $p60^{cpn\text{-}60\alpha}$ or $p60^{cpn\text{-}60\beta}$ were difficult to identify. Thus, none of the polypeptides eluted as would be expected if they were present as components of authentic plant or bacterial tetradecameric species. In fact, it appears from this analysis that the predominant $cpn60_{14}$ species present in these cells are novel hybrid molecules that contain both plant and bacterial cpn60 polypeptides. It is possible that authentic $groEL_{14}$ or authentic plant $cpn60_{14}$ species also exist in these cells. If so, their characteristic elution profiles are masked by the elution of novel species.

Implications with Respect to Functional Interdependence of cpn-60α and cpn-60β Gene Function—The successful synthesis of *B. napus* $p60^{cpn\text{-}60\alpha}$ and $p60^{cpn\text{-}60\beta}$ in *E. coli* allows the first examination of the interdependence of *cpn-60α* and *cpn-60β* gene functions. With co-expression of *cpn-60α* and *cpn-60β*, both plant gene products are efficiently assembled into $cpn60_{14}$ species. There is also efficient assembly of $p60^{cpn\text{-}60\beta}$

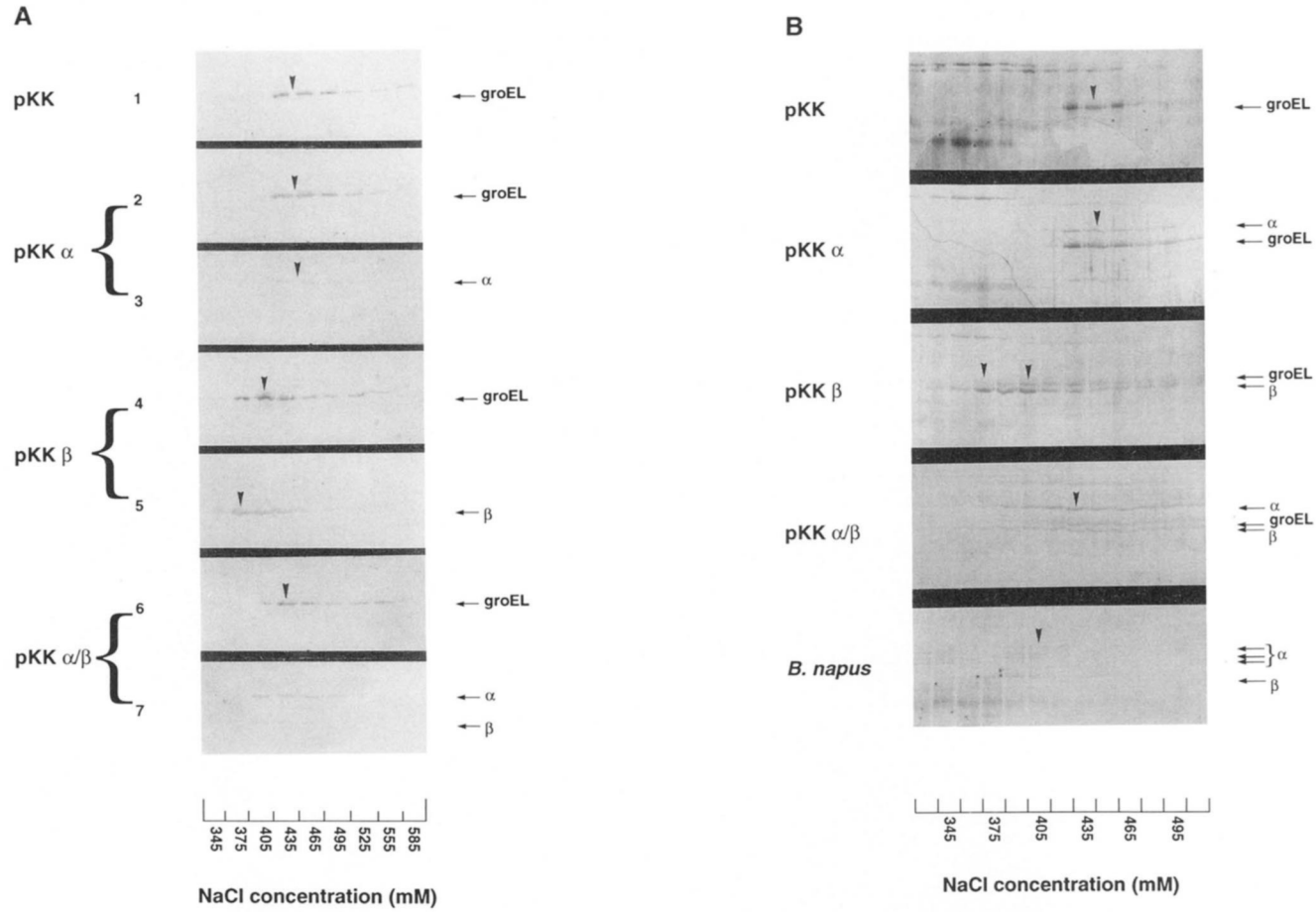

FIG. 5. **Analysis of high M_r hybrid chaperonin-60 proteins.** Soluble extracts were prepared from α, β, $\alpha\beta$, and control cells. Crude chloroplast stromal protein extracts were prepared from *B. napus*. Proteins were resolved by gel permeation chromatography. Proteins in fractions corresponding to $cpn60_{14}$ were further resolved by ion exchange chromatography. Proteins eluting at the salt concentrations indicated were analyzed by SDS-PAGE (15%). The cpn60 polypeptides present in each ion exchange fraction were identified by immunoblot analysis, and silver staining gave an independent estimate of relative quantities present. *A*, immunoblots were probed with anti-groEL (*blots 1*, *2*, *4*, and *6*), or with anti-chloroplast cpn60 (*blots 3*, *5*, and *7*). *B*, proteins were visualized by silver staining. Positions of migration of groEL, $p60^{cpn\text{-}60\alpha}$, and $p60^{cpn\text{-}60\beta}$ polypeptides are indicated with *arrows*. Fractions containing peak quantities of specific polypeptides are indicated with *arrowheads*.

in β cells. In contrast, $p60^{cpn\text{-}60\alpha}$ assembly in α cells, if it occurs, is very inefficient. It is tempting to suggest from these observations that the functions of *cpn-60α* and *cpn-60β* in the plant are not necessarily interdependent, but that $p60^{cpn\text{-}60\alpha}$ may depend on $p60^{cpn\text{-}60\beta}$ for its assembly into $cpn60_{14}$ and therefore also for its function. It is possible that $p60^{cpn\text{-}60\alpha}$ and $p60^{cpn\text{-}60\beta}$ assemble together as subunits of the same $cpn60_{14}$ specie or that some specialized function of $p60^{cpn\text{-}60\beta}$ is required to chaperone the assembly of $p60^{cpn\text{-}60\alpha}$.

There is evidence that hybrid $cpn60_{14}$ species, containing plant and bacterial polypeptides, form in β and $\alpha\beta$ cells. It is not clear whether authentic plant and bacterial $cpn60_{14}$ also form. These observations may suggest that unrestricted heterotetrameric species may form in cellular compartments that contain more than one type of cpn60 polypeptide. If so, this might be the case in the chloroplast ($\alpha_n\beta_{14-n}$). It remains possible that additional factors present in the chloroplast might permit or direct the assembly of $cpn60_{14}$ species with restricted polypeptide compositions such as α_{14}, β_{14}, or $\alpha_7\beta_7$.

Implications with Respect to the Potential Benefits of Expression of Foreign Chaperonin Genes—It has been proposed that the failed assembly of a heterologously synthesized polypeptide might be corrected by co-expression of the appropriate molecular chaperone gene. We have observed the synthesis and assembly into $cpn60_{14}$ species of high levels of foreign *cpn60* polypeptides in *E. coli* β and $\alpha\beta$ cells. However, two effects on the endogenous groEL are observed in these cells: (*a*) a proportion of the groEL is present as unassembled polypeptide and (*b*) a proportion of the groEL polypeptide found in tetradecameric species is present with plant cpn60 polypeptides in novel hybrid species. These observations raise the possibility that endogenous chaperonin functions may be affected by these interactions.

Although decreased levels of $groEL_{14}$ are present in β and $\alpha\beta$ cells, the contribution of the assembled plant polypeptides results in elevated levels of total $cpn60_{14}$. There is preliminary evidence from this study suggesting that the plant gene products may be active, since expression of *cpn-60β* is required for the efficient assembly of $p60^{cpn\text{-}60\alpha}$ into $cpn60_{14}$ species. The activity of the plant gene products synthesized in *E. coli* will be further assessed in a separate study (30).

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REFERENCES

1. Hemmingsen, S. M., and Ellis, R. J. (1986) *Plant Physiol.* **80,** 269–276
2. Martel, R., Cloney, L. P., Pelcher, L. E., and Hemmingsen, S. M. (1990) *Gene (Amst.)* **94,** 181–187
3. Hemmingsen, S. M., Woolford, C., van der Vies, S. M., Tilly, K., Dennis,

D. T., Georgopoulos, C. P., Hendrix, R. W., and Ellis, R. J. (1988) *Nature* **333,** 330–334
4. Zeilstra-Ryalls, J., Fayet, O., and Georgopoulos, C. (1991) *Annu. Rev. Microbiol.* **45,** 301–325
5. Ellis, R. J., and van der Vies, S. M. (1991) *Annu. Rev. Biochem.* **60,** 321–347
6. Barraclough, R., and Ellis, R. J. (1980) *Biochim. Biophys. Acta* **608,** 19–31
7. Gatenby, A. A., and Ellis, R. J. (1990) *Annu. Rev. Cell Biol.* **6,** 125–149
8. Ellis, R. J. (1990) *Science* **250,** 954–959
9. Roy, H., and Cannon, S. (1988) *Trends Biochem. Sci.* **13,** 163–165
10. Gatenby, A. A., Viitanen, P. V., and Lorimer, G. H. (1990) *Trends Biotech.* **8,** 354–358
11. Vierling, E. (1991) *Annu. Rev. Plant Physiol.* **42,** 579–620
12. Ellis, R. J. (1991) *Plant J.* **1,** 9–13
13. Georgopoulos, C., and Ang, D. (1990) *Semin. Cell Biol.* **1,** 19–25
14. Goloubinoff, P., Christeller, J. T., Gatenby, A. A., and Lorimer, G. H. (1989) *Nature* **342,** 884–889
15. Fayet, O., Ziegelhoffer, T., and Georgopoulos, C. (1989) *J. Bacteriol.* **171,** 1379–1385
16. Ohtaka, C., Nakamura, H., and Ishikawa, H. (1992) *J. Bacteriol.* **174,** 1869–1874
17. Zumstein, L., and Wang, J. C. (1986) *J. Mol. Biol.* **191,** 333–340
18. Amann, E., and Brosius, J. (1985) *Gene (Amst.)* **40,** 183–190
19. Hanahan, D. (1983) *J. Mol. Biol.* **166,** 557–580
20. Lubben, T. H., Donaldson, G. K., Viitanen, P. V., and Gatenby, A. A. (1989) *Plant Cell* **1,** 1223–1230
21. Lecker, S., Lill, R., Ziegelhoffer, T., Georgopoulos, C., Bassford, P. J., Jr., Kumamoto, C. A., and Wickner, W. (1989) *EMBO J.* **8,** 2703–2709
22. Buchner, J., Schmidt, M., Fuchs, M., Jaenicke, R., Rudolph, R., Schmid, F. X., and Kiefhaber, T. (1991) *Biochemistry* **30,** 1586–1591
23. Viitanen, P. V., Donaldson, G. K., Lorimer, G. H., Lubben, T. H., and Gatenby, A. A. (1991) *Biochemistry* **30,** 9716–9723
24. Mendoza, J. A., Lorimer, G. H., and Horowitz, P. M. (1991) *J. Biol. Chem.* **266,** 16973–16976
25. Laminet, A. A., Ziegelhoffer, T., Georgopoulos, C., and Plückthun, A. (1990) *EMBO J.* **9,** 2315–2319
26. Drahos, D. J., and Hendrix, R. (1982) *J. Bacteriol.* **149,** 1050–1063
27. Kochan, J., and Murialdo, H. (1982) *J. Bacteriol.* **149,** 1166–1170
28. Bahl, H., Echols, H., Straus, D. B., Court, D., Crowl, R., and Georgopoulos, C. P. (1987) *Genes & Dev.* **1,** 57–64
29. Goff, S. A., and Goldberg, A. L. (1985) *Cell* **41,** 587–595
30. Cloney, L. P., Bekkaoui, D. R., Wood, M. G., and Hemmingsen, S. M. (1992) *J. Biol. Chem.* **267,** 23333–23336