Structures of Chaperonins from an Intracellular Symbiont and Their Functional Expression in *Escherichia coli groE* Mutants

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An intracellular symbiont harbored by the aphid bacteriocyte, a specialized fat body cell, synthesizes in vivo substantially only one protein, symbionin, which is a member of the chaperonin-60 family of molecular chaperones. Nucleotide sequence determination of the symbionin region of the endosymbiont genome revealed that it contains the two-cistron operon sym. Just like the *Escherichia coli groE* operon, the sym operon was dually led by a heat shock and an ordinary promoter sequence. According to the nucleotide sequence, symbionin was 85.8% identical to GroEL of *E. coli* at the amino acid sequence level. SymS, another protein encoded in the sym operon, which is a member of chaperonin-10, was 79.6% identical to GroES. Complementation experiments with *E. coli groE* mutants showed that the chaparonin-10 and chaparonin-60 genes from the endosymbiont are expressed in *E. coli* and that they can function as molecular chaperones together with endogenous GroEL and GroES, respectively.

Prokaryotic endosymbionts harbored by the aphid bacteriocyte are inherited by the next generation at an early stage of the host's embryogenesis (2). They live only in the host cell cytoplasm, and they have no free-living stage. As a result, they cannot propagate themselves when taken out of the host cell. Aposymbiotic aphids that have lost these endosymbionts as a result of antibiotic or heat treatment are markedly undersized and sterile (14, 25). Thus, the aphid and its endosymbionts are intimately mutualistic with each other, which makes these endosymbionts unique organisms quite different from common free-living bacteria. From the nucleotide sequence data for 16S rRNA of pea aphid endosymbionts, it has been suggested that they are members of the γ subdivision of the class *Proteobacteria* and that they diverged from Escherichia coli about 420 million years ago (32).

The aphid endosymbiont synthesizes in vivo substantially only one protein, symbionin, with a molecular mass of 63 kDa (13). To elucidate the biological role of symbionin, we previously purified it (9) and partially determined its amino acid sequence, which revealed that symbionin is very similar to *E. coli* GroEL protein (8), a heat shock protein that is a member of the chaperonin-60 (cpn60) family of molecular chaperones (11). Cpn60 is widely distributed from bacteria to eukaryotic organelles and is involved in the folding (4), assembly (4, 5), and translocation (1, 19) of other polypeptides. Our previous study showed that symbionin is able to reconstitute dimeric ribulose 1,5-bisphosphate carboxylase/ oxygenase (RuBisCO) holoenzyme from its unfolded subunits in vitro, suggesting that this protein also functions as a molecular chaperone in the endosymbiont.

In *E. coli*, cpn60 (GroEL protein) requires GroES protein, another heat shock protein that is a member of the chaperonin-10 (cpn10) family, in order to function as molecular chaperone in the process of bacteriophage morphogenesis (30, 31). The two proteins are also essential for cell viability at all temperatures in *E. coli* (3). In contrast, no information is available about the involvement of cpn10 in the chaperonin function in vivo of organellar cpn60s such as mitochondrial hsp60 (15, 23, 26, 27) and the RuBisCO-binding protein of chloroplasts (11). Since, according to the endosymbiosis theory, the endosymbiont is supposed to be midway between a free-living bacterium and an organelle, it is intriguing to know whether symbionin, an endosymbiotic cpn60, requires cpn10 to function in vivo.

In the present study, to obtain additional information about the chaperonin function of symbionin, we isolated a DNA fragment encoding symbionin and its flanking regions and determined its nucleotide sequence. Also, using this cloned DNA from the endosymbiont, we performed complementation experiments with *groEL* and *groES* mutants of *E. coli* to assess the activity of endosymbiont chaperonins in heterologous cells.

MATERIALS AND METHODS

Insect materials. A long-established parthenogenetic clone of pea aphids, *Acyrthosiphon pisum* (Harris), was maintained on young broad bean plants, *Vicia faba* (L.), at 15°C in a long-day regimen with 18 h of light and 6 h of dark.

Bacterial strains. E. coli mutant strains NRK233 (groES619 zje::Tn10) and NRK117 (groEL44 zje::Tn10), which are derivatives of MC4100 [groE⁺ araD139 Δ (argF-lac)U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR], were kindly provided by K. Ito (18).

Isolation of intracellular symbionts. Primary endosymbionts of the pea aphid *A. pisum* were isolated essentially as described by Ishikawa (12) and Harrison et al. (10). Special care was taken not to damage the endosymbionts mechanically during homogenization of the insect materials. Living pea aphids (about 5 g) were sterilized with 70% ethanol, quickly washed twice with distilled water, and homogenized carefully in 6 volumes of ice-cold buffer A (35 mM Tris-HCl [pH 7.6], 0.25 M sucrose, 25 mM KCl, 1 mM MgCl₂, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). The homogenate was passed through a 90-µm-pore-size nylon mesh and centrifuged at 1,700 × g at 4°C for 20 min. The

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pellet was gently suspended in buffer A and layered over a discontinuous Percoll density gradient that contained 9, 18, 27, 36, and 45% Percoll. Percoll solutions were prepared by diluting 100% Percoll containing 1% bovine serum albumin, 5% polyethylene glycol 6000, 1% Ficoll, and 8.6% sucrose in buffer A. The gradient was centrifuged at 1,000 \times g at 4°C for 20 min. Endosymbionts concentrated at the boundary between 36 and 45% Percoll were recovered and washed in buffer A.

Preparation of endosymbiont DNA. Isolated endosymbionts were incubated in lysis buffer (0.1 M Tris-HCl [pH 8.0] containing 0.1 M NaCl, 0.1 M EDTA, 1% sodium dodecyl sulfate [SDS], and 0.1% proteinase K) at 42°C for 1 h. After lysis, ordinary phenol-chloroform extraction and ethanol precipitation were performed, and the fibrous DNA was collected with a glass rod.

DNA library construction and screening. To isolate the symbionin gene, we first performed Southern blot analysis of isolated endosymbiont DNA using an E. coli DNA fragment containing the groEL coding region (EcoRV-EcoRV 2.1-kb fragment) derived from $\lambda 648$ (17) as a probe. A 14-kb single band was detected by EcoRI digestion under low-stringency hybridization and washing conditions (data not shown). Then, a genomic library of endosymbiont DNA fragments of 12- to 16-kb EcoRI digests in a λEMBL phage vector was constructed and screened with the same E. coli DNA probe. The hybridization buffer contained 50% (vol/vol) formamide, $5 \times$ SSPE (1 × SSPE is 0.18 M NaCl, 10 mM NaPO₄, and 1 mM EDTA [pH 7.7]), 0.1% SDS, 5× Denhardt's solution, and 100 µg of denatured sperm DNA per ml, and the hybridization was performed at 38°C. The filters were washed for 30 min at 55°C with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% SDS. The insert from the positive clone (EcoRI 14-kb fragment) was subcloned into pBluescriptKS+ (pOTKS1, shown in Fig. 3c). Fragments of this DNA were subcloned into plasmid pBluescriptKS+ or SK+, and a series of overlapping deletions of these plasmids were constructed as described by Sambrook et al. (28). The nucleotide sequence was determined by the dideoxy method (29).

Plasmid construction and complementation tests. pOTKS1 was constructed by inserting the 14-kb EcoRI fragment encompassing the symS-symL region into pBluescriptKS+. pOTKS2 was constructed as follows. pOTKS1 was digested with PstI, and the resultant DNA fragments of 7.2 and 6.2 kb were ligated and introduced into the host E. coli (XL1-Blue). The direction of the ligated inserts of plasmids prepared from the transformants was determined by restriction enzyme digestion. Another DNA library of 6- to 8-kb HindIII fragments in $\lambda L47$ was screened with a probe containing only the groES region of E. coli DNA to obtain the complete symS and incomplete symL regions. The inserted DNA fragments from the positive clone were subcloned into pBluescriptSK+ (pSYHD2). E. coli NRK117 and NRK233 were transformed by recombinant plasmids at 30°C. After being cultured at 30°C overnight, the colonies which grew were spread on two ampicillin plates. One plate was incubated at 42°C, and the other was incubated at 30°C. To examine plaque-forming ability, 2×10^8 cells of each transformant were mixed with a λ EMBL phage suspension (containing about 10³ phage) in top agar and plated on L plates. Plates were incubated at 37°C overnight.

Nucleotide sequence data. The nucleotide sequence data reported here will appear in the EMBL data base under the accession number X61150.

RESULTS

Isolation and sequencing of a DNA clone for symbionin. Since our previous study had indicated that symbionin is very similar to the *E. coli* GroEL protein in amino acid sequence, we used a DNA fragment from the *groE* operon to probe the symbionin gene in an endosymbiont's genomic library and detected a positive clone. After subcloning and mapping this positive insert with restriction endonucleases, we determined the nucleotide sequence of 2,739 bp containing the symbionin-coding region (Fig. 1).

It was found that the symbionin gene, designated symL, codes for a polypeptide of 548 amino acids which apparently belongs to the cpn60 family. An additional coding region, designated symS, for a polypeptide of 96 amino acids which belongs to the cpn10 family was found upstream from symL. Therefore, the fragment sequenced contained a two-cistron operon structure, which we named the sym operon.

Characterization and homology analysis of the sym operon. The promoter region of the sym operon was very similar to that of the groE operon of E. coli, in which a heat shock and an ordinary promoter sequence are adjacent. Around 10 bp upstream of symS was a Shine-Dalgarno sequence. symL was separated from symS by a 45-bp noncoding region, in which another Shine-Dalgarno sequence was detected. Around 50 bp downstream from the end of the symL coding sequence was a sequence which may assume the stem-loop structure that terminates the transcription of the sym operon (Fig. 1).

Homology analysis (Fig. 2) revealed that SymL (symbionin) and SymS from the endosymbiont are 85.8 and 79.6% identical to GroEL and GroES of *E. coli*, respectively, at the amino acid sequence level, indicating that these endosymbiont chaperonins are the most identical to those of *E. coli* among the chaperonins sequenced so far (7).

Rescue of *E. coli groE* mutants with symL and symS expression plasmids. To examine whether SymL and SymS are expressed and function in *E. coli*, we performed complementation tests with *E. coli groE* mutants (Fig. 3). Two mutants carrying groES619 (NRK233) and groEL44 (NRK117) were used (18). These mutants exhibit temperature-sensitive cell growth and do not support growth of bacteriophage λ (30).

Each strain was transformed with pOTKS2, pSYHD2, or pBluescript, and complementation of the phenotypes of the mutants was examined (see Materials and Methods). The groEL(Ts) mutant, NRK117, regained viability at 42°C and formed plaques with λ phage when transformed with plasmid pOTKS2 expressing SymL alone. Similarly, the groES(Ts) mutant, NRK233, was complemented with plasmid pSYHD2 containing the entire symS sequence but not complete symL. Neither NRK117 nor NRK233 was rescued by the control plasmid pBluescriptKS+ (Fig. 3a and b). The results indicated that the heterologous chaperonin combinations of symbionin (cpn60 of the endosymbiont) and GroES (cpn10 of *E. coli*) and GroEL (cpn60 of *E. coli*) and SymS (cpn10 of the endosymbiont) are functional as molecular chaperones in *E. coli*.

DISCUSSION

The nucleotide sequence determination of the symbionincoding region revealed that it contains a two-cistron *sym* operon. The *sym* operon is led by heat shock and ordinary promoter sequences, which are arranged adjacently. This finding suggests that in the transcriptional initiation of the

CHAPERONINS OF ENDOSYMBIOTIC BACTERIUM 1871

10 20 30 40 50 60 70	1610 1620 1430 1440 1450 1460 1470
GCAAGTTITAITGAAAATTTTATATTATATTATTGTTTTTTAAAAAAATTACTTTTTAAAATATTTTAA	GGATTTGGTGATOGTCGTAAASCAATGTTACAAGATATTTCAATTCTTACTGGTGGTTCTGTTATCTCTG
	G F G D R R K A M L Q D I S I L T G G S V I S E
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ANTATANACTITCTTANATAAAAAAAAAAAAAAAAATAATTTTTTACCCTTCAAGTTTTAATAAATA	1480 1490 1500 1510 1520 1530 1540
-35(hs) -10(hs)	AAGAATTAGCTATGGAATTAGAAAAATCTACTTTAGAAGATTTAGGACAAGCAAAACGTGTTGTTATTAG
150 160 170 180 190 200 210	E L A H E L E K S T L E D L G Q A K R V V I S
INTERNATINGAGA AAAATATTAAATTAATTAATAATTAATTTTTAAAATAAT	
220 230 240 250 260 270 280	CAMIGACACTACTACTATTATTOGTIGGTIGTAGGAGAAAAACACTCCATTCAAAGTCGTATTAGTCAAATC
ACROGAGE ATTATCATATGAAAATTCGTCCATGCATGATCGTGTGCTTGTTAAGCGTCAAGAAGTCGAA	KDITTIIGGVGEKHSIQSRISQI
SD HKIRPLHDRVLVKRQEVE	
290 300 310 320 330 340 350	CGACAAGAAATTCAAGAAGCTACTICTGATTATGATAAAGAAAAATTAAATGAACGCTTAGCTAAACTAT
TCANAATCTGCAGGTGGTATTGTATTAACAGGATCTGCTGCAGGGAAATCGACTCGAGGAACAGTGACAG	ROBIOBATSDYDKEKLNERLAKLS
S K S A G G I V L T G S A A G K S T R G T V T A	
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CTISTIGGAAAAGGTCGIGTTTTAGATAATGGAGACACITAAACCATTAGATGIAAAAGTIGGIGAIGIIGI	U U V A V L K V U A A T E V E M K E K K R V
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TATTTTTTAATGAAGGTTATGGTGCAAAAAACAGAAAAATTGATAACGAAGAATTATTAATTCTAACTCTAAC	SURLEATEAAVEEGVVXGGGVXL
IFNEGYGAKTEKIDNEELLILTE	
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500 510 520 530 540 550 560	GTGUSTGTAGEAGGGAAAATAGETGATTTALGTGGTEAAAAATGAAGATCAGAACGTAGGTATTCGAGTTG
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SDILAIVE • • 50	
	1900 1910 1920 1930 1940 1950 1960
570 580 590 600 610 620 630	CTITIGCETIGCAATGEAAGCTCCATTACETCAAATTETTTCTAATTCTGETGAAGAACCTTCTGTAGTINC
CANATGGCCGCTAAAGATGTAAAATTTGGAAATGAAGCCCGCATTAAAATGCTTCGTGGAGTTAATGTAT	L R A M E A P L R Q I V S N S G E E P S V V T
N A A K D V K F G N B A R I K M L R G V W V L	
	1970 1980 1990 2000 2010 2020 2030
640 650 660 670 680 690 700	AAACAATGTAAAAGACGGAAAAGGTAACTATGGTTACAATGCAGCTACTGATGAATATGGTGACATGATA
TAGCAGATGCAGTAAAAGTGACTTTAGGACCAAAAGGTAGAAATGTAGTTCTAGATAAATCTTTTGGAGC	ΝΝΥΚΡΟΚΟΝΥΟΥΝΆΑΤΟΒΥΟΟΗΊ
A D A V K V T L G P K G R N V V L D K B P G A	
	2040 2050 2060 2070 2080 2090 2100
710 720 730 740 750 760 770	GATTTTUGTATATTAGATCCAACTAAAGTTACACGTTCTGCTTTACAGTATGCTGCTGCTCGTCGCTGGTC
ACCTAGTATTACTAAAGATGGTGTATCCGTAGCCCGTGAAATTGAATTAGAAGATAAATTCGAAAACATG	D F G I L D F T K V Ť R S A L Q Y A A S V Á G L
P S I T K D G V S V A R B I B L B D K F B N N	
	2110 2120 2130 2140 2150 2160 2170
780 790 800 810 820 830 540	TAATGATEACAACAGAATGTATGGTAACTGACTTGCCTAAAGAAGATAAATCTTCTGATTCTAGTTCTTC
GENECTCANATEGETANAAGANGTTECATCANAAGEAAACGATECAGCAGETEATEGTACCACAACAGCAA	
	M 1 T T E C M V T D L P K E D K S S D S S S
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$ \begin{array}{c} G \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $	H 1 T T E C M V T D L P K B D K S
G A Q M V K E V A S K A R D A A G D G T T T A T 850 860 870 880 890 900 910 CNITATIAGCACAATCIAITGAANGGAGITAAAAGCACCAGTAGAATCCAATGGATCT L L A Q S I V N E G L K A V A A G N N P H D L 920 930 940 950 960 970 980 GAAACGTGGAATTGATAAAGCTGTTACAATGGGTGTAGAACTTAAAACCAATGGATCT K R G I D K A V I S A V E B L K H L S V P C S 990 1000 1010 1020 1030 1040 1050 GAATCTAAAGCAATTACAATGGTACTATTTCTGGCAATGCAGATGGAAGTTGGTACTATTG K R G I D K A V I S A V E B L K H L S V P C S 990 1000 1010 1020 1030 1040 1050 GAATCTAAAAGCAATTACAATGGTACTATTTCTGGCAATGCAGATGGAAAGTTGGTTCTTTAATG D S K A I T Q V G T I S A N A D E K V G S L I A 1060 1070 1080 1090 1100 1110 1120 CAGAAGCAATGGAAAAAGTTGGTAATGCAGGATGTAATAAGAGGAACGGTTTACAGGATGA E A M E K V G M D G V I T V E E G T G L O D E 1130 1140 1150 1160 1170 1180 1190 ACTTGAAGTGGATGGAATGGAATTGCATATGTTGGTGATATATTTTAATGAATAAAATTCTAATAATAACAGAA I E V V K G M Q F D R G Y L S P Y F I M K P E 1200 1210 1220 1230 1240 1250 1260 ACAGGTATTGTAATTAGAAAACCAATATATTTTAATGAATAATATCTAATGTTGGTGAAT T G I V E L E M P Y I L M A D K K I S N V R E M 1270 1280 1290 1300 1310 1320 1330 TGTTACCAATATATTTAGAAAAACCAATATATTTACGAAAACAACTAATATTATCGAAGTATAATATCTAATTTAGAAGTTAGAGGA 1 B V A K G M Q F D 1300 1310 1320 1330 TGTTACCAATATATTTAGAAAAACCAATATATTTATCAATATATTATCAATGAAGTTTAGAGGGA L P I L E S V A K S G K P L L I I S E D L E G 1340 1350 1360 1370 1380 1390 1400	H 1 T T E C M V T D L P K B D K S

FIG. 1. Nucleotide sequence of the *A. pisum* endosymbiont *sym* operon. The corresponding amino acid sequences of the SymS and SymL proteins are indicated. Sequences resembling the *E. coli* consensus heat shock and ordinary promoters (6) are boxed [positions -35(hs) and -10(hs) and -35 and -10, respectively]. Potential ribosome binding site is indicated by arrows. SD, Shine-Dalgarno sequence.

sym operon, as in that of the groE operon of *E. coli* (34), alternative promoters will be involved depending on the environmental conditions. Since it is harbored by the bacteriocyte, the aphid endosymbiont may be constrained to use

EALATLVÝNSNEGIVKVAAVKAP.

the heat shock promoter preferentially, just as does E. colistressed by heat shock, which will lead to a selective production of symbionin (13).

This assumption is compatible with the previous results on

a	ercentage -	85.77 %											
	10	20	30	40	50	60	70	80	90	100	110	120	
GroEL	MAAKDVKFGNDA	RVIMLRGVNVL	ADAVKVTLGP	KGRNVVLDKS	FGAPTITEDG	SVAREIELED	CFENNGAQMV	KEVASKAND!	VAGDGTTTATV	LAQAIITEGL	KAVAAGMNP	DLKRGI	
Sympionin	MAARDVRFGNEA	RINNLRGVNVL	ADAVKVTLGP	KGRNVVLDKS	FGAPSITKDG	SVAREI ELED	KPENNGAQMV	KEVASKAND/	AGDGTTTATL	TWOILANGER	110	120	
(SymL)	10	20	30	40	50	60	70	80	90	100	110		
	130	140	150	160	170	180	190	200	210	220	230	240	
	DKAVTAAVEELKALSVPCSDSKAIAQVGTISANSDETVGKLIAEAMDKVGKEGVITVEDGTGLQDELDVVEGMQPDRGYLSPYFINKPETGAVELESPFILLADKKISNIREMLPVL												
	**** ****** *********** ******** ** **												
	DKAVISAVEELI	HLSVPCSDSKA	ITOVGTISAN	ADEKVGSLIA	EAMERVGNDG	VITVEEG TGLQ	DELEVVKGMO	FDRGYLSPY	PINKPETGIV	ELENPYILMA	KICISNVREM	LPILESV	
	130	140	150	160	170	180	190	200	210	220	230	240	
	250	260	270	280	290	300	310	320	330	340	350	360	
	AKAGKPLLIIAE	EDVEGEALATAV	VNTIRGIVKV	ANVKAPGFGD	RRKAMLODIV	ILTOGTV ISEE	IGMELEKATL	EDLGQAKRV	VINKOTTTIII	DGVGEEAA1QC	RVAQIRQQI	EEATSDY	
	** ******	*******	** ******	*********	*******	**** * ****	***** **	********	** ******	**** **	* **** *	*****	
	AKSGKPLLIISE	EDLEGEALATLV	VNSMRGIVKV	AAVKAPGFCD	RRKAMLQDIS:	LTGGSV ISEE	LAMELEKSTL	EDLGQAKRV	VISKOTTTII	GGVGEKHSIQS	RISQIRQEI	QEATSDY	
	250	260	270	280	290	300	310	320	330	340	350	360	
	370	380	390	400	410	420	430	440	450	460	470	480	
	DREKLQERVAKI	AGGVAVIKVGA	ATEVEMICEK	CARVEDALHAT	RAAVEEGVVA	GGVALI RVAS	KLADLRGQNE	DONVGIKVA	LRAMEAPLRO	IVLNCGEEPS\	VANTVKGGD	GNYGYNA	
	* *** ** ***	* ***** ****	********	********	*********	****** ***	* ********	****** **	*********	•• • ••••	* * ** *	******	
	DKEKLNERLAK	LSGGVAVLKVGA	ATEVENKEKI	CARVEDALHAT	RAAVEEGVVM	GGVALV RVAG	KIADLRGQNE	DONVGIRVA	LRAMEAPLRO	IVSNSGEEPS	/VTNNVKDGK	GNYGYNA	
	370	380	390	400	410	420	430	440	450	460	470	480	
	490	500	510	520	530	540	550						
	ATEEYGNMIDM	GILDPTKVTRSA	LQYAASVAGI	MITTECMVTD	LPKNDAADLG	NAGGMGG MGGM	iggmm						
	** *** ***	**********	********	*********	*** *	** ****	****						
	ATDEYGDMIDFO	SILDPTKVTRSA	LQYAASVAGI	MITTECMVTD	LPKEDKSSDS	SSSPAGG MGGN	EGMM 650						
	490	500	510	520	530	540	330						
b ᢪ	ercentage	- 79.59	8										
	1	.0	20	30	40	50	60						
GroES	MNIRPLHDR	VIVKRKEVE	TKSAGGIV	LTGSAAAKS	TRGEVLAV	GNGRILENG	EVKPLDVK	:					
	* ******	* *** ***	******	***** **	*** * **	* ** * **	*****						
SymS	MKIRPLHDR	VLVKRQEVE	SKSAGGIV	LTGSAAGKS	STRGTVTAV	GKGRVLDNG	DIKPLDVK	2					
	1	.0	20	30	40	50	60)					
	7	0	80	90	100								
	VGDIVIFND	GYGVKSEKI	DNEEVLIM	SESDILAIN	/EA								
	**** **** * ***************************												
	VGDVVTENEGYGAKTEKTDNEELLTLTESDILATVE												
	7	0	80	90	100								

FIG. 2. Comparison between the amino acid sequences of GroEL and symbionin (SymL) and GroES and SymS. Identical amino acid residues are indicated by asterisks. The percent identity is indicated above each alignment.

protein synthesis by the isolated endosymbiont in vitro. Under in vitro conditions, the synthesis of symbionin is markedly lowered and other numerous proteins are produced by the endosymbiont, a phenomenon which is reminiscent of a cell recovered from heat shock (13). In addition, when stressed by heat shock or other stresses such as heavy metals and ethanol, the isolated endosymbiont resumes the selective synthesis of symbionin in large amounts (22). These results suggest that the intracellular environment constrains the endosymbiont to synthesize symbionin preferentially.

As expected from the close similarity of symbionin to GroEL in amino acid sequence (Fig. 2), it was shown that symbionin is functional as an assembly factor in vitro (16) and in vivo in *E. coli*, a heterologous cell (Fig. 3). These

findings raise the possibility that in the aphid endosymbiont, symbionin also functions as an assembly factor. Actually, it has been suggested that in the bacteriocyte, many polypeptides synthesized by the cytoplasmic ribosomes are found associated with the endosymbiont (12). Moreover, when the synthesis of symbionin by the endosymbiont is somehow arrested, the endosymbiont structure in the bacteriocyte is destroyed (14, 25). All these, taken together, suggest that symbionin is a molecular chaperone like GroEL and that, just like organellar cpn60s, it is involved in the assembly of polypeptides imported into the endosymbiont from the host cell.

In the complementation experiment shown in Fig. 3, not only symL but also symS of the endosymbiont was expressed in the *E. coli* mutant cell and complemented the



FIG. 3. Restoration of viability at 42°C (a) and phage plaque formation ability (b) of temperature-sensitive E. coli groEL and groES mutants with symS and symL expression plasmids. (a) Mutants were transformed with the control and recombinant plasmids shown in panel c. 1 and 2, NRK117 (groEL)/pBluescriptKS+; 3 and 4, NRK117/pOTKS2; 5 and 6, NRK233 (groES)/pBlue-scriptKS+; 7 and 8, NRK233/pSYHD2. Plates were incubated at 42°C or 30°C. (b) Transformants were infected with the same number of PFU of λ phage. +, plaques were observed; -, plaques were not observed. (c) Physical map of the sym region. The inserted DNA fragments of the recombinant plasmids are indicated below the map. \triangle , deletion obtained by removal of the *PstI* fragment in the symS region. Hd, HindIII; E, EcoRI; Hc, HincII; P, PstI; K, KpnI.

defect in groES. This represents the first example of a functional cpn10 in any heterologous cell. As shown in this experiment, SymS acts like cpn10 in E. coli, taking the place of GroES. In addition, symbionin is able to chaperone RuBisCO in vitro, replacing GroEL only in the presence of GroES (16). In this regard, an apparent contradiction is that in the endosymbiont, the synthesis of SymS relative to that of symbionin seems to be very low (16). In E. coli (24) and cyanobacteria (33), the level of expression of cpn10 is almost equivalent to that of cpn60.

In mitochondria, no direct evidence is available about the in vivo levels of cpn10, although the presence of a cpn10-like protein has been suggested (20). One possibility is that although cpn10 may be required for some synergistic function with cpn60, cpn10 is not as essential as cpn60 because functions dependent on cpn60 alone are more numerous in organelles. Actually, even in E. coli, not all GroEL-dependent processes have been shown to require GroES (1, 19). In mitochondria and chloroplasts, although the cpn60 gene is found in the nuclear genome, the gene encoding cpn10 has



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FIG. 4. Model for gene expression of the sym operon of the aphid endosymbiont. P1, heat shock promoter; P2, ordinary promoter; T, terminator. A stem-loop structure shown below is assumed to enhance the expression of SymL selectively at the translational level. SD, Shine-Dalgarno sequence.

not been found in the same operon. In the aphid endosymbiont, the expression of cpn10 (SymS), although encoded in the same operon that encodes cpn60 (symbionin), may be repressed in vivo because of its organellelike environment. Judging from the polarity of the sym operon (symS arranged upstream of symL), the repression of symS could be at the posttranscriptional level. In this context, it may be noteworthy that a region preceding the Shine-Dalgarno sequence of symL tends to assume a stem-loop structure, which does not seem to be formed either in the Shine-Dalgarno region of symS or in the corresponding region of groE (Fig. 4). The similar structures are often observed with multicistronic mRNAs of E. coli and reported to enhance the translational level of the relevant cistron (21). Further studies of the aphid endosymbiont should provide important insights into the origin of the system in cell organelles, since the endosymbiont is thought to be an organelle at a primitive stage.

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