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Characterization of Promoter Sequences for Strong Expression of *groEx* in *Escherichia coli*.

Jung E. Lee, Ssang T. Lim and Tae I. Ahn

Department of Biology Education, Seoul National University, Seoul 151-742, Korea

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The cloned X-bacterial gene (*groEx*) which is analogous to *groE* of *E. coli* strongly expressed in *E. coli* when grown at the temperature 27°C or higher without having to add any inducers. By S1-nuclease mapping, primer extension analysis and site directed mutagenesis, we found 4 promoters in this gene. Among them two promoters located at 5′-extended region of the gene are homologous to the promoters found in *groE* family of heat-shock genes; they are, σ^{32} factor-dependent P1 promoter and σ^{70} factor-dependent P2 promoter. The other two promoters found within the coding region of *groESx* were P3, 5′-TTGGCG-(18 bases)-AATACT-3′ and P4, 5′-TTGGCA-(19 bases)-TAAAGT which overlapped within 49 bases. These unique intragenic σ^{70}-dependent promoters are the first to be cloned and characterized in *groE* analogous heat-shock genes so far. These P3 and P4 promoters appeared to be responsible for the strong expression of GroELx in X-bacteria *in vivo*.

Key words: *groE*, promoters, gene expression, symbiosis

Molecular chaperones are present in various organisms, ranging from eubacteria to eukaryotic cells, and they are involved in the assembly of oligomeric protein complexes, assisting other polypeptides to maintain or assume correct conformations. Among the chaperones, GroES and GroEL chaperonins are known to be universally conserved (14), and genes coding for chaperonins have been characterized in many organisms including archaebacteria, *Escherichia coli, Bacillus, Streptomyces, Clostridium, Coxiella, Chlamydia, Brucella, Haemophilus, Legionella,* cyanobacteria, thermophilic bacteria and symbiotic bacteria in aphids and amoebae (4).

Endosymbiotic bacteria (called X-bacteria) living inside xD amoebae initially infected the D strain of *Amoeba proteus* as parasites but became integrated as essential cell components within a few years (17). At present, each xD amoeba contains about 42,000 X-bacteria, and the host and symbionts are mutually dependent for survival as evidenced by treatments with antibiotics or by growth at an elevated temperature (2, 16). The cytoplasm of a host cell is considered to be a hostile environment for intracellular symbionts, since endosymbionts must overcome many difficulties in order to survive inside the host. X-bacteria contain a large amount of a 67-kDa protein, a GroEL analog.

It is believed that the GroES and GroEL analogs present in symbionts may perform a chaperone function similar to that of heat-shock proteins in other cells placed under a stress condition (7). We cloned the X-bacterial gene coding for the 67-kDa protein and sequenced its nucleotides (3). The gene was composed of two open reading frames (ORFs) analogous to *groES* and *groEL* of *E. coli*. Thus, the gene was named as *groEx* (4). The nucleotide sequence of the ORF for *groELx* had a greater than 70% identity with those of *groEL* genes of other bacteria sequenced. The *groELx* was strongly expressed in *E. coli* by simple culturing at the temperature 27° or over without adding any inducer. The level of GroELx expressed in *E. coli* was over 50% of the total proteins (3).

The gene products, GroESx and GroELx have functional analogy with GroES and GroEL of *E. coli*, respectively. The *groEx* gene complemented *groE* mutations in *E. coli* and enhanced the growth of transformed *E. coli* even at their usually lethal temperatures. In this gene the presence of an additional promoter within the coding region of *groESx* has been predicted (4). We demonstrated the strong activity of the putative promoter by producing over 40,000 Miller's units of β-galactosidase from pUXGPRM (GroELx-fusion vector) which was constructed by using the 5′ end fragments of the gene including the putative promoter sequences and part of

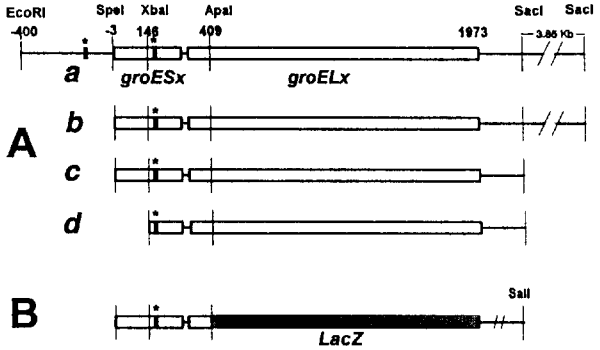

Fig. 1. Physical maps of *groEx* clones (A) and pUXGPRM-*lac* (Δ*Eco*RI-*Spe*I) (B) used for the analysis of promoters. For S1-mapping and primer extension analysis, clones containing all or part of *groEx* in pBSK vector, a; pAJX97, b; pAJX91, c; pAJX53, and d; pAJX52 were used. In site directed mutagenesis pUXGPRM-*lac*(Δ*Eco*RI-*Spe*I) was used as template. Solid bar marked with asterisk indicates the putative promoter site.

groELx coding region (21).

In order to utilize these potent promoter sequences in the development of expression plasmid vector, we determined the transcription initiation sites of the gene by S1-nuclease mapping and primer extension analysis. We found 4 promoters in this gene. Among them two promoters located in 5′-extended regions are consensus promoters found in *groE* family of heat-shock gene, that is, one σ^{32} factor-dependent P1 promoter and the other σ^{70} factor-dependent P2 promoter. The other two promoters found within the coding region of *groESx* were unique to this gene. We confirmed the −10 and −35 elements of the unique P3 and P4 promoters by site-directed mutagenesis.

Materials and Methods

Cells and materials

E. coli XL1-Blue cells containing pBSKII from Stratagene (La Jolla, CA), pAJX97, pAJX91 or their derivatives containing all or part of *groESx* (4) were used for the analysis of S1 nuclease mapping and primer extension (Fig. 1A). For site directed mutagenesis in putative unique promoters, *E. coli* CJ236 and pUXGPRM-*lac*(Δ*Eco*RI-*Spe*I) (Fig. 1B) were used as the host and template, respectively.

^{35}S-α-dATP and ^{32}P-γ-dATP were purchased from NEN (Boston, MA) and ICN (Costa Mesa, CA), respectively. Muta-gene phagemid *in vitro* mutagenesis Version 2.0 was obtained from BioRad (Richmond, VA). Restriction endonuclease, Bal31 nuclease, T4 polynucleotide kinase, sequenase 2.0 and USBioclean were purchased from USB (Cleveland, OH). AMV reverse transcriptase and RNasin were obtained from Promega (Madison, WI). Calf intestinal alkaline phosphatase (CIAP) was purchased from Pharmacia LKB (Uppsala, Sweden). For immunoblotting, anti-GroELx monoclonal antibody was obtained from Dr. Jeon's lab (7) and anti-β-galactosidase monoclonal antibodies were obtained from Sigma Chemical Co. (Saint Louis, Mo).

Preparation of RNAs

For S1-mapping and primer extension analysis, total RNAs of *E. coli* containing pAJX plasmids or pBSKII were prepared by hot phenol method (1) or guanidium method (6). All solutions were prepared in DEPC-treated water. Final preparation of RNA was dissolved in water containing RNasin (1.25 U/μl).

S1 nuclease mapping

In order to approximate the sites for transcription initiation of *groEx*, S1 nuclease mapping was performed according to Abia *et al.* (1). For the preparation of probe DNA, purified 0.8 kb subclone derived from pAJX97 was digested with *ApaI*. After treating with CIAP, *ApaI*-end of the DNA was labeled by T4 polynucleotide kinase using ^{32}P-γ-dATP. Then, unlabeled ^{32}P-γ-dATP was removed by Sephadex G-50 spin column, and the DNA was digested with *Eco*RI and electrophoresed in 1% agarose gel. A fragment of the gel containing excised 0.8kb DNA including *groESx* and 5′-flanking region of the gene was cut out and the DNA was extracted by using US-Bioclean. Ethanol precipitate of mixture containing 50 ng of probe DNA (5×10^7 cpm) and 135 μg of total RNAs was dissolved in 30 μl of hybridization buffer (80% formamide, 0.4 M NaCl, 20 mM HEPES pH 6.5). After denaturing the mixture for 10 min at 75℃, hybridization was processed for 14 hr at 37℃. Then, the reaction was incubated with 300 U S1-nuclease for 15-30 min at 37℃ and stopped by phenol extraction. Finally, the products were recovered by ethanol precipitation.

Primer extension analysis

Transcription initiation sites of *groEx* were determined by primer extension analysis by following Ausubel *et al* (5). In the analysis of the transcription initiation site of the heat-shock consensus promoter, a 17-mer nucleotide complementary to nt (nucleotide number, starting nucleotide for *groESx* was assigned as nt 1) 50-66 was used as a primer. In the analysis of the unique putative promoter predicted within the coding region of *groESx*, we used 40-mer oligonucleotide that was complementary to nt 304-343 as a primer. 5′-ends of the primer DNAs were labeled by T4 polynucleotide kinase using ^{32}P-γ-dATP. For primer extension, an ethanol precipitated mixture of 5 ng of labeled primer and 50 μg of total RNAs

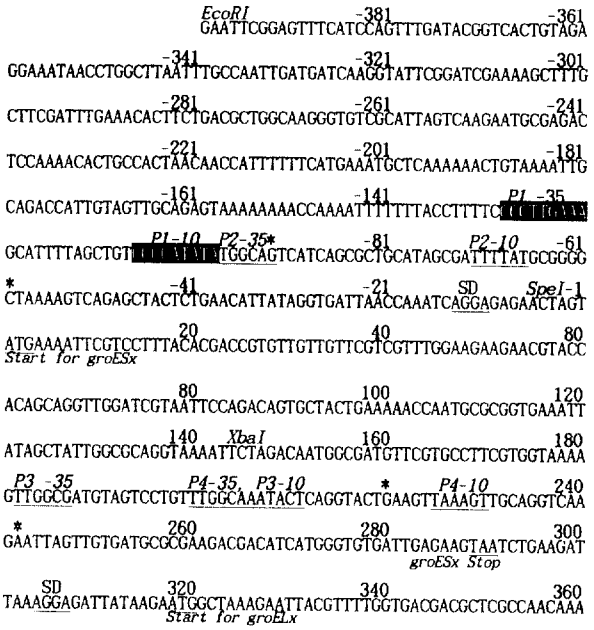

Fig. 2. Part of nucleotide sequences of *groEx* gene including the promoter signal region. Start codon of *groESx* was designated as nt number 1. The transcription-initiation sites at nt −93, −60, 220 and 242 as determined by S1 mapping (Fig. 3) and primer-extension analysis (Fig. 4) are marked with asterisks on top. The nucleotides for heat-shock factor σ^{32}-dependent P1(−129~−99) promoter are shown with reversed characters. σ^{70}-dependent P2 promoter (−98~−67), the intragenic P3 (182-211) and P4 (200-230) promoters of *groEx* are underlined. The start and stop codons for *groESx*, *groELx* and the consensus ribosome-binding sites(SD) are also underlined. Complete nucleotide sequences of *groEx* are deposited with GenBank (Accession No. M86549).

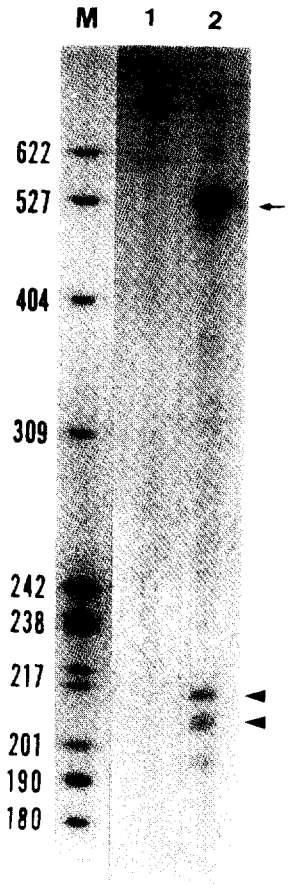

Fig. 3. S1-nuclease mapping for *groEx* transcripts. The arrow indicates the position of a transcript from heat-shock consensus promoter and the arrow head indicates the second transcript initiated within *groESx* coding region. Lane M, pBR322 DNA digested with MspI as molecular size marker; Lane 1, 5′-end labeled *Eco*RI/*Apa*I probe DNA; Lane 2, S1 nuclease treated probe DNA after hybridization with 5′-end labeled *Eco*RI/*Apa*I fragment and RNAs extracted from *E. coli* transformed with pAJX97.

dissolved in 30 μl of buffer (3 M NaCl, 1 mM EDTA, 0.5 M HEPES pH 7.5) was denatured for 10 min at 70℃ and hybridized for 15 hr at 30℃. Then, the primer was extended for 90 min at 42℃ by using 40 units of AMV reverse transcriptase. After stopping the reaction by adding EDTA, template RNAs were digested with RNase A (DNase-free) and the reactants were extracted with phenol. A sequencing ladder was made using the same primer and run paralleled with the primer extended reactants.

Site-directed mutagenesis

In order to confirm the nucleotide sequences of predicted putative promoters, site directed mutagenesis was carried out using pUXGPRM-*lac*(ΔEcoRI-SpeI) (21) as template. The 26 to 32-mer primers with 3 to 4 mutated nucleotides corresponding to −35 and −10 sequences of P3 and P4 promoters of *groEx* were synthesized from Korean Basic Science Research Center. Site-directed mutagenesis was performed according to Kunkel (18), and mutations were confirmed by DNA sequence analysis. The effect of mutation on the expression of reporter gene was analyzed by SDS-PAGE and β-galactosidase assay.

DNA sequence analysis

Plasmid DNAs were purified by alkaline method (22). DNA sequencing was performed by dideoxy-chain termination method using Sequenase version 2.0 kit (20).

Assay of β-galactosidase

E. coli was inoculated in A media and cultured up to OD_{600} 0.3-1. Enzyme activities were quantitated by Miller's method (24) using 0-nitrophenyl-β-D-galactopyranoside as a substrate.

SDS-PAGE and Western blotting

Proteins expressed from *E. coli* containing *groEx*, reporter genes or its mutants were analyzed by SDS-

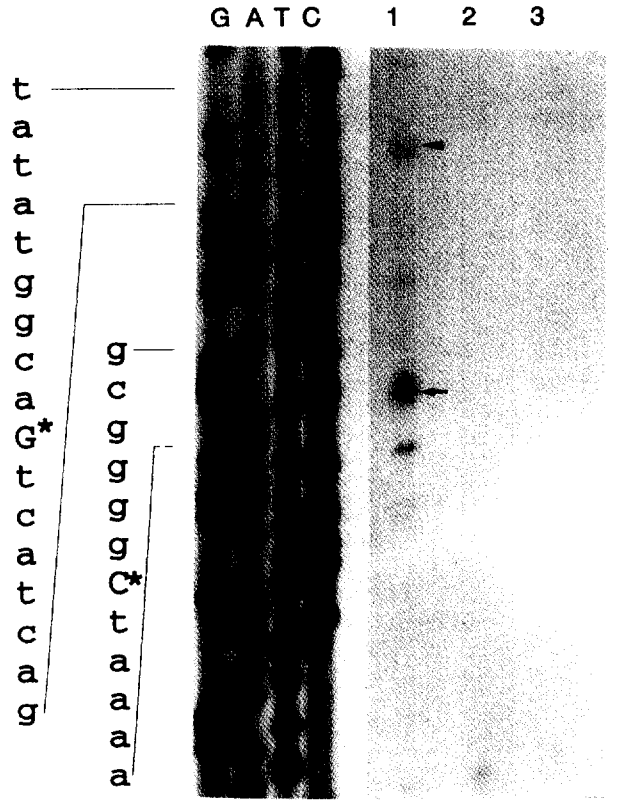

Fig. 4. Primer extension analysis for heat-shock consensus promoters of *groEx*. The two extended ends are marked with an arrowhoad and an arrow. The sequencing ladder is shown in lanes GATC. Lanes 1 and 2; the total RNAs from *E. coli* transformed with pAJX97 and pAJX91, respectively. Lane 3; total RNAs from *E. coli* transformed with the pBSKII vector alone for comparison. Lane 1 shows two extended reverse transcripts that are not present in lane 2 and 3. The nt sequences of the ladder (listed on the left) match with sequences of *groEx* from nt −102 to −55 (Fig. 2). Two transcription start points (G at nt −93, and C at nt −60) are marked with asterisks.

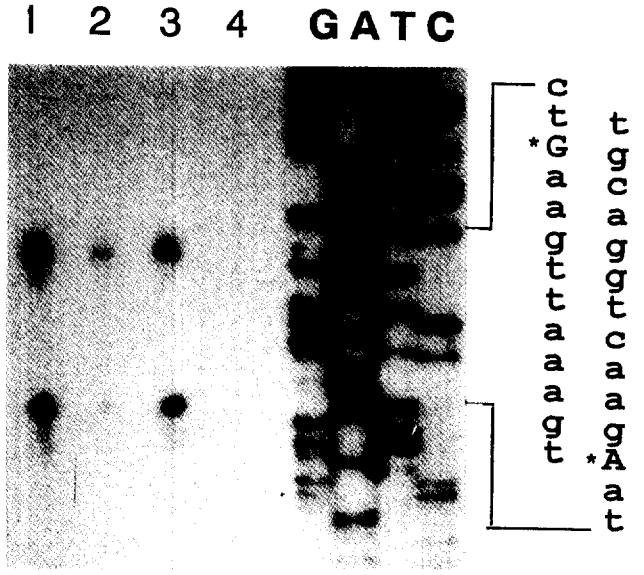

Fig. 5. Primer extension analysis for intragenic promoters of *groEx*. The sequence ladder is shown in lanes GATC; Lane 1, 2 and 3 represents the total RNAs from XL1 Blue of *E. coli* transformed with pAJX97, pAJX91 and pAJX53 plasmid, respectively. In lane 4, the total RNAs from *E. coli* transformed with pBSKII vector alone was used as a control. Lanes 1-3 show two extended reverse transcripts that are not present in lane 4. The nt sequences of the ladder (listed on the right) match with the sequences of *groEx* from nt 214 to nt 247 (Fig. 2). Two transcription start points (G at nt 220, and A at nt 242) are marked with asterisks.

PAGE (19) and Western blotting as described previously (4).

Results

In the analysis of S1-nuclease mapping using 5′-end ^{32}P-labeled *Eco*RI/*Apa*I fragment of *groEx* as probe, 3 mRNAs different in length of 5′-extended ends were identified (Fig. 3). 5′-ends of these transcripts were 504, 210 and 195 bases apart from *Apa*I end of the gene. The nucleotide at 504 bases upstream from *Apa*I end is close to the predicted heat-shock consensus promoter sequences of the gene. Those nucleotides 210 and 195 bases upstream from *Apa*I were within the coding region of *groESx* and close to the predicted putative promoter region of the gene (Fig. 2).

Primer extension analysis using 17-mer corresponding nt 50-66 of the gene as a primer revealed two transcription initiation sites, G at nt −92 and C at nt −60 (Fig. 4). These two transcription initiation sites were shown in pAJX97 clones containing full sequences and 5′-extended regions of the gene. pAJX91 clone or the subclones which were lacking 5′-extended regions and 5′-half of *groESx* coding regions did not show the products. The G is 6 bases downstream from the predicted −10 element of the heat-shock consensus promoter. Thus, the G is the σ^{32} factor-dependent transcription initiation site for the heat-shock consensus promoter. On the other hand, the C is 39 bases downstream from −10 element of heat-shock consensus promoter.

In primer extension analysis for intragenic promoters, all of those *groEx* clones expressing GroELx showed G at nt 214 and A at nt 242 as transcription initiation sites (Fig. 5). These two sites were not shown in *E. coli* containing pBSKII plasmid as control. By examining the nucleotide sequences ahead of these 2 sites, we could locate two putative promoter sequences, P3; TTGGCG-(18 bases)-AATACT (nt 182-211) and P4; TTGGCA-(19 bases)-TAAAGT (nt 200-230) (Fig. 2).

In order to confirm −10 and −35 elements of P3 and P4 promoters, site-directed mutagenesis were carried out using pUXGPRM-*lac*(Δ*Eco*RI-*Spe*I) as template.

P3 -35	P4 -35	P3 -10	P4 -10	Miller Units
AGTTG	GCGAT	GTAGT	CCTGT TTGGC	AAATA CTCAG GTACT GAAGT TAAAG T	54, 142
..C.C	T...G	.....	....	....	125
.....	..... .A.T.	.....	..... .TGC.	....	25
.....	....	C	ACTAG T....	....	160
.....	....	.....	....	.... ACT.. .	5

Fig. 6. Summary of site-directed mutagenesis in P3 and P4 promoter regions of *groEx* in pUXGPRM-*lac*(Δ*Eco*RI-*Spe*I). Note that any mutation in P3 or P4 promoter sequences strongly reduced the expression of *lacZ*.

The plasmid has P3, P4 promoters and the structural gene of *lacZ* fused to N-terminal part of *groELx*. By mutagenesis, various base substitutions in the putative promoter sequences were obtained as confirmed by sequence analysis. Effects of these mutations were assayed by comparing the expressed level of β-galactosidase. The results are summarized in Fig. 6. Any base substitution in −35 or −10 element of both P3 and P4 considerably reduced the expression of *lacZ*. Among the mutant clones, 3 base substitution at −10 sequences of P3 and P4, AATACT and TAAAGT to TACACT and ACTAGT, respectively, were the most affected. With these results we confirmed the P3 and P4 sequences as intragenic promoters.

Discussion

In this study it was shown that *groEx* has 4 different transcripts. Among them, two transcripts encoding for both GroESx and GroELx are initiated by consensus promoters (P1 and P2). The other two transcripts encoding for GroELx are initiated by intragenic promoters (P3 and P4). The presence of intragenic promoters is the first shown in *groE* family.

The *groEx* has transcription initiation site G at nt −92 and C at nt −60 from the *groESx* start codon. The G is 6 bases downstream from the −10 element of the heat-shock consensus promoter. In the alignment of the heat-shock consensus promoter of *groEx* with those of known heat-shock genes in *E. coli*, the sequences of −35 and −10 elements, 5′-CTTGAA-(13 bases)-CCCCATAT-3′, are well conserved (Table 1). In *groE* of *E. coli*, the transcription initiation site A is 8 bases apart from the −10 element and 72 bases upstream of the initiating AUG for *groES* (8). In *Synecoccus* sp., transcription initiation site C is located at −81 base from *groES* start codon (28). Thus, the transcription initiation site G of *groEx* appeared to be transcription initiation site by the heat-shock consensus promoter.

In *E. coli*, a heat shock (42℃) induces the expression of *rpoH* and then its product, σ^{32}, which recognizes the heat-shock consensus promoter sequences as a transcription factor (11) replaces factor σ^{70} to enhance the expression of *groE*. Expressions of at least 13 heat-shock genes (*dnaK, groEL*, etc.) are under the control of positively acting *rpoH* (also known as *htpR*) in *E. coli* (26). Another novel sigma factor, σ^{E}, has also been identified in *E. coli* to act on the *rpoH* promoter at high temperature (9). Since the *groEx* cloned from the symbiotic X-bacteria has the sequences homologous to heat-shock consensus promoters of *E. coli* (Table 1) and transcription products as shown in S1-mapping (Fig. 3) and primer extension analysis (Fig. 4), expression of the gene by heat-shock consensus promoter may be under the control of an analogous gene to *rpoH*. Thus, an alternate RNA polymerase σ subunit may be involved in transcriptional regulation of heat-shock inducible genes.

The transcription initiation site C of *groEx* is 39 bases downstream from −10 element of heat-shock consensus promoter. This transcript is 28 bases shorter than that was initiated from G site. An examination of DNA sequence from the C site of the *groEx* revealed a sequence resembling the σ^{70}-dependent promoter consensus having the sequence TGGCAG in the −35 region, a 20 bp spacer, and a −10 region sequence of TTTTAT. This appears to be a homologue of the second promoter found in *groE* of *E. coli* (29). In *E. coli*, the second *groE* mRNA species that is initiated 25 to 30 bases downstream of the σ^{32}-dependent transcript. The second σ^{70} factor-dependent promoter TGGTCA (17 bp spacer) TAAGCT of *groE* was predicted by S1 mapping and *in vitro* transcription (29). This second promoter was found responsible for the basal-level synthesis of GroESL as an additional mechanism controlling the synthesis of the heat-shock protein.

By primer extension analysis and site directed mutagenesis we confirmed two additional potent promoters present within the coding region of *groESx*. The two promoter sequences, P3; 5′-TTGGCG (18 bases) and AATACT-3′ (nt 182-211), and P4; 5′-TTGGCA (19 bases) TAAAGT (nt 200-230) are overlapped within 49 bases (Fig. 2). These intragenic promoters are the first shown in *groE* analogous heat-shock genes that have been cloned and characterized so far. As shown in the alignment with known promoter sequences of *E. coli* (Table 2), these unique promoters are homologous to σ^{70}-dependent promoters. In addition, we were able to obtain transcripts using *E. coli* RNA polymerase holoenzyme containing σ^{70} factor and *Spe*I-*Apa*I fragment of *groEx* as template *in vitro* (data not shown).

The nucleotide sequences of P4 promoter of *groEx* match with previously known promoters, but those of P3 did not match with any known promoters (13). TTG-

Table 1. Comparisons of P1 promoter of *groEx* with known σ^{32}-dependentheat-shock promoters in *E. coli*

Genes	−35		−10	+1
groEx P1	TTTCCCCCTTGAA	AGCATTTTAGCTG	CCCCATATATGGCA	G
groE	TTTCCCCCTTGAA	GGGGCGAAGCCAT	CCCCATTTCTCTGGTCAC	
dnaK P1	TCTCCCCCTTGAT	GACGTGGTTTACGA	CCCCATTTAGTAG	TCAA
dnak P2	TTGGGCAGTTGAA	ACCAGACGTTTCG	CCCCTATTACAGACTCAC	
C62.5 P1	GCTCTCGCTTGAA	ATTATTCTCCCTTGT	CCCCATATCTCCCACATC	
rpoD Phs	TGCCACCCTTGAA	AAACTGTCGATGTGG	GACGATATAGCAG	ATAA
Consensus	TttCcCcCTTGAA	13-15 bp	CCCCATtTa	

Table 2. Comparisons of P2, P3 and P4 promoters of *groEx* with σ^{70}-dependent promoters in *E. coli*

Genes	−35	−10	+1
groEx P2	TGGCAG tcatcagcgctgcatagcga	TTTTAT gcggg	gCt
groEx P3	TTGGCG atgtagtcctgtttggca	AATACT caggtac	tGa
groEx P4	TTGGCA aatactcaggtactgaagt	TAAAGT gcaggtcaa	gAa
*groE**	TGGTCA ccagccgggaaaccacg	TAAGCT ccggcgtcacccat	
*uvrB P1***	TTGGCA taattaagtacgacgag	TAAAAT taca	tAc
*spot42 RNA***	TTTCTG aactgaacaaaaaagag	TAAAGT tagtcg	cGt
*glnS***	TTGTCA gcctgtcccgcttataa	GATCAT acgccGt	tAt
*malP***	ATGAGG aaggtcaacatcgagctgg	CAAACT agcgataacgttgt	
*argP***	TTGCAA atgaataattacacata	TAAAGT gaattttaattcaa	
*argI***	TTGCAA atgaataatcatccata	TAAATT gaattttaattcat	
Consensus	tgtTTGACa t (15-21: 17± 1)	TAtAaT (5-8: 7)	cAt

*: Zhou *et al.* (29), **: Hawley and McClure (13)

GCA, the −35 element of P4 matches with that of P1 promoter of *uvrB* gene of *E. coli* which has 3 promoters; 2 promoters in about 50 bp and the other one is several hundred nucleotides separate (27). P4 sequences of *groEx* are also shown in *Legionella pneumophila htpAB*, an analogous gene to *groEx*, but the promoter activity has not been reported (15).

In the previous work (4) we confirmed the promoter activity of the P3 and P4 promoter of *groEx* by constructing *groEx-lacZ* fusion plasmid and examining the expression of fusion protein in *E. coli*. In the *E. coli* grown at 37℃, the level of β-galactosidase expressed by the P3 and P4 promoter was about 10 times the level expressed by *tac* promoter. High-level expression of the GroELx in transformed *E. coli* was achieved without the heat-shock consensus P1 and P2 promoters of *groEx* and at a much lower temperature (27℃) than the usual heat-shock temperature for *E. coli* (3). These intragenic promoters may be responsible for the large accumulation of GroELx *in vivo* (7).

Some prokaryotes have somewhat different mechanism for the expression of heat-shock gene. Instead of heat-shock consensus promoter, inverted repeat motifs are used as temperature-sensitive component. In *Clostridium acetobutylicum*, *groES* and *groEL* organized in bicistronic operon has promoter structure which is highly homologous to the consensus promoter sequence of gram-positive bacteria as well as *E. coli*. In this gene an 11-bp inverted repeat, located between promoter and transcription start site of *groES* was to play an important role in the regulation of heat-shock gene expression (25). In *Bacillus subtilis* (23) the same promoter is utilized in the expression of *groESL* under normal and heat-shock condition. This promoter has consensus −35 and −10 regions similar to those recognized by typical σ^A-containing RNA polymerase. Around the transcription start site, there are three inverted repeat sequences which are relatively short and AT-rich. These repeat sequences are postulated to serve as binding site for positive factor required to transcribe the heat-shock genes.

In *E. coli*, GroEL has been shown to form a complex with several secretory proteins including pro-OmpA, pre-PhoE and pre-β-lactamase, and to maintain the export competence of these proteins (23). In addition, the export of *lacZ*-hybrid proteins was facilitated by overproduced GroEL in *E. coli* (10). The fact that *groESx* and *groELx* can complement *groES* and *groEL* mutations of *E. coli* indicates that both GroESx and GroELx are functional as molecular chaperonins. However, there is no evidence that GroESx is expressed in X-bacteria themselves. Only GroELx was accumulated large quantities in X-bacteria

isolated from xD amoebae. This could be due to the strong activities of P3 and P4 promoters. Optimal temperature for growing xD amoebae harboring the endosymbionts is 24℃ at which the heat-shock consensus P1 promoter of *groEx* may not be active. The bacterial endocellular symbionts in aphid having *symSL* as a *groE* analogue do not express *symS in vivo*. They express and accumulate large amount of GroEL analogous SymL while they are in symbiosis (12). However, the presence of intragenic promoter has not been reported in this gene.

Since the inside of a living cell is a potentially hostile environment, it is not surprising that endosymbionts contain an additional promoter for the expression of a specific chaperonin. We assume that the GroEL chaperonins in endosymbionts play some roles, such as enhancing transport and stabilizing imported proteins. The pathogenic *Legionella pneumophila* residing within the phagosomes of alveolar macrophages also express large amount of 60 kDa HtpB protein that is an analogous to GroEL on the surface. The protein is the immunodominant and expressed on the surface of intracellular bacteria (15). Thus, the nature of the exerted stress, the regulatory mechanism for the expression of GroEL analogue and chaperonin function of GroEL proteins in endosymbionts or pathogenic bacteria may be different from the well-documented heat-shock response in *E. coli*.

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