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Continuous Synthesis of *Escherichia coli* GroEL at a High Temperature

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GroEL is a typical molecular chaperone. GroEL synthesis patterns at various culture temperatures in *Escherichia coli* were investigated in this study. No significant differences in the amount of GroEL produced from the chromosome were found at 30 and 37°C. However, GroEL production increased 3.4-fold at 42°C. GroEL synthesis was not transient but continuous at 42°C, although most heat shock gene expression is known to be transient. To understand the role of the *groEL* structural gene, a *groE* promoter-*lacZ* fusion was constructed. Interestingly, while transcriptional fusion is not thermally inducible, it is inducible by ethanol, suggesting that the secondary structure of the *groEL* transcript is involved in thermal regulation of the *groEL* gene. Secondary structures of *groE* mRNA at 37 and 42°C were compared using the computer program RNAdraw. Distinct structures at the two temperatures were found, and these structures may be related to a high level of GroEL expression at 42°C.

Key words: *Escherichia coli, groE* promoter-*lacZ* fusion, GroEL, RNA secondary structure

One of the typical heat shock proteins, GroEL, plays important roles in protein folding (2) and assembly (3), and secretion (9). In *Escherichia coli*, the *groEL* gene is preceded by the *groE* promoter and *groES* gene in the *groE* operon (4). When cells are heat-shocked, GroEL synthesis increases (5). Most of the heat-stress response is known to be transient (11). However, in some cases the response is continuous, rather than transient (10). Whether or not GroEL synthesis in *E. coli* is continuous when cells are cultured at a high temperature was determined in this study.

GroEL amounts in cells cultured at 30 and 37°C were similar. However, GroEL amounts increased continuously at 42°C. Continuous synthesis of GroEL for 20 h, at 42°C was also observed.

To obtain a clear understanding of the role of the structural segment of *E. coli groEL*, *groE* promoter was fused into the *lacZ* gene as a marker gene, instead of the *groE* structural gene. When cells containing *groE* promoter-*lacZ* fusion were heat-shocked, there was no significant difference in β-galactosidase levels at 30 and 42°C. This suggests that the element in the *groE* structural gene possibly affects thermo-regulation.

Increase of GroEL synthesis at a high temperature can be partly explained by a high level of transcription (1). Translational regulation in the *groEL* gene of *Streptomyces albus* was also reported (10). Differences of RNA secondary structures at low and high temperatures might be involved in thermo-regulation of the *E. coli groE* gene at the translational level. The difference of *E. coli* groE mRNA secondary structures at 37 and 42°C can be distinguished by using a computer program, RNAdraw. RNA secondary structure containing +130 nt at 42°C was different from that at 37°C, suggesting that different RNA secondary structures might be involved in different levels of GroEL production.

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Materials and Methods

Overproduction of GroEL at various temperatures

For overproduction and comprasion of the amount of GroEL, *E. coli* BL21 was cultured at 30, 37, or 42°C from 8 h to 20 h. *E. coli* BL21 was also transformed with plasmid pGroESL (3) containing *groEL*, and the transformant BL21 (pGroESL) was also cultured in the same conditions.

Quantitation of GroEL production

Harvested *E. coli* BL21 or BL21(pGroESL) was broken by sonication, and the lysates were separated by centrifugation. Total soluble protein was run on 12% SDS-PAGE, and the gel was stained with Coomassie Brilliant Blue R-250. Densitometry analysis of the SDS-PAGE gel was performed by Scion Image Software (Scion Corp.

MD, USA).

Construction of groE promoter-lacZ fusion

To understand the role of the *groEL* structural gene, the segment was replaced by *lacZ*, a reporter. *groE* promoter was amplified from *E. coli* DH5α strain using two primers (7). The amplified product was ligated into *Eco*RI and *Bam*HI sites of plasmid pRS415. The plasmid containing the *groE* promoter and ampicillin resistant gene was named pRSgro-lac. In pRSgro-lac, *lacZ* expression is under the control of the *groE* promoter. Transcriptional fusion of the *groE* promoter-*lacZ* was transferred from pRSgro-lac to *E. coli* MC4100 (*lacZ* deletion strain), using homologous recombination of phage λRZ5 and plasmid pRSgro-lac. *E. coli* MC 4100 was lysogenized by the recombinant phage. The strain containing the *groE* promoter-*lacZ* fusion in the chromosome was named *E. coli* YH24 (7).

Measurement of stress induction

E. coli YH24 containing *groE* promoter-*lacZ* fusion was cultured in LB at 37°C for 14 h. It was diluted 100-fold in new LB, and cultured again to the exponential growth phase. At this phase, the culture was stressed with 3% ethanol. *E. coli* YH24 was cultured at 30°C, and then heat-shocked at 42°C. β-galactosidase activity of stressed cells was determined using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate (8).

Computer analysis of secondary structure of groE mRNA

The full sequence of the *groEL* gene of *E. coli* was used in analyzing its mRNA structure. The promoter sequence is 5' TTTCCCCCTTGAAGGGGCGAAGCCTCATCCC-CATTTCTC TGGTCA 3', and the last 'A' at the 3'-end is the transcriptional start site (1) and designated +1. RNA folding program, RNAdraw, was run to predict secondary structure of *groE* mRNA at 37°C and 42°C.

Results and Discussion

GroEL overproduction at various culture temperatures

Since protein unfolding occurs at a high temperature and GroEL plays a role in solving unfolding problems, GroEL overproduction in the cell is expected at that temperature. GroEL production at 30, 37, and 42°C was investigated in this study. GroEL was produced from the *E. coli* BL21 chromosome or plasmid pGroESL containing the *groEL* gene. GroEL amounts produced from *E. coli* BL21 cells cultured for 12 h at 30, 37, and 42°C were 1.5, 1.7, and 5.1% of total cellular proteins, respectively (Fig. 1, Table 1). There was no significant difference in GroEL amounts at 30 and 37°C. However, GroEL production sharply increased at 42°C, which is 3.4-fold. When *E. coli* BL21 (pGroESL) containing *groEL* was cultured at 30, 37, and 42°C for 12 h, GroEL amounts reached levels of 8.1, 8.0, and 12.9% of total cellular proteins, respectively (Fig. 1, Table 1). Like GroEL production from the chromosome, there was no significant difference in GroEL levels at 30 and 37°C. However, a sharp increase of GroEL synthesis at 42°C was again observed, suggesting an increase of unfolded proteins which need GroEL for their folding.

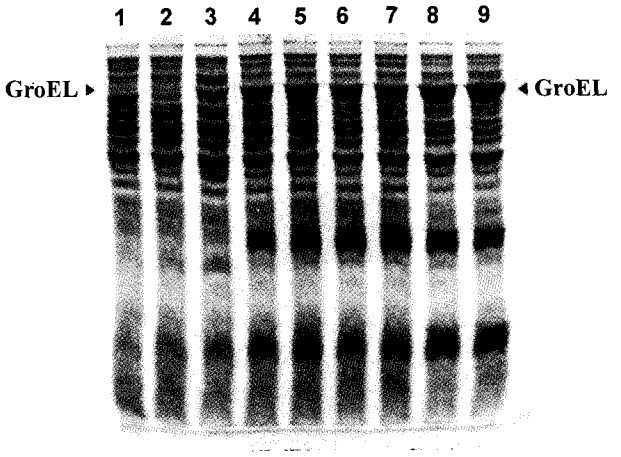

Fig. 1. Effect of culture temperature on GroEL production. Total cellular proteins of broken cells were run on SDS-PAGE. *E. coli* BL21 cells cultured for 12 h, at 30 (lane1), 37 (lane 2), 42°C (lane 3) were run with BL21(pGroESL) cultured for 12 h at 30 (lanes 4-5), 37 (lanes 6-7), 42°C (lanes 8-9). The arrow indicates GroEL position.

Most heat shock gene expression is transient (11). However, GroEL production in *streptomyces albus* is known to be continuous at 42°C under post-transcriptional regulation (10). It is interesting to determine whether or not GroEL synthesis at 42°C in *E. coli* is transient as in most of heat shock proteins. In *E. coli* BL21 cultured at 42°C for 8, 12, 16, and 20 h, GroEL amount were 6.3, 6.5, 6.6 and 7.2% of total cellular proteins, respectively (Fig 2, Table 1). GroEL amounts in BL21 (pGroESL) cells cultured for 8, 12, 16, and 20 h, were 10.9, 12.6, 13.6, and 14.6% of total cellular proteins, respectively. Thus, GroEL synthesis at 42°C in *E. coli* was not transient but con-

Table 1. Determination of the percentage GroEL in total cellular proteins.

% \ Lane	1	2	3	4	5	6	7	8	9
Fig. 1 lanes	1.5	1.7	5.1	7.8	8.4	8.2	7.8	12.7	13.0
Fig. 2 lanes	6.3	6.5	6.6	7.2	10.9	12.6	13.6	14.6	

Densitometry analysis of lanes in Figs 1 and 2 of the SDS-PAGE gel was performed using Scan Image Software

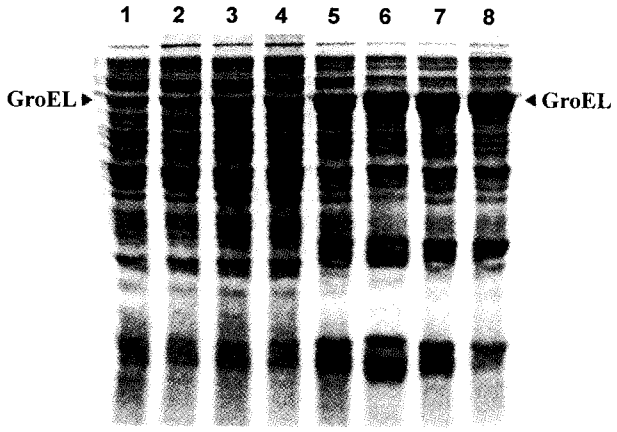

Fig. 2. GroEL production at 42°C. *E. coli* BL21 cells cultured at 42°C for 8 (lane 1), 12 (lane 2), 16 (lane 3), and 20 h (lane 4) were run on SDS-PAGE with BL21(pGroESL) cells cultured at 42°C for 8 (lane 5), 12 (lane 6), 16 (lane 7), and 20 h (lane 8). The arrow indicates GroEL position.

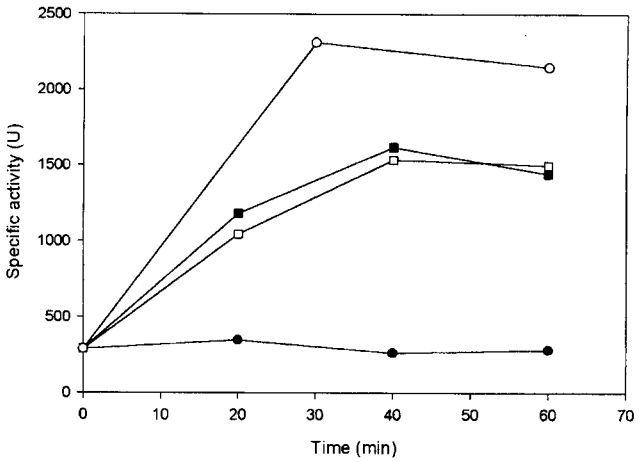

Fig. 3. Ethanol and heat stress. *E. coli* YH24 which is lysogenic for *groE* promoter-*lacZ* fusion was stressed with 3% ethanol (○), heat-shocked (■) at the exponential phase or cultured at 30°C (□). β-galactosidase activities were measured at each intervals. *E. coli* strain MC4100 containing the *lacZ* gene without its native *lac* promoter in the chromosome was used as a control (●). The results were obtained after three sample analyses.

tinuous although the synthesis rate was slow.

There is a previous report that chloramphenicol acetyltransferase (CAT) production was promoted five-fold by increasing the culture temperature of *Escherichia coli* from 37 to 42°C (6). The *cat* promoter is not a heat shock promoter (6). RpoH expression is also increased at 42°C (12). Thus, high expression levels of proteins at a high culture temperature is not a rare phenomenon.

Role of groEL structural gene

There is a report that transcriptional fusion of the *Streptomyces albus groEL* promoter with a reporter gene resulted in constitutive expression independent of heat shock, and in a translational fusion, expression of a marker gene was thermally inducible (10).

The effect of heat shock on *E. coli groE* promoter-*lacZ* transcriptional fusion was investigated in this study. Surprisingly, β-galactosidase expression at 30 and 42°C was not significantly different, suggesting constitutive expression of the *lacZ* gene (Fig. 3). When the *groE* promoter-*lacZ* fusion was stressed with 3% ethanol, clear induction of the enzyme was observed (Fig. 3). Thus, transcriptional fusion is inducible by ethanol, although it is not thermally inducible. The fact that the synthesis of β-galactosidase was not inducible at 42°C in the fusion suggests that the RNA secondary structure of the *groEL* structural gene is involved in thermal regulation of the *groEL* gene. At 30°C, the RNA secondary structure of the *groE* structural gene may repress its translation, and at 42°C, the changed *groE* structure may allow efficient translation. Constitutive expression in the *groE* promoter-*lacZ* fusion can be explained in this sense.

Other factors such as GroEL degradation rate or stability at 42°C might be related to the thermal regulation of the *groEL* gene, and it will be needed to determine whether or not GroEL degradation is reduced at 42°C.

In RpoH (σ32) expression, a translation enhancer region was repressed by a region containing the negative element, and high level of RpoH expression at 42°C was explained by destroying the mRNA secondary structure formed at 30°C (12).

Possible secondary structure of groE mRNA

GroEL amounts in *E. coli* BL21(pGroESL) cells cultured at 42°C increased to 12.8% of total cellular proteins, compared with GroEL amounts (8.0%) at 37°C (Fig. 1, Table 1). It is possible that high culture temperature increased its translation efficiency by changing the secondary structure of *groE* mRNA.

Whether or not the secondary structure of *groE* mRNA at 37 and 42°C can be distinguished was tested using the computer program, RNAdraw. The secondary structures of *groE* mRNA carrying +50~330 nt from transcription start site at 37 and 42°C were compared by gradually extending 10 nts. This approach might be significant in explaining mRNA synthesis in the cell, since its synthesis and folding occur at the same time *in vivo*. There were no big differences in *groE* mRNA structure containing +50~+120 nt at the two temperatures (data not shown). However, in *groE* mRNA with +130 nt, profound differences were found (Fig. 4). Expected free energies needed to form the structure at 37 and 42°C were 73 and 60 kJ, respectively. Before the initiation codon, the same stem-loops were observed. However, after the codon, there were broad base pairings between +70 and 125 nt at 37°C, not at 42°C. The extra 8 base pairings at 37°C which are not found at 42°C might play roles in inhibiting efficient translation of *groE* mRNA in the cell (Fig. 4). The-

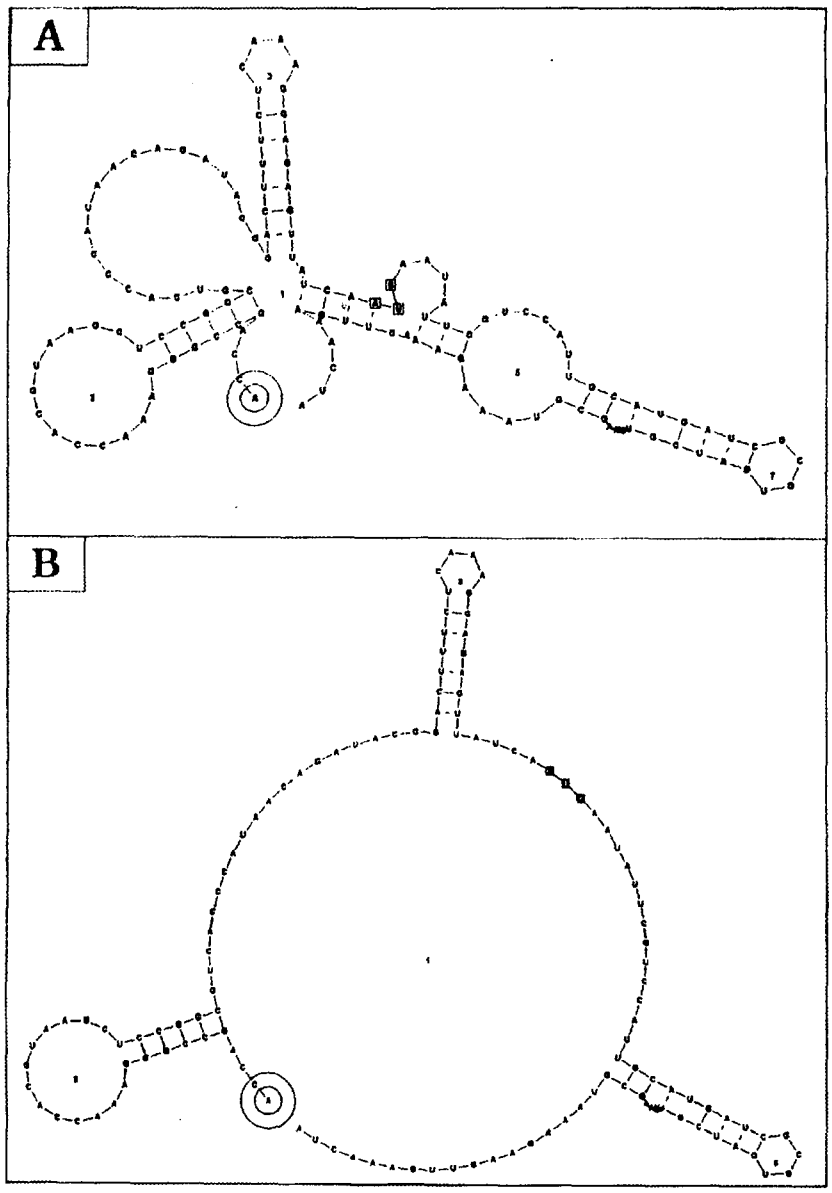

Fig. 4. The predicted structure of *groE* mRNA. Putative secondary structure of mRNA running from transcription start site to +130 nucleotide at 37 (A) and 42°C (B) were predicted. Dual circles indicate 5'-end of *groE* mRNA, and the translation initiation codon is boxed.

oretical analysis using a computer program in this study does not prove differences of RNA secondary structure at the two temperatures, but it can be useful in interpreting different levels of GroEL production at the two temperatures. Different RNA secondary structures at 37 and 42°C were also observed in *cat* mRNA analysis by RNAdraw (6). As in GroEL expression, high level of CAT expression in the cell was achieved at 42°C (6).

Construction of translational fusion of the long segment of the *groEL* structural gene and *lacZ* is needed to prove thermal regulation of the *E. coli groEL* gene at the translational level.

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