***Streptomyces lividans* groES, groEL1 and groEL2 genes**

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The *Streptomyces lividans* groES/EL1 operon and groEL2 gene were cloned and their respective DNA sequences determined. The sequenced DNA comprised the genes and their respective regulatory regions in both cases. Transcription of both groES/EL1 and groEL2 seemed to be subjected to temporal control at 30 °C. At 45 °C the amount of the groEL2 transcript increased considerably in comparison to that of groES/EL1. Among the proteins synthesized under heat shock by *S. lividans*, a fraction enriched in GroEL2 showed the presence of a ring-shaped structure that resembles that of other chaperonins and was active in a rhodanase folding assay.

Keywords: *Streptomyces lividans*, GroEL-like chaperones, heat shock

INTRODUCTION

Streptomyces is a genus that includes filamentous, Gram-positive bacteria with a complex life cycle that involves spore production and mycelium formation (Chater, 1984). Antibiotics, extracellular degradative enzymes and other secondary metabolites of broad industrial application begin to be produced at the transition between vegetative growth and the development of aerial mycelium (Berdy, 1980). Knowledge about physiology and growth control in streptomycetes is essential to fully understand and gain control of the production of these compounds of biotechnological importance.

All micro-organisms respond to an increase in the external temperature by inducing the expression of a reduced number of genes or sets of genes, which is known as the heat shock response. So far, very little is known about the overall regulation of the heat shock response in streptomycetes. GroEL-like proteins are heat-inducible, highly conserved from bacteria to eukaryotes and their function as molecular chaperones is indispensable (Craig *et al.*, 1993). In *S. albus* and *S. coelicolor* the existence of two heat-inducible groEL genes has been reported (Mazodier *et al.*, 1991; Duchene *et al.*, 1994a). Among the streptomycetes, *S. lividans* is

almost exclusively used as a host for the cloning and expression of exogenous DNA because of the lack of an endonuclease restriction system (Kieser & Hopwood, 1991). *S. lividans* is able to produce extracellular proteins in high amounts (Strickler *et al.*, 1992) and glycosylate proteins (Hu *et al.*, 1993), and has been broadly used for the production of proteins both of prokaryotic and eukaryotic origin. Successful overproduction of homologous and heterologous proteins might require a concomitant increase of production levels of cellular chaperones to properly stabilize the newly made polypeptides. In an attempt to further characterize the heat shock response and groEL genes, we cloned and sequenced the groEL genes from *S. lividans* and analysed their transcription level at different phases of cellular growth in both heat-shocked and untreated cultures.

METHODS

Bacterial strains, plasmids and culture methods. *S. lividans* TK21 (Hopwood *et al.*, 1985) was used as source of chromosomal DNA. The plasmid pUC19 (Norrand *et al.*, 1983) was propagated in *Escherichia coli* DH5 α (Hanahan, 1983) for DNA sequencing. Plasmid pRM1, a pRM1cat derivative from which the cat gene had been removed (Parro *et al.*, 1991), was used as the vector to propagate the *S. lividans* chromosomal DNA in *E. coli* NM514 (Murray, 1983). Procedures for the growth and manipulation of *Streptomyces* and general recombinant DNA manipulation were as described by Hopwood *et al.* (1985) and Sambrook *et al.* (1989). For *Streptomyces* vector selection, thiostrepton (a gift of S. J. Lucania, Squibb Institute for Medical Research, Princeton,

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NJ, USA) was used at 50 µg ml⁻¹ in agar and 10 µg ml⁻¹ in broth cultures. For Southern blot hybridization, DNA probes were labelled with [α -³²P]dATP using the Random Primed DNA Labelling Kit from Boehringer Mannheim, following the manufacturer's instructions. The radioactive probes were used to hybridize *S. lividans* genomic DNA fragments transferred to Hybond-N membrane (Amersham).

DNA sequencing. Sequencing of DNA was by the dideoxy chain-termination method (Sanger *et al.*, 1977), using the 7-deaza-dGTP reagent kit from USB following the manufacturer's recommendations. DNA ladders for the analysis of the S1 nuclease mapping experiments were sequenced by the same method.

In vitro transcription and RNA manipulations. RNA was isolated from *S. lividans* liquid cultures growing exponentially in NMMP medium at 30 °C or heat-shocked at 45 °C for 15 min (Marco *et al.*, 1992) and prepared as described previously (Parro *et al.*, 1991; Pulido *et al.*, 1987). The cultures growing at 30 °C made the transition to stationary phase around 40–42 h after inoculation. RNA was extracted from cultures at 30 °C or after 15 min heat shock at 45 °C at times coinciding with the four different growth phases identified for *Streptomyces* spp. (Puglia *et al.*, 1995), designated RG1 (19 h), T (23 h), RG2 (27 h) and S (42 h). Hybridization conditions and S1 nuclease digestion were as described by Sambrook *et al.* (1989) and Barthelemy *et al.* (1986). The size values given for the S1 nuclease protected fragments were corrected by differential migration compared with the sequence ladder (Sollner-Webb & Reeder, 1979). Total RNA for the colony hybridization was labelled with [γ -³²P]ATP in the presence of T4 polynucleotide kinase as described by Favaloro *et al.* (1980).

Computer analysis of sequences. The DNA sequence was analysed for ORFs using CODONPREFERENCE (University of Wisconsin Genetic Computer Group), with a *Streptomyces* codon usage table made from 64 sequenced genes (Wright & Bibb, 1992). Amino acid sequences were analysed using programs from the University of Wisconsin package (version 8.0, September 1994). Sequences were compared with the EMBL database.

GroEL purification. Cells were grown under standard conditions (see above), heat-shocked at 45 °C for 15 min during exponential growth, lysed and processed for GroEL purification as described by Carrascosa *et al.* (1990) and Marco *et al.* (1992), except that after lysis with alumina, the supernatant was batch incubated for 1 h at 4 °C with DEAE-cellulose equilibrated in buffer A (1:1, v/v) and the resin eliminated by low speed centrifugation prior to ammonium sulphate precipitation followed by 5–20% (w/v) sucrose gradient centrifugation (Marco *et al.*, 1992). Fractions from the gradient were tested by SDS-PAGE and electron microscopy to detect protein aggregates.

Western blotting and protein sequencing. Samples were fractionated by SDS-PAGE (Laemmli, 1970) in 10% (w/v) polyacrylamide and transferred to an Immobilon-P membrane as described by Timmons & Dunbar (1990). The transferred material was incubated with a 1:10000 dilution of rabbit polyclonal antibody raised against purified GroEL obtained from *E. coli* grown at 47 °C (Carazo *et al.*, 1991). The bands reacting with the antibody were further revealed by incubation with ¹²⁵I-labelled protein A from *Staphylococcus aureus* (Amersham).

To obtain internal protein sequences, 5 µg purified protein

was subjected to SDS-PAGE, stained with Coomassie Blue and the 62 kDa band was excised and digested with 1 µg trypsin for 4 h as described by Rosenfeld *et al.* (1992). The resulting peptides were separated on a Vydac C18 (250 × 2.1 mm) reverse phase column (Stone & Williams, 1993) and selected fractions were then applied onto polybrene-coated glass fibre filters and subjected to sequencing using an ABI 473A pulsed-liquid phase protein sequencer (Applied Biosystems). To determine the N-terminal protein sequence, 0.2 ml purified protein (about 0.5 mg ml⁻¹) was applied onto a PVDF membrane using a ProSpin cartridge (Applied Biosystems). After washing, the membrane was used directly for sequencing, employing the modified cycles according to manufacturer's instructions.

Folding activity measurement. Chaperonin folding activity was measured using the rhodanase assay as described by Llorca *et al.* (1994). Briefly, rhodanase (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1) from bovine liver (Sigma) was denatured in 8 M guanidinium chloride and diluted 50-fold in buffer A, containing the samples to be analysed, 2 mM ATP, 15 mM MgCl₂, 5 mM KCl, 1 mM DTT and 70 mM Na₂S₂O₈. Incubation was carried out at 37 °C. *E. coli* GroEL and GroES (0.2–0.3 mM each) were incubated under the same conditions as a positive control of the folding reaction. Rhodanase activity was measured as described by Westly (1981).

Electron microscopy. Samples were adhered to carbon-coated collodion grids and negatively stained with 2% (w/v) uranyl acetate. Micrographs were taken in a JEOL 1200EX II electron microscope at 120 kV and 50000 × magnification.

RESULTS

Cloning and sequencing of *groES/EL1* and *groEL2* genes

Sets of oligonucleotides, derived from the conserved regions of the known DNA sequences of the *S. coelicolor* *groES/EL1* operon (Duchene *et al.*, 1994a), were used to synthesize specific DNA fragments from *S. lividans*

Fig. 1. Restriction map and genetic organization of the *groES/EL1* (pPLVG1) and *groEL2* (pPLVG2) regions. Inserts used for transcription initiation mapping, cloned into plasmids pUC18 or pUC19, are shown. Restriction sites employed for subcloning are indicated: A, *AvaI*; B, *BamHI*; H, *HindIII*; P, *PstI*; Pv, *PvuI*; R, *RsaI*; S, *SalI*; Sm, *SmaI*; Sp, *SphI*.

Fig. 2. Comparison of *groES/EL1* (a) and *groEL2* (b) regulatory regions of *S. lividans* (1) with that of *S. coelicolor* (2; Duchene et al., 1994a) and *S. albus* (3; Mazodier et al., 1991) from nt 102 (*groES/EL1*) and nt 1 (*groEL2*) from the *S. coelicolor* sequences as they appear in Duchene et al. (1994a). Identical nucleotides are indicated in bold. Putative -35 and -10 regions relative to the *S. lividans* apparent transcription initiation sites (+1), as determined by S1 mapping, are indicated. CIRCE motifs are underlined with dashed arrows. Translation start codons are underlined. Asterisks indicate gaps in the sequence alignment.

genomic DNA by several cycles of PCR amplification. The oligonucleotides used were GRO-NC (5' GACCG-CCGCAAGGCGATG 3') in combination with GRO-RC (5' GCCCTCCTCGACCGCGGC 3') located at positions 1612-1629 and 1978-1995, respectively, in the case of *groEL2*, and R-PES (5' GGACGAGGTACTC-CTCGC 3') in combination with N*-PES (5' GCATT-GGCACTCCGCTTG 3') or N-PES (5' CCAAGGTTG-CCATCAAGC 3'), located at positions 580-597, 151-168 and 343-360, respectively, in the case of *groES/EL1*. Each oligonucleotide was constructed with *Hind*III restriction sites at each 5' end. The amplified fragments were used as probes to screen a genomic library of *S. lividans* made in *E. coli*. Both the *groES/EL1* operon and the *groEL2* gene were cloned and their respective DNA

sequences determined. A physical map of the cloned regions is shown in Fig. 1. The sequenced DNA contained the genes and their respective regulatory regions in both cases. The sequence of the regulatory regions is shown in Fig. 2.

Southern analysis

Different DNA probes were used in Southern blot hybridization experiments to look for the possible presence of more *groEL*-like genes in the *S. lividans* genome. The 217 bp B2-A1 DNA fragment from plasmid pPLVG1 (Fig. 1) was used as a probe specific for *groES*. The 244 bp Sm-A1 DNA fragment from plasmid pPLVG2 (Fig. 1) was used as a probe specific for *groEL2*.

Fig. 3. Genomic blot hybridization. DNA probes specific for *groES* (lane 1), *groEL2* (lane 2) and *groEL1-groEL2* (lane 3) were used to hybridize *S. lividans* genomic DNA restricted with *Sal*I. Radioactively labelled *Hind*III fragments from bacteriophage λ DNA were used as size markers (lanes M). Numbers on the right indicate the molecular size of the DNA fragments giving positive hybridization signals.

A third probe specific for both *groEL1* and *groEL2* was the 374 bp DNA fragment resulting from several cycles of PCR amplification of the *S. lividans* genome using the oligonucleotides Gro-1 (CCGATCGACGAGAAGTC-CGAC) and Gro-2 (GGAGAGCGCCTCGCCCTCCA-GG) as primers. *Sal*I was chosen to restrict *S. lividans* genomic DNA. The results of the Southern blot hybridization are shown in Fig. 3. Unique DNA fragments of 2820 and 1241 bp (Fig. 3, lanes 1 and 2) hybridized to the *groES*- and *groEL2*-specific probes, respectively; the same two DNA fragments also hybridized to the probe specific for both *groEL1* and *groEL2* (Fig. 3, lane 3), strongly suggesting that *S. lividans* DNA does not contain more than two *groEL*-like genes.

Transcription initiation

Transcription initiation sites for *groES/EL1* and *groEL2* were determined by high resolution S1 nuclease protection experiments. S1 mapping experiments were performed with appropriate single-stranded probes, using total RNA extracted as described in Methods.

Transcription initiation from *groES/EL1* was mapped using the single-stranded *Hind*III-*Ava*I fragment (H1-A1 in pPLVG1b, Fig. 1), purified as described by Barthelemy *et al.* (1986) and labelled at its *Ava*I 5' end, as a probe. This fragment was obtained from the digestion of a PCR fragment (obtained from the amplification of *S. lividans* genomic DNA using oligonucleotides N*-PES and R-PES as primers; H1-H2 in

pPLVG1a, Fig. 1) with *Hind*III and *Ava*I. Transcription initiation from *groEL2* was mapped using the single-stranded *Sma*I-*Ava*I fragment (Sm-A1, Fig. 1), purified as described by Barthelemy *et al.* (1986) and labelled at its *Ava*I 5' end, as a probe.

Transcription initiated from unique start sites at 197 nt from the 5'-labelled *Ava*I end in the case of *groEL2* (Fig. 4b) and at 166 nt from the 5'-labelled *Ava*I end in the case of *groES/EL1* (Fig. 4a). Transcription was subjected to temporal control at 30 °C, as it decreased when the culture entered stationary phase. Under heat shock conditions the amount of the *groEL2* transcript was approximately tenfold higher than that of *groES/EL1*, as determined by densitometry of the S1-protected radioactive fragments, corrected by the exposure time of the gels. In addition, transcription from *groEL2* also showed a dramatic increase at 45 °C, about tenfold higher than transcription at 30 °C (Fig. 4c).

Partial characterization of the heat-induced GroEL complex

After a 15 min heat shock, exponentially growing *S. lividans* TK21 cells were lysed and most of the soluble protein adhered to DEAE-cellulose (Fig. 5, lanes 1–3). The main component that remained unbound to the resin had a relative mobility very similar to the GroEL peptide (60 kDa) from *E. coli*. When this material was subjected to centrifugation in a sucrose gradient, fractions corresponding to a sedimentation coefficient around 20S showed a protein pattern composed mainly of this peptide (Fig. 5, lane 4), suggesting that it is assembled in a multimeric complex.

The 62 kDa peptide from the gradient-enriched sample showed a clear cross-reaction against a polyclonal serum specific for GroEL from *E. coli* (Fig. 6). The specific reactivity of the *S. lividans* peptide was similar to that of the homologous GroEL and clearly different from a control protein of similar molecular size, glutamine synthetase (EC 6.3.1.2; Fig. 6).

Partial sequencing of the purified protein and chaperonin folding activity

As an additional characterization of the *S. lividans* 62 kDa fraction, a sample from the sucrose-gradient-purified material was subjected to trypsin digestion. Two resulting peptides were sequenced and the resulting amino acid sequences compared to those derived from the nucleotide data from *S. lividans groEL1* and *groEL2* coding sequences. In both cases the alignment matched clearly with GroEL2, strongly suggesting that the fraction produced under heat shock conditions, predominantly enriched in the 62 kDa polypeptide, was actually derived from the GroEL2 protein of *S. lividans* (Fig. 7).

Chaperonin folding activity from the 62 kDa peptide from the gradient-enriched sample was measured as

Fig. 4. Determination of transcriptional start sites of the *groES/EL1* operon (a) and *groEL2* (b) by S1 mapping. Total RNAs were isolated from cultures at various times of growth: 19 h (lanes 1 and 5), 23 h (2 and 6), 27 h (3 and 7) and 42 h (4 and 8) at 30 °C (1–4) or after a 15 min shift to 45 °C at the same times (5–8). Unique transcription initiation start sites are indicated by arrows. Sequence ladders, run in parallel, from the known DNA sequence of pPLVG1a (starting at H1 and progressing to H2; Fig. 1) are shown. (c) The variation in the different *groEL* transcripts during the culture growth. Values given correspond to arbitrary integration units (U) of the S1-protected fragments corrected by the exposure time of the autoradiographs. ●, ○, *groES/EL* transcription at 45 and 30 °C, respectively. ■, □, *groEL2* transcription at 45 and 30 °C, respectively.

Fig. 5. GroEL2 purification. Polyacrylamide gel (12.5%, w/v) showing the protein analysed at different stages of purification. Lanes: 1, lysed culture; 2, lysed culture supernatant; 3, protein unbound to DEAE-cellulose; 4, sucrose gradient fraction containing the purified protein; 5, GroEL protein from *E. coli*. The arrow indicates the presence of a 62 kDa protein.

Fig. 6. Protein blotting and parallel electrophoresis. A fraction from the sucrose gradient containing the 62 kDa protein from *S. lividans* (lane 2) was analysed by SDS-PAGE together with glutamine synthetase (lane 1) and *E. coli* GroEL (lane 3). They were subjected to a Western blotting assay using antibodies raised against *E. coli* GroEL; the reactions with glutamine synthetase (lane 4), *S. lividans* 62 kDa protein (lane 5) and *E. coli* GroEL (lane 6) are shown.

described in the legend to Table 1. The 62 kDa fraction (GroEL2 from *S. lividans*) exhibited a folding activity very similar to that obtained with GroEL and GroES from *E. coli*, even though in the *S. lividans* GroEL2

fraction there was no noticeable co-chaperonin. This behaviour is different to that of the *E. coli* GroEL, the folding activity of which is fully dependent of the presence of the co-chaperonin GroES. Interestingly, when the *E. coli* GroES was added to the *S. lividans*

GroEL1 <i>S. lividans</i>	1..MAKILK F DEDARRAL..15
GroEL2 <i>S. lividans</i>	1..MAK I IA F DELARRGL..15
peptide A	1..... I IA F DXXA.....8
GroEL1 <i>S. lividans</i>	54..IARE E VE E DPYENLG..68
GroEL2 <i>S. lividans</i>	55..IAK E IELE D PYEKIG..69
peptide B	1..... E IELE D PYEK....10

Fig. 7. Comparison of the *S. lividans* GroEL1 and GroEL2 deduced amino acid sequences with those obtained from internal peptides of the 62 kDa purified protein. Numbers indicate the relative amino acid positions within the sequences. Identical amino acids are indicated in bold.

Table 1. Chaperonin-like folding activity of the fraction enriched in the 62 kDa protein

Chaperonin folding activity was assayed at 37 °C for 40 min and measured as described by Llorca *et al.* (1994). Values represent the mean of three measurements.

Sample	Activity (OD units/ %)
<i>E. coli</i> GroEL + <i>E. coli</i> GroES	0.151/100
<i>E. coli</i> GroEL	0.001/0.7
<i>S. lividans</i> GroEL2	0.155/103
<i>S. lividans</i> GroEL2 + <i>E. coli</i> GroES	0.052/34

GroEL2 fraction, the folding activity dropped almost by 60%.

Structure of the heat-induced GroEL complex

Samples from the sucrose gradient fractions enriched in the 62 kDa protein were analysed by electron microscopy. Micrographs revealed the presence of two main particles (Fig. 8). The most abundant one had a rectangular shape with dimensions 15 × 10 nm, showing lateral indentations and a stain-penetrated striation along the centre of the particle. The other had a doughnut shape, with a central stained region and a diameter around 15 nm. Due to their similar dimensions, both images could be derived from the same particle, corresponding to lateral and end-on views, respectively, as seen in the Hsp60 GroEL particles from *E. coli* and *Bacillus subtilis* (Martin & Hartl, 1997; Carrascosa *et al.*, 1990). Nevertheless, the morphology and dimensions of the *S. lividans* particle are more similar to the single ring particle produced by the *E. coli* GroEL mutant SR1 (Weissman *et al.*, 1996) than to the usual two-ring structure that is characteristic of the common Hsp60

GroEL. The single toroidal structure is also reminiscent of the morphology of the mammalian chaperonin 60 (Viitanen *et al.*, 1992).

DISCUSSION

In *S. lividans* there are two *groEL* homologues, a characteristic that seems to be shared by other actinomyces including *S. albus* and *S. coelicolor* (Mazodier *et al.*, 1991; Duchene *et al.*, 1994a) as well as by some *Mycobacterium* and *Synechocystis* species (Rinke de Wit *et al.*, 1992; Lehel *et al.*, 1993). More than two *groEL* homologues have been described for *Sinorhizobium* (*Rhizobium*) *meliloti* (Rusanganwa & Gupta, 1993) and *Rhizobium leguminosarum* (Wallington & Lund, 1994). The nitrogen fixing soybean root nodule bacterium, *Bradyrhizobium japonicum*, possesses a multigene family consisting of five *groESL*-like genes and this bacterium may control its GroES and GroEL content in response to specific environmental and physiological needs (Fischer *et al.*, 1993), a feature that might be shared by the other bacteria.

In response to a temperature shift, transcription of both *groES/EL1* and *groEL2* from *S. lividans* occurs at high temperature, but the level of *groEL2* transcription seems to be considerably higher. The upstream regulatory regions from both *groES/EL1* and *groEL2* showed 98% homology to those of *S. coelicolor* (Duchene *et al.*, 1994a). The regions upstream from *groES/EL1* and *groEL2* are also similar to those of *S. albus* (Mazodier *et al.*, 1991) as the homology between them is 93% and 85%, respectively (Fig. 2). It has been reported that the vegetative σ factor, HrdB, would be the regulatory protein involved in controlling the expression of the *S. coelicolor* *groEL* genes (Duchene *et al.*, 1994b). In the case of *S. lividans*, both promoters could also be subjected to control by the equivalent *S. lividans* HrdB since both of them have -10 and -35 regions which are homologous to those of *S. coelicolor* and to the consensus sequence for vegetative promoters in *Streptomyces* (Strohl, 1992; Fig. 2).

The highly conserved inverted repeat GCACTCN₉GA-GTGC, designated CIRCE, has also been described upstream of *groEL* and *dnaK* genes of many bacteria including *groESL4* from *B. japonicum* (Babst *et al.*, 1996; Zuber & Schumann, 1994), suggesting that CIRCE elements may play a role in heat shock regulation of these genes. This region appears to be a DNA recognition site, acting as an operator for the *dnaK* operons of *B. subtilis* and *S. coelicolor* (Shulz & Schumann, 1996; Segal & Ron, 1996). If a similar mechanism operated in *S. lividans* to allow transcription of the *groEL* genes after the temperature shift, another regulatory element such as an activator may be required to account for the tenfold increase of *groEL2* transcription at 45 °C. Post-transcriptional regulation may also occur, either through direct control of mRNA stability or by regulation of translation. Post-transcriptional regulatory signals needed for heat-induced

Fig. 8. Representative electron micrograph of a field of samples from the sucrose gradient containing the 62 kDa protein. The arrowhead points to the front view and the arrow to the side view. Bar, 50 nm.

GroEL1 synthesis have been mapped within the *groEL1* structural gene of *S. coelicolor* (Servant *et al.*, 1994) and *S. albus* produces three GroEL-like proteins, Hsp56 (GroEL2), Hsp58 (GroEL1) and Hsp18 whose N-terminal amino acid sequence is identical to that of Hsp58 (Guglielmi *et al.*, 1993). Both *groEL* genes from *S. albus* have been mutagenized by gene disruption in the chromosome and the conclusion of this experiment is that the N-terminal part of *groEL1* encoding Hsp18 and the whole of *groEL2* encoding Hsp56 seem to be essential for growth (Servant *et al.*, 1993). GroEL1 post-transcriptional regulation is likely to occur in *S. lividans* as well, as deduced from the conserved amino acid sequences of the *S. lividans*, *S. coelicolor* and *S. albus* GroEL1 proteins.

Among the proteins synthesized by *S. lividans* under heat shock, we were able to isolate a fraction enriched in a 62 kDa protein. This protein cross-reacted with antibodies raised against GroEL from *E. coli*, and two internal peptides produced by trypsin digestion matched with sequences of GroEL2 from *S. lividans*. The sedimentation behaviour of this fraction revealed that the protein was assembled as a multimeric particle with a morphology that closely resembled that of the single

ring Hsp60 chaperonins (Viitanen *et al.*, 1992; Weissman *et al.*, 1996). The GroEL2 enriched fraction showed a chaperonin-like peptide folding activity, suggesting that the GroEL2 chaperonin activity may not require the presence of a co-chaperonin. Alternatively, possible non-detectable contaminant amounts of co-chaperonin present in the fraction may be sufficient for the folding reaction. In any case, the folding activity was significantly reduced in the presence of the GroES co-chaperonin from *E. coli*, perhaps indicating a possible competitive interaction between the *E. coli* co-chaperonin and the substrate for the *S. lividans* 62 kDa chaperonin. The presence of a genuine co-chaperonin from *S. lividans*, although not detected in our analysis, cannot be ruled out; therefore, competitive interaction between *E. coli* and *S. lividans* co-chaperonins might also lead to a decrease in folding activity. A more highly purified fraction and a higher yield of the 62 kDa protein, as well as purified GroES from *S. lividans* are needed to characterize properly both the folding activity (and ATP dependence) and the final structure of the *S. lividans* GroEL2 chaperonin. Comparative studies involving GroEL1 and GroEL2 should shed more light on the physiological role played by the two chaperonins in streptomycete cellular metabolism.

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REFERENCES

- Babst, M., Hennecke, H. & Fisher, H. M. (1996). Two different mechanisms are involved in the heat-shock regulation of chaperonin gene expression in *Bradyrhizobium japonicum*. *Mol Microbiol* 19, 827–839.
- Barthelemy, I., Salas, M. & Mellado, R. P. (1986). *In vivo* transcription of bacteriophage ϕ 29 DNA: transcription initiation sites. *J Virol* 60, 874–879.
- Berdy, J. (1980). Recent advances in and prospects of antibiotic research. *Process Biochem* Oct/Nov, 28–35.
- Carazo, J. M., Marco, S., Abella, G., Carrascosa, J. L., Secilla, J. P. & Muiyal, M. (1991). Electron microscopy study of GroEL chaperonin: different views of the aggregate appear as a function of cell growth temperature. *J Struct Biol* 106, 211–220.
- Carrascosa, J. L., Abella, G., Marco, S. & Carazo, J. M. (1990). Three-dimensional reconstruction of the sevenfolded form of *Bacillus subtilis* GroEL chaperonin. *J Struct Biol* 104, 2–8.
- Chater, K. F. (1984). *Morphological and Physiological Differentiation in Streptomyces*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Craig, E. A., Gambill, B. D. & Nelson, R. J. (1993). Heat shock proteins: molecular chaperones of protein biogenesis. *Microbiol Rev* 57, 402–414.
- Duchene, A.-M., Kieser, H. M., Hopwood, D. A., Thompson, C. J. & Mazodier, P. (1994a). Characterization of two *groEL* genes in *Streptomyces coelicolor* A3(2). *Gene* 144, 97–101.
- Duchene, A.-M., Thompson, C. J. & Mazodier, P. (1994b). Transcriptional analysis of *groEL* genes in *Streptomyces coelicolor* A3(2). *Mol Gen Genet* 245, 61–68.
- Favaloro, J., Treiman, R. & Kamen, R. (1980). Transcription maps of polyoma virus-specific RNA: analysis by two dimensional S1 gel mapping. *Methods Enzymol* 65, 718–749.
- Fischer, H. M., Babst, M., Kaspar, T., Acuña, G., Arigoni, F. & Hennecke, H. (1993). One member of a *groESL*-like chaperonin multigene family in *Bradyrhizobium japonicum* is co-regulated with symbiotic nitrogen fixation genes. *EMBO J* 12, 2901–2912.
- Guglielmi, G., Duchene, A.-M., Thompson, C. & Mazodier, P. (1993). Transcriptional analysis of two different *Streptomyces albus* *groEL*-like genes. In *Industrial Microorganisms: Basic and Applied Molecular Genetics*, pp. 17–24. Edited by R. H. Baltz, G. D. Hegeman & P. L. Skatrud. Washington, DC: American Society for Microbiology.
- Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166, 557–580.
- Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P., Ward, J. M. & Schrepf, H. (1985). *Genetic Manipulation of Streptomyces: a Laboratory Manual*. Norwich: John Innes Foundation.
- Hu, P., Chase, T., Jr & Eveleigh, D. E. (1993). Cloning of *Microbispora bispora* cellobiohydrolase gene in *Streptomyces lividans*. *Appl Microbiol Biotechnol* 38, 631–637.
- Kieser, T. & Hopwood, D. A. (1991). Genetic manipulation of *Streptomyces*: integrating vectors and gene replacement. *Methods Enzymol* 204, 430–458.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Lehel, C., Los, D., Wada, H., Gyorgyei, J., Horváth, I., Kovács, E., Murata, N. & Vigh, L. (1993). A second *groEL*-like gene, organized in a *groESL* operon is present in the genome of *Synechocystis* sp. PCC6803. *J Biol Chem* 268, 1799–1804.
- Llorca, O., Marco, S., Carrascosa, J. L. & Valpuesta, J. M. (1994). The formation of symmetrical GroEL–GroES complexes in the presence of ATP. *FEBS Lett* 345, 181–186.
- Marco, S., Parro, V., Carrascosa, J. L. & Mellado, R. P. (1992). *Streptomyces lividans* possesses a GroEL-like chaperonin. *FEMS Microbiol Lett* 93, 127–132.
- Martin, J. & Hartl, F. U. (1997). Chaperone assisted folding. *Curr Opin Struct Biol* 7, 41–52.
- Mazodier, P., Guglielmi, G., Davies, J. & Thompson, C. J. (1991). Characterization of the *groEL*-like genes in *Streptomyces albus*. *J Bacteriol* 173, 7382–7386.
- Murray, N. E. (1983). Phage lambda and molecular cloning. In *Lambda II*, pp. 395–431. Edited by R. W. Hendrix, J. W. Roberts, F. W. Stahl & R. A. Weisberg. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Norrander, J., Kempe, T. & Messing, J. (1983). Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. *Gene* 26, 101–106.
- Parro, V., Hopwood, D. A., Malpartida, F. & Mellado, R. P. (1991). Transcription of genes involved in the earliest steps of actinorhodin biosynthesis in *Streptomyces coelicolor*. *Nucleic Acids Res* 19, 2623–2627.
- Puglia, A.-M., Vohradsky, J. & Thompson, C. J. (1995). Developmental control of the heat-shock stress regulon in *Streptomyces coelicolor*. *Mol Microbiol* 17, 737–746.
- Pulido, D., Jimenez, A., Salas, M. & Mellado, R. P. (1987). A *Bacillus subtilis* phage ϕ 29 transcription terminator is efficiently recognized in *Streptomyces lividans*. *Gene* 56, 277–282.
- Rinke de Wit, T. F., Bekelie, S., Osland, A., Miko, T. L., Hermans, P. W. M., Van Soolingen, D., Drijfhout, J. W., Schöningh, R., Janson, A. A. M. & Thole, J. E. R. (1992). Mycobacteria contain two *groEL* genes: the second *Mycobacterium leprae* *groEL* gene is arranged in an operon with *groES*. *Mol Microbiol* 6, 1995–2007.
- Rosenfeld, J., Capdeville, J., Guillemot, J. C. & Ferrara, P. (1992). In-gel digestion of proteins for internal sequence analysis after one or two-dimensional gel electrophoresis. *Anal Biochem* 203, 173–179.
- Rusanganwa, E. & Gupta, R. S. (1993). Cloning and characterization of multiple *groEL* chaperonin-encoding genes in *Rhizobium meliloti*. *Gene* 126, 67–75.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74, 5463–5467.
- Segal, G. & Ron, E. R. (1996). Regulation and organization of the *groE* and *dnaK* operons in Eubacteria. *FEMS Microbiol Lett* 138, 1–10.
- Servant, P., Thompson, C. & Mazodier, P. (1993). Use of new

- Escherichia coli*/*Streptomyces* conjugative vectors to probe the functions of the two groEL-like genes of *Streptomyces albus* G by gene disruption. *Gene* 134, 25–32.
- Servant, P., Thompson, C. J. & Mazodier, P. (1994).** Post-transcriptional regulation of the groEL1 gene of *Streptomyces albus*. *Mol Microbiol* 12, 423–432.
- Shulz, A. & Schumann, W. (1996).** hrcA, the first gene of the *Bacillus subtilis* dnaK operon encodes a negative regulator of Class I heat shock genes. *J Bacteriol* 178, 1088–1093.
- Sollner-Webb, B. & Reeder, R. H. (1979).** The nucleotide sequence of initiation and termination sites for ribosomal RNA transcription in *X. laevis*. *Cell* 18, 485–499.
- Stone, K. L. & Williams, K. R. (1993).** Enzymatic digestion of proteins and HPLC peptide isolation. In *A Practical Guide to Protein and Peptide Purification for Microsequencing*, pp. 43–69. Edited by P. Matsuda. New York: Academic Press.
- Strickler, J. E., Berka, T. R., Gorniak, J., Fornwald, J., Keys, R., Rowland, J. J., Rosenberg, M. & Taylor, D. P. (1992).** Two novel *Streptomyces* protein protease inhibitors. *J Biol Chem* 267, 3236–3241.
- Strohl, W. R. (1992).** Compilation and analysis of DNA sequences associated with apparent streptomycete promoters. *Nucleic Acids Res* 20, 961–974.
- Timmons, T. M. & Dunbar, B. S. (1990).** Protein blotting and immunodetection. *Methods Enzymol* 182, 679–688.
- Viitanen, P. V., Lorimer, G. H., Seetharam, R., Gupta, R. S., Oppenheim, J., Thomas, J. O. & Cowan, N. J. (1992).** Mammalian mitochondrial chaperonin 60 functions as a single toroidal ring. *J Biol Chem* 267, 695–698.
- Wallington, E. J. & Lund, P. A. (1994).** *Rhizobium leguminosarum* contains multiple chaperonin (cpn60) genes. *Microbiology* 140, 113–122.
- Weissman, J. S., Rye, H. S., Fenton, W. A., Beechem, J. M. & Horwich, A. L. (1996).** Characterization of the active intermediate of a GroEL–GroES-mediated protein folding reaction. *Cell* 84, 481–490.
- Westly, J. (1981).** Thiosulfate:cyanide sulfurtransferase (rhodanase). *Methods Enzymol* 77, 285–291.
- Wright, F. & Bibb, M. J. (1992).** Codon usage in the G+C rich *Streptomyces* genome. *Gene* 113, 55–65.
- Zuber, U. & Schumann, W. (1994).** CIRCE, a novel heat shock element involved in regulation of heat shock operon dnaK of *Bacillus subtilis*. *J Bacteriol* 176, 1359–1363.

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