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Large-scale gene expression profiling reveals physiological response to deletion of chaperone *dnaKJ* in *Escherichia coli*

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ABSTRACT

Chaperone DnaK and its co-chaperone DnaJ plays various essential roles such as in assisting in the folding of nascent peptides, preventing protein aggregation and maintaining cellular protein homeostasis. Global transcriptional changes *in vivo* associated with deletion of *dnaKJ* were monitored using DNA microarray to elucidate the role of DnaKJ at the transcriptional level. Microarray profiling and bioinformatics analysis revealed that a few chaperone and protease genes, stress-related genes and genes involved in the tricarboxylic acid cycle and oxidative phosphorylation were up-regulated, whereas various transporter genes, pentose phosphate pathway and transcriptional regulation related genes were down-regulated. This study is the first to systematically analyze the alterations at the transcriptional level *in vivo* in deletion of *dnaKJ*. Fatty acid methyl esters analysis indicated that the amount of unsaturated fatty acid sharply increased and subcellular location prediction analysis showed a marked decrease in transcription of inner-membrane protein genes, which might have triggered the development of aberrant cell shape and susceptibility for some antibiotics in the Δ*dnaKJ* strain.

1. Introduction

The basic biological role of molecular chaperones is to prevent unfolded proteins from aggregation and to assist proteins in folding into their correct conformation. Multiple chaperones engage in concerted and well-regulated activities in the crowed cellular environment to maintain protein homeostasis (proteostasis) *in vivo* (Powers et al., 2009; Hartl et al., 2011). As a member of the Hsp70 chaperone family and an ATP-dependent chaperone, DnaK, together with cellular ribosome-bound chaperone trigger factor (TF) and chaperonin GroEL/ES, assists the newly synthesized proteins to correctly fold in prokaryotes (Hartl and Hayer-Hartl, 2002). Co-chaperone DnaJ recruits non-native substrates into DnaK. The hydrolysis of ATP to ADP in DnaK is accelerated by DnaJ, resulting in stable substrate binding. After binding of GrpE and release of ADP followed by DnaJ dissociation, the substrate is released for folding or transferred to other chaperones *in vivo* (Mayer, 2010; Hartl et al., 2011). In *Escherichia coli*, at least ~700 cytosolic proteins whose folding processes are associated with DnaK have been identified by quantitative proteomics (Calloni et al., 2012).

The functional relationships among these chaperones are very complex. Chaperone-depletion in cell results in protein aggregation due to the defects of protein exporting. DnaK-deficient cells grow normally and do not exhibit folding defects between 30 °C and 37 °C (Hesterkamp and Bukau, 1998). However, DnaK is essentially required for *E. coli* to survive at temperature stress conditions such as >40 °C or <18 °C (Bukau and Walker, 1989a). When cultured at 42 °C, at least 150 proteins species aggregate as a result of dysfunctional DnaK in *E. coli* (Mogk et al., 1999). TF-deleted cells are viable at any temperature and no obvious protein folding defect is detectable in Δ*tig* strains (Deuerling et al., 1999; Teter et al., 1999). These studies show that individual DnaK or TF is not essential for

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E. coli survival. However, the combined deletion of DnaK and TF is synthetically lethal for *E. coli* at temperature above 30 °C (Deuerling et al., 1999; Teter et al., 1999; Genevaux et al., 2004). The cells in simultaneous deletion of DnaK and TF could only grow at a very narrow temperature range (<30 °C) and exhibit a higher level of protein aggregation compared to Δ*tig* or Δ*DnaK* cells (Genevaux et al., 2004). Unlike DnaK or TF, chaperonin GroEL/GroES is essential for the growth of *E. coli* at all temperatures (Fayet et al., 1989). Although the function of DnaK and TF overlap, the defect in DnaK could not be compensated by the TF overexpression, as it could not rescue the heat-sensitive phenotype in Δ*dnaK* mutant strains and it was even lethal for the Δ*dnaK* mutant at 30 °C (Schaffitzel et al., 2001). Furthermore, increasing expression of some chaperones such as GroEL/GroES, SecB, or Hsp33 could partially rescue the temperature-sensitive phenotype and protein folding defective of Δ*dnaK*Δ*tig* mutants (Genevaux et al., 2004; Vorderwulbecke et al., 2004). At the proteome level, some substrates that were shared by DnaK and TF and involved in multiple cellular processes have been identified through the analysis of aggregated proteins in Δ*tig* cells or in Δ*dnaK*Δ*tig* cells by two-dimensional gel electrophoresis. DnaK associates with 77% of TF-binding peptides; therefore, DnaK and TF had highly similar substrates populations *in vivo* (Tomoyasu et al., 2001; Deuerling et al., 2003; Calloni et al., 2012).

To date, all investigations on DnaK substrates were done using proteome analysis and have been focused on the specific changes in strains without DnaK and/or TF. Considering spatial and temporal features of regulatory chaperones *in vivo*, it is necessary for assessing the transcriptional changes in *dnaK*-depleted strains to get more in-depth understanding on chaperones regulation network. In the present study, we utilized expression microarrays to examine the global transcriptional response upon deletion of DnaKJ in *E. coli* to establish the transcriptional network affected by DnaKJ. In parallel, we characterized the components of the cell membrane that were responsible for developing an aberrant cell shape in DnaKJ-depleted strains. We summarized genes involving in different biological pathways that were likely regulated and controlled by DnaKJ; and further analyzed the regulatory roles of DnaKJ on prokaryotic gene expression.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The *E. coli* strains Z125 (Δ*dnaK*/*dnaJ*::*kan*) were constructed as previously described (Datsenko and Wanner, 2000; Zhou et al., 2010). Briefly, the primer pair K-K*J*1 (5′-acaaccacatgatgaccgaatatatagtggagacgtttagatgattgaacaagatggattgc-3′)/K-K*J*2 (5′-ttttacccaggcctgcccacgggcaggcttttggggaggttagaagaac-tcgtcaagaaggc-3′) whose 5′ region was homologous to the flanking regions of *dnaK*/*dnaJ* was used to amplify a kanamycin resistance gene (*kan*) from the pKD4 plasmid. The *E. coli* BW25113 harboring vector pKD46 was then transformed using the PCR products by electroporation to replace its *dnaK*/*dnaJ*. The *kan*$^+$ clones were screened on LB plates containing kanamycin and then characterized by PCR by using the primer pair, GK*J*1 (5′-agtcaaccgcagtgagtga-3′)/GK*J*2 (5′-cactttacaggtgctcgcat-3′), which targeted the upstream and downstream regions of *dnaK*/*dnaJ* coding region, respectively. A 980-bp PCR product in 1% agarose gel was observed, which indicate *dnaK*/*dnaJ* was replaced by *kan*. The absence of *dnaK*/*J* in the resultant strain that was named as Z125 was further confirmed by western blotting using an anti-DnaK monoclonal antibody. No band was observed for Z125 which was a *dnaK* clean deletion mutant. Both the wild strain BW25113 and Z125 were cultured at 37 °C, 220 rpm in LB medium. Until culture density of them reached 0.6 at a wavelength of 600 nm, the cells were harvested for RNA extraction and fatty acid methyl ester analysis.

2.2. RNA preparation and cDNA synthesis

Total RNA was isolated from strains using a NucleoSpin RNAII kit (Macherey-Nagel Corporation, Düren, Germany) following the manufacturer's instructions. Firstly, the quality and integrity of extracted RNA was evaluated by electrophoresis on 1% formaldehyde denaturing agarose gel. Distinct 23S and 16S RNA bands were observed in agarose gel by electrophoresis for both BW25113 and Z125. The ratios of OD_{260}/OD_{230} and OD_{260}/OD_{280} for RNA varied from 1.97 to 2.18 and from 2.11 to 2.16, which were indicative of adequate quality RNA for microarray assay. RNA concentration was indirectly measured by absorbance at a wavelength of 260 nm.

To perform microarray testing, cDNA was synthesized from the isolated total RNA by using a PrimeScript RT reagent kit (Takara). The cDNA from BW25113 and Z125 were labeled with Cy5- and Cy3-dCTP fluorescent dye (GE Healthcare), respectively, by using a Klenow enzyme labeling strategy prior to hybridization.

2.3. Microarray operation and data analysis

Triplicate *E. coil* Genome Oligo genome 70-mer oligonucleotide microarray (CapitalBio Corporation, Beijing, China) was used in the present study. The microarray consisted of 9308 oligonucleotide probes (Operon). The cDNAs from BW25113 and Z125 were purified by using a PCR NucleoSpin Extract II Kit (Macherey-Nagel Corporation, Düren, Germany) and resuspended in an elution buffer, then labeled with fluorescent dye Cy5 and Cy3, respectively. Hybridization and scanning were conducted according to a standard protocol (Guo et al., 2008). All the microarray experiments had been performed for three times.

The transcription data were deposited in the NCBI Gene Expression Omnibus (GEO) database and assigned accession number GSM1712366 and platform identification number GPL8850.

In the original microarray data, genes displaying statistically significant differential expression between BW25113 and Z125 were screened according to following criteria: average up-fold change ≥2 or down-fold change ≤0.5 and q-values of quality control ≤1%. Genes exhibiting differential expression were grouped and annotated based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (www.genome.jp/kegg) and Uniprot website (www.uniprot.org).

2.4. Bioinformatics analysis

Bioinformatics and statistical analyses of the physicochemical properties of deduced protein sequence from altered genes in microarray were performed as detailed in the following DnaK, DnaJ and an up-regulated small RNA gene were excluded from all bioinformatics analyses. The molecular weight, isoelectic point, and average hydrophobicity value of deduced proteins were determined by ProtParam Tool (*Protein Identification and Analysis Tools on the ExPASy Server*). The solubility upon *in vitro* translation of deduced proteins was calculated from eSOL database (solubility database of all *E. coli* protein) (Niwa et al., 2009). The subcellular localization of deduced proteins was predicted with TargetP 1.1 (Emanuelsson et al., 2007). All the *E. coli* protein sequences were downloaded from Uniprot website. Statistical analysis was performed with the PROMPT software (Schmidt and Frishman, 2006).