FEBS Letters 589 (2015) 4047–4052

journal homepage: www.FEBSLetters.org

GroEL to DnaK chaperone network behind the stability modulation of σ^{32} at physiological temperature in *Escherichia coli*

Monobesh Patra, Sourav Singha Roy, Rakhi Dasgupta, Tarakdas Basu *

Department of Biochemistry and Biophysics, University of Kalyani, Kalyani 741235, West Bengal, India

ARTICLE INFO

Article history:
Received 24 July 2015
Revised 24 October 2015
Accepted 27 October 2015
Available online 9 November 2015

Edited by Stuart Ferguson

Keywords:
Stability of σ^{32}
Physiological temperature
DnaK
GroEL
Chaperone network
E. coli

ABSTRACT

The stability of heat-shock transcription factor σ^{32} in *Escherichia coli* has long been known to be modulated only by its own transcribed chaperone DnaK. Very few reports suggest a role for another heat-shock chaperone, GroEL, for maintenance of cellular σ^{32} level. The present study demonstrates in vivo physical association between GroEL and σ^{32} in *E. coli* at physiological temperature. This study further reveals that neither DnaK nor GroEL singly can modulate σ^{32} stability in vivo; there is an ordered network between them, where GroEL acts upstream of DnaK.

1. Introduction

Accurate maintenance of cellular proteome integrity is the prime criteria for survival of every cell; otherwise, aggregation of proteins causes many harmful diseases [1,2]. Significant numbers of proteins acquire their three-dimensional structure according to the Anfinsen's model [3,4]. Others (20–30%) need co- and/or post-translational help of chaperones to acquire their biologically functional conformation [5,6]. Such chaperones were initially identified in cells under heat stress. Heat stress induces not only chaperones, but also proteases and other accessory proteins. At physiological temperature, alterations of protein conformation occur also as a consequence of different environmental stresses other than heat. Thus, tight regulation of the expression of chaperones and proteases are crucial to maintain the cellular protein folding environment at physiological as well as under a number of stressed conditions [7–9].

Escherichia coli heat-shock genes for chaperones and proteases are transcribed by RNA polymerase with the alternative sigma factor σ^{32}. The cellular level of σ^{32} (10–30 copies/cell) increases by 12–15-fold upon shifting the cells from physiological temperature (30 °C) to a heat-stress one (45 °C), with transient induction of chaperones and proteases [7–11]. However, over-expression of σ^{32} is toxic to the cells [12]. A complex negative feedback pathway is known to regulate tightly the cellular level of σ^{32} by its own transcribed gene products viz., DnaK chaperone system and FtsH protease [7–9,12–16]. The existing model for regulation of σ^{32} stability is the 'protein titration model', that depicts unfolded proteins and σ^{32} competing for binding with DnaK; accumulation of unfolded proteins relative to the basal level titrates away DnaK from σ^{32} and consequently σ^{32} becomes stable and active, resulting in overproduction of DnaK, which binds free σ^{32} to make the σ^{32} accessible to FtsH attack and thereby σ^{32} becomes further unstable [7,8,12,15,16].

The important chaperone systems in *E. coli* are the GroE (consisting of the chaperone GroEL with its co-chaperone GroES) and DnaK (consisting of the chaperone DnaK with its co-chaperones DnaJ and GrpE) [7,8,12]. According to the current knowledge, for attainment of an active conformation, 18–20% cytosolic proteins require assistance of the DnaK system, 6–8% proteins require the GroE system and about 10% proteins require both the DnaK and GroE systems where GroEL acts downstream of DnaK [17–20].

Author contributions: MP contributed substantially in designing and performing the experiments. In acquisition, analysis and interpretation of data SSR contributed physically and RDG contributed intellectually during experimentations. TB contributed by conceptualizing the original problem, discussing the results time to time and finally preparing the manuscript. All authors read and approved the final manuscript.

* Corresponding author. Fax: +91 33 25828282.
E-mail address: tarakdb@yahoo.com (T. Basu).

http://dx.doi.org/10.1016/j.febslet.2015.10.034
0014-5793/

The concept of σ^{32} as the obligate substrate of DnaK, as per the protein titration model, faces question from the findings that (i) depletion of GroEL/S in *E. coli* causes stabilization of σ^{32} and increases σ^{32}-dependent transcription, (ii) over-expression of GroEL decreases σ^{32} activity, (iii) induction of GroEL-specific substrate increases σ^{32} activity and (iv) GroEL binds σ^{32} in vitro, inhibiting σ^{32}-dependent transcription [12,21]. All this evidence signifies involvement of both DnaK and GroE chaperones in the maintenance of σ^{32} stability and activity. However, the mechanism of GroEL function in the regulation of σ^{32} stability is still obscure. This study provides experimental evidence for the first time in favor of the in vivo physical association of σ^{32} with GroEL and DnaK chaperones, as well as the essential requirement for both the chaperones in modulation of σ^{32} stability in a sequential manner, where GroEL acts before DnaK.

2. Materials and methods

2.1. Bacterial strains, plasmids and antibodies

The *E. coli* strains, plasmids and antibodies, used in this study, were as follows: (1) MC4100 and MC4100 *ΔrpoH* [22] as *wild type* (*wt*) strain and its *rpoH* (σ^{32} gene) mutant respectively, were obtained as generous gift from Dr. Franz Narberhaus, Ruhr University, Germany; (2) MG1655 and MG1655 Δ*KJ* [23] as (*wt*) strain and its *dnaKdnaJ* mutant respectively, were obtained from Dr. C.K. Dasgupta, Jadavpur University, India; (3) *E. coli groEL* mutant LG6, a derivative of MG1655, wherein the chromosomal *groE* operon was placed downstream of *lac* promoter [24], was gifted by Dr. S.C. Mande, National Centre for Cell Science, India; (4) plasmid pKJE7 containing the *dnaK*, *dnaJ*, *grpE* genes and plasmid pGro7 containing the *groEL*, *groES* genes [25] were kind gift from Dr. S.N. Gummadi, IIT-Madras, India; (5) plasmid pHis-IbpB containing the his-tagged *ibpB* gene under the control of *lac* promoter [26] was obtained from Dr. Krzysztof Liberek, University of Gdansk, Poland; (6) primary antibodies of *E. coli* proteins, such as monoclonal anti-DnaK antibody raised in mouse was purchased from MyBioSource, USA; polyclonal antibody to GroEL raised in rabbit from Sigma–Aldrich, USA; monoclonal antibody of σ^{32} raised in mouse from Neoclone, USA; polyclonal anti-His antibody raised in rabbit from Biobharati Life Science Pvt. Ltd., India; and HRP-conjugated goat anti-rabbit IgG and rabbit anti-mouse IgG were procured from Genei, India.

2.2. Co-immunoprecipitation assay

Bacterial cells were grown in MOPS medium [27] at 30 °C until the OD_{600nm} value attained around 0.3. The centrifuged pellet from equal number of 5–10 mL grown cells (by OD_{600nm}) was subjected to native lysis and the lysate was made free of cell debris, as described in Kourennaia et al. [28]. Prior to the co-immunoprecipitation step, 2–3 μL of required primary antibody and 30 μL of protein A-CL agarose beads (Genei, India) were taken in 1.0 mL W (wash)-buffer [28], allowed to incubate at 4 °C overnight in a 'Roto-spin' and washed by the W-buffer through centrifugation at 10000 rpm at 4 °C. To the bead-antibody pellet, 1.0 mL cell lysate (pre-cleared with protein A-CL agarose beads) was added and allowed immunoprecipitation for overnight in a 'Roto-spin' at 4 °C. The immunoprecipitate, after washing with W-buffer, was subjected to western blot analysis [29] by anti-σ^{32}/anti-GroEL/anti-DnaK/anti-His primary antibody separately or by a mixture as needed. The band intensities were analyzed by 'Image Master' software (2D Platinum 6.0) of the gel imaging system Typhoon 9210 (GE Health Care).

2.3. Radioactive pulse-chase and immunoprecipitation experiment

The experiment was performed just as described in Jana et al. [30]. Here, cells were initially grown up to the log phase ($OD_{600nm} \approx 0.3$) at 30 °C and radio-labeled with ^{35}S-methionine (BRIT, India) at a final concentration of 30 μCi/mL. For the pulse-chase experiment, labeling was done for a pulse of 1 min and then chased with cold methionine; in addition, L-arabinose was added at a final concentration of 0.2%, so that the GroE and DnaK were induced in the transformed cells (MG1655 Δ*KJ* + pGro7) and (LG6 + pKJE7) respectively. At different instants of chasing up to 30 min, a 1–3 mL cell aliquot was withdrawn and proteins were extracted. Protein extracts were then subjected to immunoprecipitation, using anti-σ^{32} primary antibody and proteinA-CL agarose beads. The immunoprecipitates were run in 12% SDS–polyacrylamide gel, the gel was dried, phosphorimaged in Typhoon 9210 and analyzed by its 'Image Master' software (2D Platinum 6.0).

3. Results and discussion

3.1. Physical association of chaperones with σ^{32}

From some previous indirect experimental observations it was predicted that there might be a GroE–DnaK chaperone network controlling the cellular level of σ^{32} in *E. coli* [12,21]. Although there were reports concerning in vitro interactions between σ^{32} and DnaK/GroEL, an in vivo interaction was evident only for DnaK [12,31,32]. Here we show for the first time the evidence for in vivo physical association between σ^{32} and GroEL, by co-immunoprecipitation experiments. Native lysate of *E. coli* (*wt*) cells MC4100 or MG1655, grown at physiological temperature (30 °C), was immunoprecipitated with anti-σ^{32} antibody and the immuno-complex was subjected to western blot analysis using anti-σ^{32}, anti-GroEL and anti-DnaK antibodies. Presence of GroEL, DnaK and σ^{32} bands in the blots (Fig. 1A and Fig. S1) implied that in the immunoprecipitate, both GroEL and DnaK were bound to σ^{32}, forming either a ternary complex GroEL–σ^{32}–DnaK or binary complexes GroEL–σ^{32} and DnaK–σ^{32}. To investigate the exact

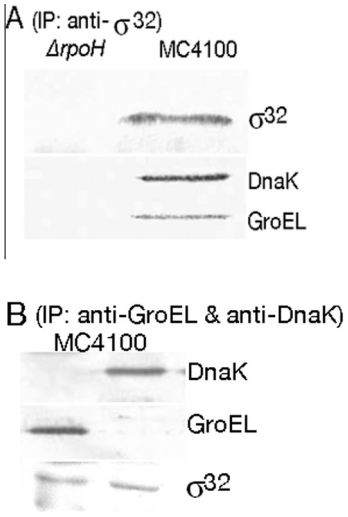

Fig. 1. In vivo association of GroEL and DnaK chaperones with σ^{32} in *E. coli* cells. (A) Co-immunoprecipitation of native cell lysate was done with anti-σ^{32} antibody and subsequent western blot was done using anti-σ^{32} antibody and (anti-GroEL + anti-DnaK) antibody mix separately with lysates of MC4100 cells at 30 °C and of MC4100 *ΔrpoH* cells at 20 °C. (B) Co-immunoprecipitation of native cell lysate was done by anti-GroEL/anti-DnaK antibody, followed by western blot using anti-σ^{32}, anti-GroEL and anti-DnaK antibodies separately at 30 °C in MC4100 cells.