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Role of *Escherichia coli* DnaK and DnaJ chaperones in spontaneous and induced mutagenesis and their effect on UmuC stability

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Abstract

The frequency of spontaneous as well as induced reversions of auxotrophic mutations in *Escherichia coli* AB1157 and its Δ*dnaK* and Δ*dnaKdnaJ* derivatives was estimated. The obtained results demonstrate that both mutants tested are characterized by elevated frequency of spontaneous reversions compared to their AB1157 parent. In contrast, the frequency of reversions induced by UV and MMS, i.e. agents inducing the SOS response, is reduced in Δ*dnaJ* and Δ*dnaKdnaJ* mutants, pointing to the possible defect of these mutants in error prone repair. Due to the fact that UmuC protein is one of the main players executing the error prone repair, its stability in Δ*dnaJ* and Δ*dnaKdnaJ* mutants was also studied. Reduced UmuC stability was demonstrated only in the Δ*dnaKdnaJ* mutant.

Keywords: DnaK; DnaJ; Mutagenesis; UmuC protein; *Escherichia coli*

1. Introduction

Escherichia coli protein DnaK (Hsp70) and its co-operating factor DnaJ (Hsp40) are members of the DnaK – DnaJ – GrpE chaperone team. They are classified as stress proteins because they are induced under such stresses as heat shock conditions, although they are present and have essential functions at low temperatures [1,2]. As molecular chaperones, these proteins can bind other proteins and influence their proper folding, oligomeric assembly, stabilization, transport across membranes and degradation [3,4]. Moreover, both proteins play a very important role in negative regulation of the heat shock response [5]. Information about the cellular functions of DnaK and DnaJ proteins originated mainly from the analysis of point and null mutants [6,7]. The lack of or the presence of mutations in *dnaK* and *dnaJ* genes lead to the impairment of cellular processes executed mainly by protein complexes, such as replication of bacterial chromosome, plasmids and phages [8–10], gene expression [11,12] and antibiotic resistance [13,14].

The frequency either spontaneous mutations or those induced by physical, chemical and biological agents depends on the activity of various repair systems. The majority of these systems require the participation of protein complexes [15] and therefore potentially can be influenced by chaperones. The amount of spontaneous mutations arising after incorporation of non-complementary bases is low, 1 per 10^9 polymerized nucleotides. Such misincorporations are repaired mainly by the

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exonuclease 3′ → 5′ activity of polymerase III ε subunit and also by specialized repair systems, e.g. mismatch repair system depending on Dam methylase in which proteins MutH, MutS and MutL are the most important [16], and the VSP system [17]. MutT protein is also involved in the repair of particular kind of spontaneously arising mistakes [18] and its biological role is to remove 8-oxo-7,8-dihydro-2′-dGTP (8-oxodGTP) from the cellular nucleotide pool [19].

The mechanisms repairing DNA induced damage usually reconstitute the original nucleotide sequence except for one that is error-prone and is known as the UmuCD-dependent mutagenic pathway. The *umuC* and *umuD* genes are part of the SOS regulon which is derepressed by the introduction into DNA of changes that block replication, e.g. thymine dimers [20]. UmuD, after self-cleavage into the shorter UmuD′ [21,22], forms a complex with UmuC [23]. $UmuD'_2C$ – *E. coli* DNA polymerase V, called an "error prone" polymerase, is able to catalyze the bypass of abasic lesions [24] by inserting primarily dAMP [25] or even to bypass non-DNA sequences [26]. The process of translesion DNA synthesis executed by lesion-replicating DNA polymerases is conserved from bacteria to humans [27]. It has been recently shown that only two co-factors are needed for efficient DNA polymerase V-mediated lesion bypass: DNA substrate onto which β-clamp is stably loaded and an extended RecA/A filament [28]. The involvement of GroEL and GroES chaperonins in UmuDC-dependent mutagenesis and UmuC stability is well established [29,30]. It has also been shown that the Hsp70 complex and Hsp60 complex participate in the sequential folding of UmuC [31].

In this paper, we present data on the participation of DnaK and DnaJ chaperones in spontaneous and induced mutagenesis obtained by examination of the frequency of auxotrophic marker reversion in Δ*dnaKdnaJ* and Δ*dnaJ* mutants. An elevated frequency of spontaneous and reduced frequency of UV and MMS-induced reversion was observed. The latter result suggests the participation of DnaK and DnaJ in error-prone repair. The involvement of DnaK and DnaJ in UmuC stability has also been proved.

2. Materials and methods

2.1. Bacterial strains and genetic manipulations

Escherichia coli K12 strains used in this work are listed in Table 1. All strains tested were derivatives of the multiple auxotroph AB1157. The strategy of Δ*dnaJ* and Δ*dnaKdnaJ* mutants construction was to create deletion in a recombinant plasmid by Kan^R cassette replacement and then to cross these mutations into the *E. coli* chromosome. Deletions of *dnaJ* and *dnaKdnaJ* were transferred to AB1157 strains using P1 transduction. The preparation of P1 and transduction were carried out following standard protocols [32]. The details of genetic manipulations were described previously [14]. Due to the fact that *dnaJ* and *dnaK* mutants easily generate secondary mutations able to suppress their defects, bacterial cultures were kept frozen at −70 °C in a large number of small aliquots in order to avoid restreaking. Strain AB1157 bearing Δ*umuDC* mutation originally constructed by Woodgate [33] was purchased from Dr. E. Grzesiuk, Department of Molecular Biology, Institute of Biochemistry, Polish Academy of Sciences.

The genetic characteristics of plasmid pJW14 complementing the defect of Δ*dnaKdnaJ* mutant strains obtained from Dr. J. Wild, University of Wisconsin have been described by Wolska et al. [14]. The operon bearing the wild type *dnaK* and *dnaJ* allele was cloned under the *lacUV5* promoter allowing to use of isopropyl-β-D-thiogalactopyranoside (IPTG) at 0.2 mM as an inducing agent. Stability of UmuC was estimated in the strains transformed with the pair of plasmids – pGW1-2 (Cm^RKm^R) containing gene encoding T7 RNA polymerase under control of the p*L*λ promoter and reporter plasmid, pGW2030 (Ap^R) containing *umuC* expressed from Φ10T7 promoter. Plasmid pGW1-2 was constructed by

Table 1
Bacterial strains and plasmids used in this study

Strain	Relevant genotype	Source or reference
AB1157	*thr-1 ara-14 leuB6* Δ(*gpt-proA*) *lacY1 ts-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kgdK51 xyl-5 mtl-1 argE3 thi-1*	Bachmann [45]
KW200	AB1157 Δ*dnaJ*/Kan^R	This study
KW198	AB1157 Δ*dnaKdnaJ*/Kan^R	This study
KW243	KW198 (pJW14)/Kan^RCm^R	This study
EC2413	AB1157 Δ*umuDC595::cat*/Cm^R	Grzesiuk and Janion [46]
KW253	AB1157 (pGW1-2, pGW2030)/$Km^RCm^RAp^R$	This study
KW257	KW198 (pGW1-2, pGW2030)/$Km^RCm^RAp^R$	This study
pJW14	ori p15A p*lacUV dnaKdnaJ lacI*^{q} (Cm^R)	Wild et al. [47]
pGW2030	ori pBR322 pΦ10T7 *umuC* (Ap^R)	Donnelly and Walker [29]
pGW1-2	ori p15A gene*1*T7 λcI857 (Km^RCm^R)	This study