

The DnaK Chaperone Is Necessary for α -Complementation of β -Galactosidase in *Escherichia coli*

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We show here the involvement of the molecular chaperone DnaK from *Escherichia coli* in the in vivo α -complementation of the β -galactosidase. In the *dnaK756*(Ts) mutant, α -complementation occurs when the organisms are grown at 30°C but not at 37 or 40°C, although these temperatures are permissive for bacterial growth. Plasmid-driven expression of wild-type *dnaK* restores the α -complementation in the mutant but also stimulates it in a *dnaK*⁺ strain. In a mutant which contains a disrupted *dnaK* gene (Δ *dnaK52::Cm*^r), α -complementation is also impaired, even at 30°C. This observation provides an easy and original phenotype to detect subtle functional changes in a protein such as the DnaK756 chaperone, within the physiologically relevant temperature.

The α -complementation of *Escherichia coli* β -galactosidase, which occurs both in vivo and in vitro, is the most well-known example of protein fragment complementation in which two segments of a protein associate noncovalently to form a functional structure. In the case of β -galactosidase, which is a homotetramer, each monomer (1,023-amino-acid residue) can be divided into two segments, a small (7,500-Da) α -fragment, or α -donor peptide, and a larger α -acceptor composing the remainder of the 116-kDa monomer. The latter is also called the M15 protein because it is the product of the *lacZ* Δ M15 allele, a spontaneous partial deletion that does not disturb the open reading frame; the M15 protein, which lacks residues 11 through 41, is an inactive dimer (4 M15 \rightarrow 2 M15₂') (10). In the presence of the α -fragment, it forms a tetramer with enzymatic activity (11, 29) according to the following multistep process determined in vitro (17): 2 M15₂' + 4 α \rightarrow 2 M15₂ α ₂' \rightarrow 2 M15₂ α ₂ \rightarrow (M15₂ α ₂)₂.

Since the molecular chaperone HSP70, the *dnaK* gene product from *E. coli*, catalyzes specific inter- and intramolecular protein interactions (8, 14), among the myriad of functions assigned to it, it was tempting to look at its possible involvement in the α -complementation of β -galactosidase in vivo.

α -Complementation in two isogenic strains [*dnaK*⁺ and *dnaK756*(Ts)] of *E. coli* grown at 30, 37 and 40°C. The *dnaK756*(Ts) allele (5) from strain AL19 (25) was readily transduced into the strain JM83 (*lacZ* Δ M15) (33), taking advantage of the genetic marker *thr::Tn10*(Tet^r) adjacent to *dnaK* and therefore selecting the P1 transductants for tetracycline resistance at 30°C; eighteen *dnaK756*(Ts) and seven *dnaK*⁺ strains were obtained, in accordance with the known cotransduction frequency (around 60%). All of them were transformed to ampicillin resistance (Amp^r) with plasmid pUC18 (23) and then checked for α -complementation (see below for

description of methods) on plates containing ampicillin, IPTG (isopropyl- β -D-thiogalactopyranoside) and X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside); when grown at 37 and 40°C, all of the *dnaK*⁺ transductants gave blue colonies and all of the *dnaK756*(Ts) ones gave colorless (i.e., white) colonies, whereas at 30°C, both alleles produced blue colonies, showing an unambiguous correlation between the presence of the *dnaK756*(Ts) allele and the lack of α -complementation at 37 and 40°C (but not at 30°C).

Two strains chosen among the transductants, TR14 (*lacZ* Δ M15, *dnaK*⁺) and TR20 [*lacZ* Δ M15, *dnaK756*(Ts)], were transformed to either kanamycin resistance (Kan^r) with plasmid pWSK129 (30) or ampicillin resistance with plasmid pUC18, which both encode the *lacZ* α -peptide, and the results of the α -complementation are shown in Fig. 1 and Table 1.

Cultures of TR14 (*dnaK*⁺) pUC18 and TR20 [*dnaK756*(Ts)] pUC18 were incubated overnight at 30°C in Luria-Bertani (LB) medium containing 1 mM IPTG and 100 μ g of ampicillin per ml and diluted 10⁶-fold with 10mM MgSO₄. Then, 0.1 ml of the diluted bacteria was spread on three LB plates containing 100 μ g of X-Gal per ml, 1 mM IPTG, and 100 μ g of ampicillin per ml and incubated for 48 h at 30, 37, or 40°C. Five hundred colonies were screened for blue versus white color, and the numbers of colonies with each color were reported as percentages.

Similarly, cultures of TR14 pWSK129 and TR20 pWSK129 were incubated overnight at 30°C in LB medium containing 25 μ g of kanamycin per ml, diluted as described above, spread on three LB plates containing X-Gal, IPTG, and 25 μ g of kanamycin per ml, and incubated for 48 h at 30, 37, or 40°C. Likewise, cultures of TR14 pWSK129 pBR322, TR14 pWSK129 pKP31, TR20 pWSK129 pBR322, and TR20 pWSK129 pKP31 were incubated overnight at 30°C in LB medium containing X-Gal, IPTG, kanamycin, and ampicillin, diluted as described above, spread on LB plates as described in Table 1, footnote a, and incubated for 48 h at 30, 37, or 40°C. The Ts phenotype was checked by streaking an aliquot of the undiluted cultures on an LB plate at 44°C. The results are shown in Table 1.

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TABLE 1. β -Galactosidase α -complementation in isolated colonies of *dnaK*⁺ and *dnaK756*(Ts) strains plated at 30, 37, and 40°C^a

Strain	Plasmid	<i>dnaK</i> allele	Ts phenotype ^b	α -Complementation in colonies grown at ^c :					
				30°C		37°C		40°C	
				Blue	White	Blue	White	Blue	White
TR14	pUC18	Wt	+	100	0	99	1	99	1
TR20	pUC18	756(Ts)	-	99	1	0	100	0	100
TR14	pWSK129	Wt	+	100	0	100	0	100	0
TR20	pWSK129	756(Ts)	-	100	0	0	100	0	100
TR14	pWSK129 pBR322	Wt	+	100	0	100	0	100 ^d	0
TR14	pWSK129 pKP31	Wt	+	100	0	99	1	100 ^e	0
TR20	pWSK129 pBR322	756(Ts)	-	100	0	0	100	0	100
TR20	pWSK129 pKP31	756(Ts) and wt	+	100	0	100	0	100 ^e	0

^a Cultures were incubated overnight at 30°C, diluted, spread on LB plates containing IPTG, X-Gal, and the appropriate antibiotics, and incubated for 48 h at 30, 37, or 40°C.

^b Ts phenotype was checked by streaking an aliquot of the undiluted cultures on an LB plate at 44°C.

^c Five hundred colonies were screened for blue versus white color. The numbers of white and blue colonies are reported as percentages.

^d Colonies were pale blue.

^e Colonies were dark blue.

As described above, the *dnaK*⁺ strains TR14 pWSK129 and TR14 pUC18 gave blue colonies at all the temperatures tested (30, 37, and 40°C), but the *dnaK756*(Ts) strains TR20 pWSK129 and TR20 pUC18 produced blue colonies only when grown at 30°C and produced white colonies after 24 h when grown at 37 and 40°C. We conclude that the lack of α -complementation in *dnaK756*(Ts) strains grown at 37 and 40°C is independent of the type of plasmid used, since pUC18 and pWSK129 differ in copy numbers (100 to 200 and 6 to 8, respectively), replication origins (ColE1 and pSC101), genetic markers (Amp^r and Kan^r), and amino acid sequences of the *lacZ* α products (Table 2). After 48 h, all of the TR20 pWSK129 and TR20 pUC18 colonies grown at 40°C were still perfectly white, but those grown at 37°C turned to a very pale blue color, suggesting that, as far as α -complementation is concerned, the *dnaK756*(Ts) gene product is completely inactivated at 40°C but still has some residual activity at 37°C. On the other hand, for both strains, the growth rate is not affected by temperature (at 30, 37, and 40°C) in antibiotic-supplemented LB medium, as thermosensitive (Ts) growth of the *dnaK756* strains appears only at 42 and 44°C (results not shown).

TR20 [*dnaK756*(Ts)] pWSK129 cells transformed to ampicillin resistance with the compatible (ColE1) plasmid pKP31 overexpressing *dnaK*⁺ (21) completely recovered their α -complementation capability when grown at 37 and 40°C but not when transformed with the empty vector pBR322 (23), confirming that the lack of α -complementation at those temperatures was due to the *dnaK756* gene product and showing that *dnaK756* behaves in a recessive manner towards *dnaK*⁺ for this novel phenotype (Fig. 1 and Table 1). Remarkably, a dependence of the α -complementation on the level of DnaK can also be observed in a *dnaK*⁺ strain such as TR14 pWSK129, which produced dark blue colonies when transformed with plasmid pKP31 overexpressing *dnaK*⁺ and grown at 40°C but produced pale blue colonies when transformed with the control plasmid pBR322 (Table 1). However, this dark blue color is observed only with growth at 40°C and not at 30 or 37°C (Table 1), suggesting that DnaK is limiting in strain TR14 pWSK129 pBR322 growing at 40°C, possibly because these plasmids recruit a large amount

of DnaK to maintain their negative supercoiling against thermal stress (18).

The *dnaK*-dependent α -complementation phenotype observed on the plates can be quantified by measuring the β -galactosidase activity in crude extracts of the different bacterial strains grown overnight in liquid cultures. The results (Fig. 2) show a clear dependence of the levels of the α -complemented β -galactosidase on the *dnaK* allele [the wild-type (wt) allele versus *dnaK756*(Ts)], the temperature (37 versus 30°C), and the overexpression or lack of overexpression of DnaK from plasmid pKP31. Almost no β -galactosidase activity is found in the *dnaK756* strain (TR20) grown at 37°C (ten times less than in the same strain grown at 30°C or in the wt strain grown at 37°C) (Fig. 2A), whereas the coproduction of DnaK from plasmid pKP31 restores the β -galactosidase activity to the level found in the wt strain under the same conditions (Fig. 2B). As described in Table 1, even in the wt strain (TR14 pWSK129) grown at 37°C, the overproduction of DnaK from plasmid pKP31 leads to a sixfold increase in the α -complemented β -galactosidase level, but this level also depends on the plasmid copy number (compare TR14 pUC18 and TR14 pWSK129 in Fig. 2A), showing that the intracellular concentrations of both the α -donor peptide and DnaK are important for driving α -complementation. However, a massive production of the α -peptide (from pUC18) cannot compensate for a deficient DnaK chaperone (TR20 pUC18 at 37°C), demonstrating (i) the necessity for an active DnaK for α -complementation and (ii) the thermo-inactivation of the *dnaK756* gene product at 37°C, as far as its role (direct or indirect) in α -complementation is concerned. No phenotype has so far been available to monitor such a mild-temperature-driven functional inactivation of DnaK756.

α -Complementation correlates with the inducible expression of DnaK. Strains TR20 [*dnaK756*(Ts)] pUC18 and TR20 pWSK129 were transformed at 30°C to chloramphenicol resistance by the compatible plasmid pARKJ, in which the *dnaKdnaJ* operon is under control of the P_{BAD} promoter inducible by arabinose, or by the corresponding empty vector (pART3) (22). Overnight cultures of the resulting strains in the presence of the appropriate antibiotics and various concentrations of arabinose (from 0 to 0.02%) were grown at 37°C

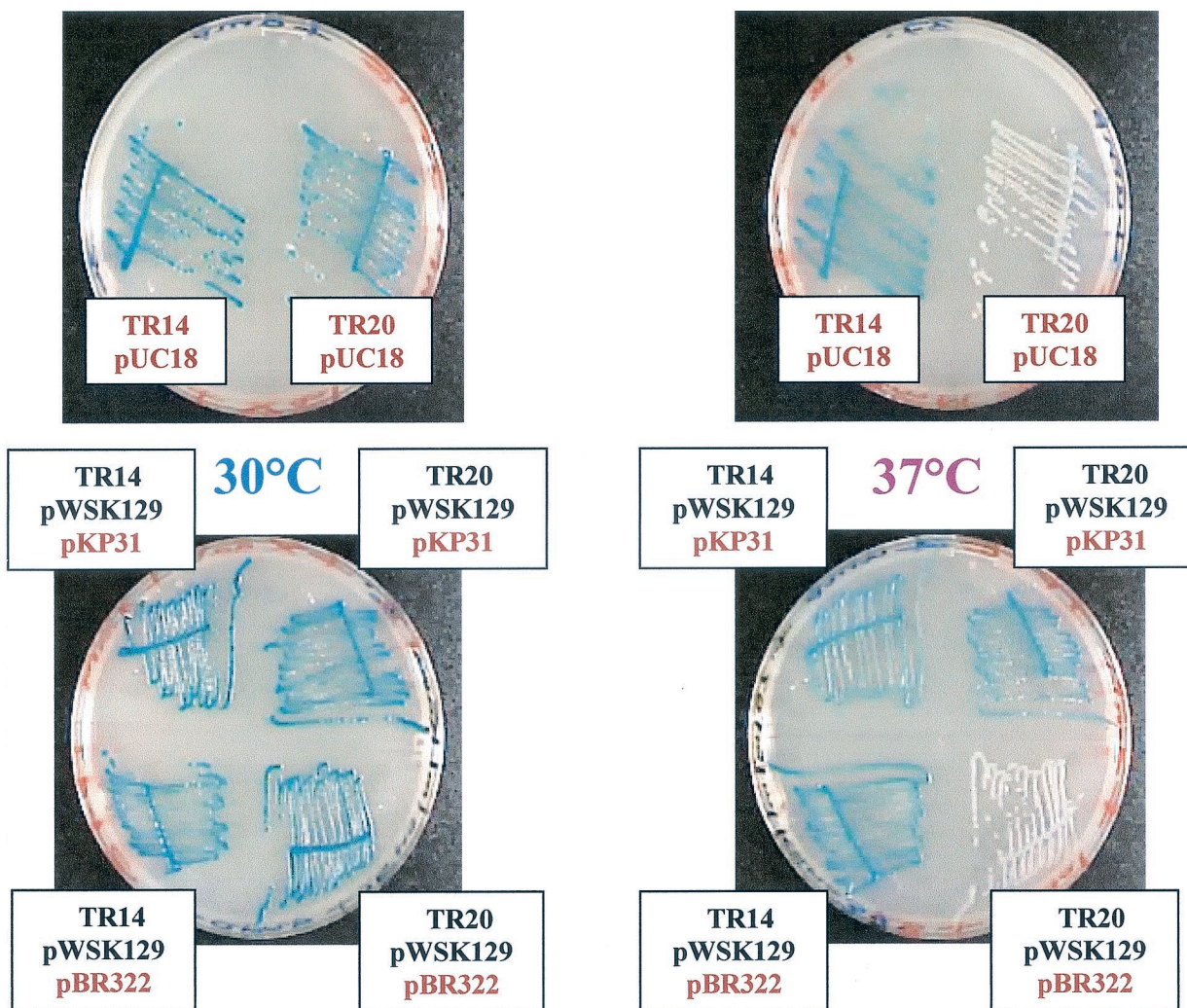


FIG. 1. α -Complementation of β -galactosidase in *dnaK756*(Ts) and *dnaK*⁺ strains plated at 30 and 37°C. Strains TR14 (*dnaK*⁺) pUC18 and TR20 [*dnaK756*(Ts)] pUC18 were streaked on two LB plates containing 100 μ g of X-Gal per ml, 1 mM IPTG, and 100 μ g of ampicillin per ml and incubated for 48 h at 30 or 37°C. Similarly, strains TR14 pWSK129 pBR322, TR14 pWSK129 pKP31, TR20 pWSK129 pBR322, and TR20 pWSK129 pKP31 were streaked on two LB plates containing X-Gal, IPTG, ampicillin, and 25 μ g of kanamycin per ml and incubated for 48 h at 30 or 37°C.

(nonpermissive conditions for α -complementation). Because of the known leakiness of the P_{BAD} promoter, cultures of the same strains without arabinose and with 0.2% glucose, a strong repressor, were also included. Aliquots of the cultures were used for β -galactosidase activity measurements, for quantitative immunoblot analysis of DnaK (results not shown), and to determine the Ts⁺ phenotype of the bacteria (at 44°C). For both strains TR20 pUC18 pARKJ and TR20 pWSK129 pARKJ, the results (Fig. 3) show a good correlation between the levels of α -complemented β -galactosidase and the proportion of thermoresistant bacteria, which both increase in parallel as a function of the added concentrations of arabinose. As expected, bacteria containing the empty vector pART3 exhibited a negligible increase in thermoresistance or in β -galactosidase activity. Because the chromosomally encoded DnaK756 protein as well as the minute amount of plasmid-encoded wt DnaK is not distinguished by immunoblot analysis, the proportion of thermoresistant bacteria in the cultures was a better

TABLE 2. Amino acid sequences of the α -peptides found in native β -galactosidase and those encoded by the *lacZ α* genes within plasmids pUC18 and pWSK129

α -Peptide	Sequence ^a
Native	LORRDWENPGVTQLNRLAAHPPFASWRNSEE ARTDRPPSQQL
pUC18-encoded	mtmitnssvpgdlpesterhaslslawLORRDWENPGVTO LNRLAAHPPFASWRNSEEARTDRPPSQQLrsl ngewrlmryfllthlgishriwctlsticssdaa
pWSK129-encoded	mtmitpsaqlltltkgnksxsstavaaalelydppgernsisslsipstrgg pvpnspysesyarsslaLQRRDWENPGVTQLNRLA AHPPFASWRNSEEARTDRPPSQQLrslngewdapp sgalsaaagvvttrsvtatlasalapapfaffpsflatfagfprqalnrglplg rrfsalrhldpkld

^a α -Peptides encoded by the *lacZ α* genes carried by the pUC18 and pWSK129 plasmids contain 110 and 190 amino acids, respectively. Those written in capital letters are the same as those found in native β -galactosidase; those written in lowercase underlined letters are translated from the polylinker sequences.

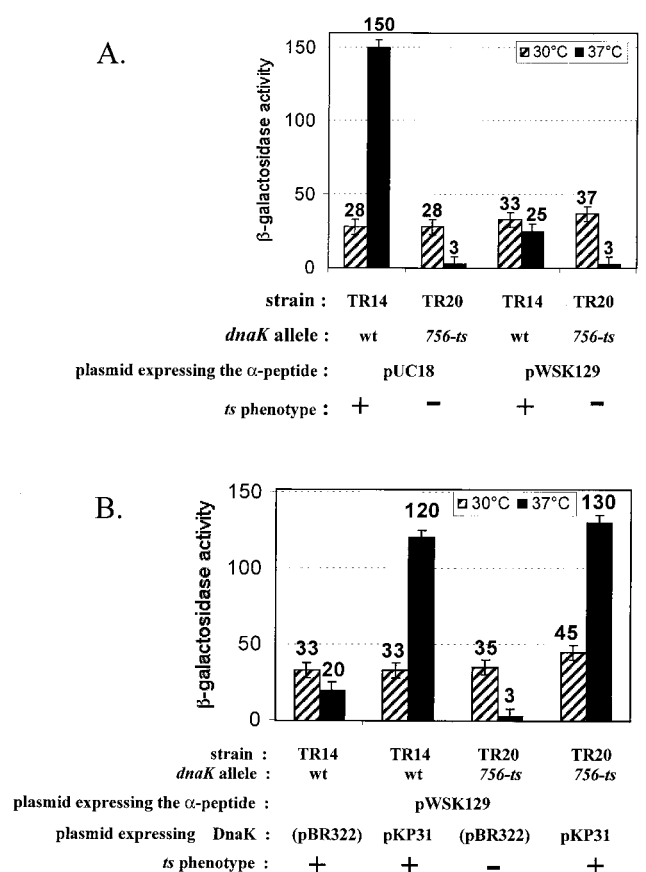


FIG. 2. α -Complemented β -galactosidase activities in *dnaK*⁺ (TR14) and *dnaK756(Ts)* (TR20) strains grown at either 30 or 37°C and overproducing or not overproducing DnaK. (A) Bacteria in overnight cultures of the different strains at 30 (hatched bars) or 37°C (black bars) in LB medium containing 1 mM IPTG and the relevant antibiotics were harvested, and the β -galactosidase assay in bacterial crude extracts was performed as described previously (16). Hydrolysis of *o*-nitrophenyl- β -D-galactopyranoside was performed at the same temperature (30, 37, or 40°C) as that used for cultivating bacteria in order to maintain the DnaK756 chaperone in the same state (active or heat-denatured) as it was in vivo. Results are expressed as 10⁻¹ Miller units, since the specific activity of the α -complemented β -galactosidase in a wt (*dnaK*⁺) strain is about 5% of that of a native β -galactosidase (in a fully induced *lacZ*⁺ strain). One tenth of a Miller unit under the conditions of this study corresponds to an A_{420} of 0.2 U/ml for 1 ml of crude extract made from a culture at a cell density corresponding to an A_{600} of 2 U/ml after 1 h of color production at 30°C. (B) The same experiment described in the legend for panel A was performed with strains carrying a second plasmid and overproducing (pKP31) or not overproducing (pBR322) DnaK. The *Ts* phenotype was checked as described in the footnote to Table 1.

indication of the level of wt DnaK than was immunodetection. Figure 3 also shows that the level of expression of DnaK was already high in the absence of arabinose, but this is an expected result ascribable to the well-known leakiness of the P_{BAD} promoter and can be reduced to zero by the addition of 0.2% glucose. Finally, an overproduction of DnaK induced by 0.02% arabinose leads, in the case of strain TR20 pWSK129 pARKJ, to a drop in α -complementation and to impaired cell growth at 44°C (Fig. 3B), but this toxic effect of overexpressed DnaK, even in *dnaK*⁺ cells, has been well documented (4, 25). The

reason that this effect is not seen with the other strain (TR20 pUC18 pARKJ) (Fig. 3A) is probably related to the very different plasmid copy numbers in these two strains (100 to 200 copies of pUC18 and 6 to 8 copies of pWSK129) and, as previously mentioned, to the recruitment of a large amount of DnaK to maintain plasmid negative supercoiling in the case of the pUC18-containing strain (18).

Immunoblot analysis of LacZ Δ M15 in *dnaK*⁺ and *dnaK756(Ts)* strains grown at 30 and 40°C, in the presence or absence of overproduced DnaK. The four strains TR14 (*dnaK*⁺) pWSK129 pBR322, TR14 (*dnaK*⁺) pWSK129 pKP31, TR20 [*dnaK756(Ts)*] pWSK129 pBR322, and TR20 [*dnaK756(Ts)*] pWSK129 pKP31 were grown at both 30 and 40°C. Quantitative immunoblot analysis of bacterial crude extracts with anti-DnaK and anti- β -galactosidase antibodies was used to determine the levels of DnaK and LacZ Δ M15 in the eight samples (Fig. 4). As expected, plasmid pKP31, when present, produced an increase in DnaK of three to four times at both temperatures. The level of LacZ Δ M15 was almost the same in the four strains when grown at 30°C (Fig. 4A), but in the cultures grown at 40°C, there were significant differences depending on the *dnaK* allele present [*dnaK*⁺ or *dnaK756(Ts)*] and on the overexpression (from pKP31) or lack of overexpression (control pBR322) of DnaK (Fig. 4B). It is clear that the intracellular concentrations of LacZ Δ M15 correlate with those of functional DnaK in a way similar to that of the α -complemented β -galactosidase activities already measured in these strains (Fig. 2). All of these data are assembled in Fig. 4B.

The decreased level of LacZ Δ M15 observed in the *dnaK756(Ts)* strain (TR20 pWSK129 pBR322) grown at 40°C could be either the cause or the consequence of the strong decrease in α -complemented β -galactosidase activity. Therefore, a similar immunoblot analysis of DnaK and LacZ Δ M15 was carried out with the four strains TR14 (*dnaK*⁺) pBR322, TR14 pKP31, TR20 [*dnaK756(Ts)*] pBR322, and TR20 pKP31, which do not contain the α -complementing plasmid pWSK129. In all of the cultures grown at 30 and 40°C, the intracellular concentrations of DnaK and those of LacZ Δ M15 (measured by quantitative immunodetection) were similar to those depicted in Fig. 4A and B; i.e., they reveal an unambiguous correlation of the relative levels of LacZ Δ M15 with those of DnaK (data not shown). That this correlation is independent of α -complementation therefore suggests a role for DnaK in the control of either the synthesis, the degradation, or the solubility of the LacZ Δ M15 protein (and perhaps that of the α -peptide too). This conclusion, however, does not preclude the possible participation of DnaK in the process of α -complementation itself.

α -Complementation in a Δ *dnaK* strain at 30°C. To study the possible DnaK dependence of α -complementation at 30°C, strain BB1553 (7, 21), which contains a disrupted *dnaK* gene (Δ *dnaK52::Cm*^r), was used. This allele was transduced from BB1553 into the strain JM83 (*lacZ Δ M15*) (33) with selection at 30°C on LB plates containing 50 μ g of chloramphenicol per ml without sodium citrate. A P1 transductant which was temperature-sensitive (at 40°C) and cold-sensitive (Cs) (at 20°C) (7) was retained and named JM83 Δ . This strain, after being transformed to kanamycin resistance with plasmid pWSK129, was unable to sustain α -complementation when streaked on an LB plate containing IPTG, X-Gal, and kanamycin, since all of the

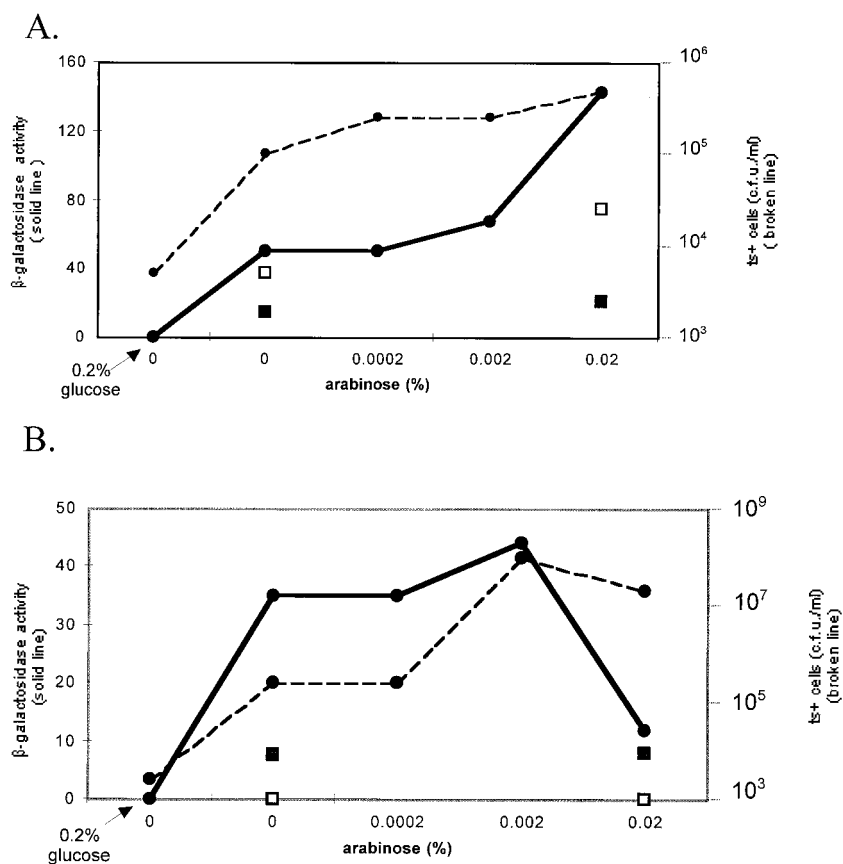


FIG. 3. Effect of arabinose-controlled expression of *dnaK*⁺ on α -complementation of β -galactosidase and on the thermoresistance phenotype of TR20 [*dnaK756*(Ts)] strains at 37°C. (A) Overnight cultures of strains TR20 pUC18 pARKJ (*dnaK*⁺) (full circles) and TR20 pUC18 pART3 (control) (squares) were grown at 37°C in LB medium containing 1 mM IPTG, 100 μ g of ampicillin per ml, and 25 μ g of chloramphenicol per ml with the indicated concentrations of arabinose. One of the cultures of TR20 pUC18 pARKJ lacked arabinose but contained 0.2% glucose. (B) Overnight cultures of strains TR20 pWSK129 pARKJ (*dnaK*⁺) (full circles) and TR20 pWSK129 pART3 (control) (squares) were grown at 37°C in LB medium containing IPTG, kanamycin, and chloramphenicol with the indicated concentrations of arabinose. One of the cultures of TR20 pWSK129 pARKJ lacked arabinose but contained 0.2% glucose. The proportion of Ts⁺ bacteria was measured at 44 and 30°C by plating serial dilutions of the cultures on LB plates containing the same concentrations of antibiotics and arabinose (or glucose) as those in the corresponding liquid cultures (broken lines and empty squares). The β -galactosidase assay in bacterial crude extracts was performed at 37°C (solid lines and full squares).

isolated colonies were white after 48 h at 30°C, although the high-cell-density portion of the streak was pale blue (Fig. 5A). α -Complemented β -galactosidase activities in crude extracts of JM83 Δ pWSK129 and JM83 Δ pUC18 bacteria grown in liquid cultures at 30°C were negligible and strongly decreased, respectively (Fig. 5B), indicating that even at 30°C, DnaK is implicated in some way in the α -complementation of β -galactosidase in vivo.

Conclusions. The two main conclusions of this study are as follows. (i) With regard to the participation, directly or indirectly, of the chaperone DnaK in the α -complementation of β -galactosidase in vivo, α -complementation is strongly reduced in both mutants *dnaK756*(Ts) (at 37°C) and Δ *dnaK* (at 30°C), whereas it is stimulated in a *dnaK*⁺ strain by overproduction of plasmid-encoded DnaK at 37 or 40°C.

(ii) The functional thermo-inactivation of the chaperone DnaK756 at 37°C is revealed only by this original phenotype. The impairment in both mutants *dnaK756*(Ts) and Δ *dnaK52::Cm* is specific to the α -complementation process,

since the rate of synthesis of native β -galactosidase is not affected in a *dnaK756*(Ts) mutant, even at 42°C (19). The same is true in the mutant Δ *dnaK52::Cm* at 30°C (12). However, the synthesis of β -galactosidase induced in a mutant Δ *dnaK dnaJ* is significantly decreased at 30 and 37°C and is abolished at 42°C (32), but this is due to the extreme instability of a positive regulator of the *lac* operon, namely, the cyclic AMP receptor protein (CRP), at 42°C in the absence of DnaJ (20).

The lack of α -complementation in *dnaK* mutants could be due to one of the following reasons. (i) Synthesis of one or both of the partners (Lac Δ M15 protein and α -peptide) may be inhibited. This possibility is unlikely, since synthesis of native β -galactosidase is not affected.

(ii) The second possible reason is their accelerated degradation. Our data of immunoblot analysis of Lac Δ M15 favor this hypothesis. However, degradation in a *dnaK756* mutant of the nonsense *lacZ-X90* allele product, a known unstable polypeptide, has been found to be three- or fourfold accelerated at

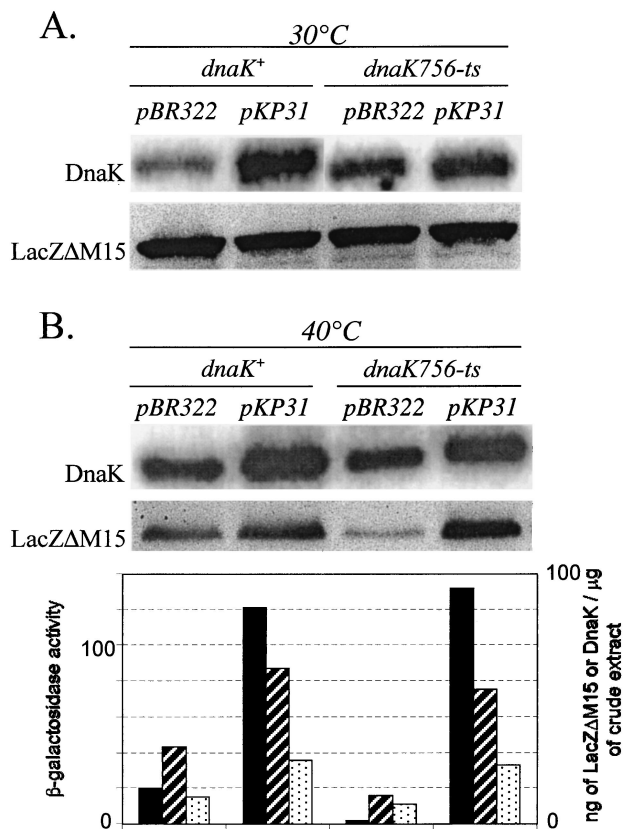


FIG. 4. Levels of DnaK, LacZΔM15, and α -complemented β -galactosidase activities in *dnaK*⁺ and *dnaK756(Ts)* strains grown at 30 or 40°C and overproducing or not overproducing DnaK. Overnight cultures of the four strains TR14 (*dnaK*⁺) pWSK129 pBR322, TR14 (*dnaK*⁺) pWSK129 pKP31, TR20 [*dnaK756(Ts)*] pWSK129 pBR322, and TR20 [*dnaK756(Ts)*] pWSK129 pKP31 grown in LB medium containing IPTG, kanamycin, and ampicillin at either 30 (A) or 40°C (B) were harvested. Immunoblot analysis of levels of DnaK and LacZΔM15 expression in the crude extracts of the different strains grown at the indicated temperatures was performed as described previously (23). Sonicated extracts of stationary-phase bacterial cells were centrifuged, and protein samples that were prepared by boiling aliquots of the supernatants with sodium dodecyl sulfate gel-loading buffer were resolved by electrophoresis on sodium dodecyl sulfate-polyacrylamide (8%) gels. Samples of known amounts of commercially available DnaK (Stressgen) and native β -galactosidase (Fluka) were also loaded. Serial dilutions of the samples allowed quantification of the signals in the linear range. The blots were probed with anti-*E. coli* HSP70 (DnaK) (Dako) or anti-native β -galactosidase (Rockland) antibodies, and in both cases reactive bands were visualized with ¹²⁵I-labeled protein A (Amersham). Protein concentrations in the bands were quantified using a PhosphorImager and expressed in ng/μg of crude extract. The relative levels of LacZΔM15 (hatched bars) and DnaK (dotted bars) measured in the different strains are derived from the immunoblots shown in B. Those of the α -complemented β -galactosidase activities (black bars) in the same strains are taken from Fig. 2.

30°C relative to that in the isogenic wt strain, but by contrast, it was inhibited 20-fold at 42°C (26).

(iii) Their insolubilization or aggregation (in inclusion bodies) is also a possible explanation. Aggregation in inclusion bodies of the pre-S2-S'- β -galactosidase, a tripartite fusion protein consisting of the 55-residue pre-S2 domain and the 30-residue hydrophobic S' domain of the hepatitis B surface an-

tigen followed by β -galactosidase, was shown to be due only to the S' domain of this hybrid β -galactosidase, and there is no effect of the *dnaK756* mutation on the accumulation of β -galactosidase activity at 30 and 37°C. Only at 42°C does localization in an inclusion-body (insoluble) form increase (27). However, co-overproduction of plasmid-encoded DnaK-DnaJ facilitates the proper folding and assembly of the tetrameric fusion protein, leading to higher yields of active pre-S2-S'- β -galactosidase (28).

(iv) A step (tetramerization?) may be absent in the process of α -complementation itself. In yeast, LacZΔM15 has been found associated with the molecular chaperone HSP90 (1). In vitro, denatured β -galactosidase forms active tetramers upon addition of HSP70 (9). Our data on the stimulation of α -complementation in a *dnaK*⁺ background (strain TR14) by overproduction of plasmid-encoded DnaK are also in favor of a possible implication of DnaK in the process of α -complementation itself. There are many examples in which DnaK plays a role in protein quaternary structure changes, including disentanglement of the λ DNA- λ O- λ P-DnaB preprimosomal complex found at the origin of λ DNA replication (34); multimerization of the C protein, a positive regulator of bacteriophage μ late transcription (24); monomerization of the replication initiator protein RepE of the mini-F plasmid, mostly present as RepE dimers (15); monomerization of the replication initiator protein RepA of P1 plasmid, mostly present as RepA dimers (31); and dissociation of hydrophobic protein aggregates by DnaK/DnaJ/GrpE, alone or in cooperation with ClpB (3).

Previous studies on the in vitro thermodenaturation of the DnaK756 protein by biophysical techniques are in complete agreement with the data described here, since they showed that the secondary structure of DnaK756 at 20°C is similar to that of DnaK at 55°C and that DnaK756 protein starts to denature at temperatures between 35 and 40°C (2).

The observation of a role, either direct or indirect, for DnaK in α -complementation raises many questions. Which of the three genetic alterations in DnaK756 (5) is responsible for the impaired α -complementation at 37°C? This question will be easily answered, since the amino-terminal G32D and the carboxy-terminal G455D and G468D substitutions have recently been separated (5). Does DnaK act in α -complementation alone or via the general DnaK/DnaJ/GrpE pathway? This question is of particular interest regarding GrpE, since genetic alteration of residue 32 in DnaK756 was found to be solely responsible for the decreased ability of DnaK756 to bind GrpE (6). Are other chaperone machines (e.g., GroEL/GroES) implicated in α -complementation? Another important task is to determine whether DnaK also plays a role in in vitro α -complementation. If it does, dissection of the still mysterious mechanism of α -complementation (13) might become possible, and a very simple colorimetric assay for DnaK could be developed.

Finally, the phenotype described here could be a useful tool for selecting extragenic suppressors of *dnaK756(Ts)* at mild temperatures (37 to 40°C). It may also be profitable to extend the study made here with the *dnaK756(Ts)* allele to other examples of protein fragment complementation, such as the *E. coli* maltose binding protein, the yeast mitochondrial ribosomal protein MrpS28, the yeast phosphoglycerate kinase, and the isoleucyl-tRNA-synthetase.

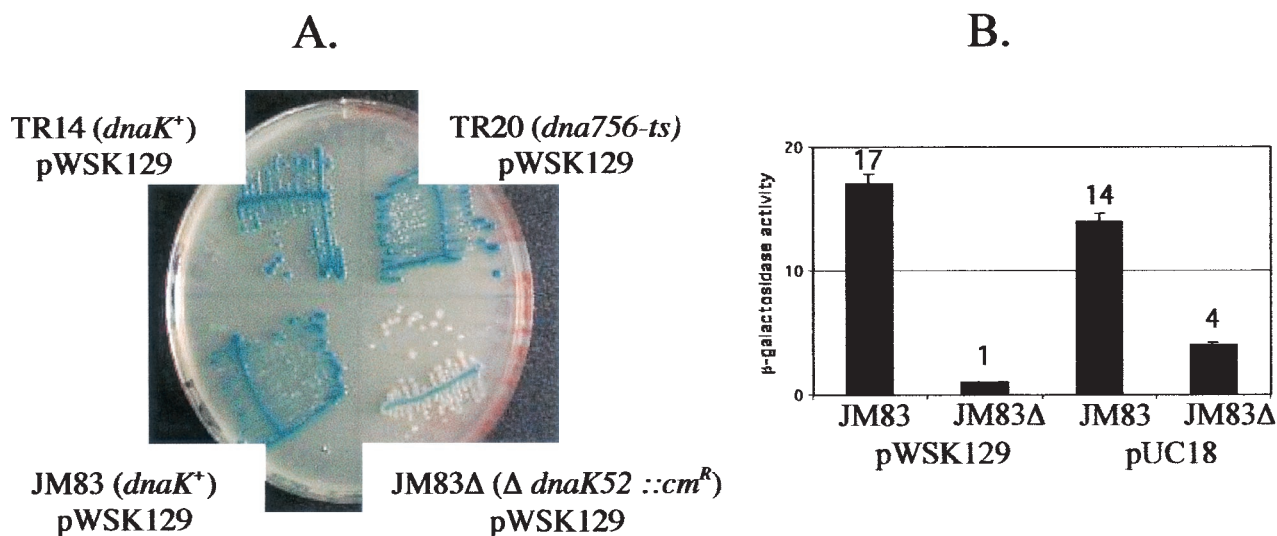


FIG. 5. α -Complementation of β -galactosidase in a $\Delta dnaK$ strain at 30°C. (A) Strains TR14 (*dnaK*⁺), TR20 [*dnaK756(Ts)*], JM83 (*dnaK*⁺), and JM83Δ (*Δ dnaK52::Cm^R*), each carrying the plasmid pWSK129, were streaked on a plate containing X-Gal, IPTG, and kanamycin and incubated for 48 h at 30°C. As expected, JM83Δ pWSK129 displayed a chloramphenicol resistance and Cs (on LB medium with kanamycin at 20°C) and Ts phenotype (on LB medium with kanamycin at 40°C). (B) Bacteria which were grown at 30°C and reached the beginning of the stationary phase in cultures of strains JM83 pWSK129 and JM83Δ pWSK129 in LB medium containing IPTG and kanamycin and of strains JM83 pUC18 and JM83Δ pUC18 in LB medium containing IPTG and ampicillin were harvested, and the β -galactosidase was measured in bacterial crude extracts.

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