Chaperone-assisted Excisive Recombination, a Solitary Role for DnaJ (Hsp40) Chaperone in Lysogeny Escape*

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Background: Site-specific recombination is involved in the temperate phage lysogenic cycle.
Results: DnaJ is recruited by a protein specific for excisive recombination.
Significance: Stress response in E. coli contributes to mobilization of temperate phages.

Temperate bacteriophage lytic development is intrinsically related to the stress response in particular at the DNA replication and virion maturation steps. Alternatively, temperate phages become lysogenic and integrate their genome into the host chromosome. Under stressful conditions, the prophage resumes a lytic development program, and the phage DNA is excised before being replicated. The KplE1 defective prophage becomes lysogenic and integrates its genome into the host chromosome. Under stressful conditions, the prophage has long served as a model system for studies of regulated site-specific recombination (3). Indeed, temperate bacteriophages, such as λ, may choose between lytic and lysogenic cycles for their propagation in the bacterial host (for a review, see Ref. 4). Under favorable conditions for bacterial growth, the phage genome is inserted into the host genome by an integrative recombination reaction, which takes place between DNA attachment sites called attP and attB in the phage and bacterial genomes, respectively. As a result, the integrated prophage DNA is bound by hybrid attachment sites termed attL and attR. In response to changes in the physiological state of the bacteria, mainly in response to stress conditions, such as DNA damage, phage DNA is excised from the host chromosome. This excisive reaction allows attL and attR to recombine and restores the attP and attB sites on the circular phage and host genomes. Although the phage-encoded integrase (Int) protein catalyzes both integrative and excisive reactions, it requires the assistance of several accessory proteins depending on the direction of the reaction. The host-encoded integration host factor (IHF) is required for both integration and excision, whereas the phage-encoded excisionase (Xis) is necessary for excision only and prevents reintegration (5, 6). Excisionase binds and bends DNA, assists the formation of the intasome, and controls the directionality of the reaction toward excision; therefore, these proteins were also named recombinase directionality factors (RDFs) (7). Moreover, directionality of the excisive reaction is conferred by the irreversibility of multiple reaction steps (8).

The KplE1 prophage (also named CPS-53) is one of the 10 prophage regions present in Escherichia coli K12 MG1655 (9). It is a defective prophage integrated into the argW tRNA gene, and the remaining genome (10.2 kb) of the prophage contains 16 open reading frames (ORFs) bordered by a duplicated core sequence of 16 nucleotides (CTCGAGGGGACACCAT).

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The abbreviations used are: Int, integrase; Hsp, heat shock protein; IHF, integration host factor; RDF, recombinase directionality factor; SSR, site-specific recombination; Q-PCR, quantitative PCR; Xis, excisionase; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.
Despite the small remnant genome, the KplE1 prophage can be excised in vivo (10, 11). Analysis of the KplE1 recombination module has shown that the prophage contains all the elements required for site-specific recombination to occur, including RDF and integrase genes as well as the attL and attR recombination regions (12). This recombination module is highly conserved in several enterobacteria phage genomes, such as HK620 and Sf6 that infect E. coli strain TD2158 and Shigella flexneri, respectively (13, 14). In vivo recombination studies are made easier by the defective nature of the KplE1 prophage; indeed, the exclusive recombination and its regulation can be dissected in vivo independently of prophage induction.

The torl gene is the last gene of the KplE1 prophage genome and was originally identified using a genetic multiplicity approach as a negative regulator of the torCAD operon that encodes the trimethylamine-oxide reductase respiratory system in E. coli (15). Despite its role as an inhibitor of the TorR response regulator, later work has shown that Torl is in fact a bona fide RDF for KplE1 phage excision (10). Torl shares several properties with other RDFs, such as a small size and a basic pI, and despite no primary sequence homology, its solution structure is highly similar to that of well characterized excisionases, such as Xis and TorI-Xis (16, 17). In vitro, Torl controls directionality of KplE1 site-specific recombination mediated by the IntS tyrosine recombinase (12), and its expression in E. coli rapidly leads to the complete excision of KplE1 prophage from the host chromosome (10). Indeed, IntS is permanently expressed in E. coli cells at a sufficient level to allow exclusive recombination as soon as the Torl RDF is produced, meaning that in vivo excision of KplE1 relies on RDF gene expression (18).

Molecular chaperones form a large family of cellular machines, which facilitate protein folding mainly by protecting non-native proteins from misfolding and aggregation (19). They generally act through cycles of binding and release of hydrophobic polypeptide segments that are often buried in the native form of proteins. Alternatively, chaperone “clients” can be found in native complexes, and in this case, molecular chaperones orchestrate specific remodeling, leading to various cellular activities (20). As the successful proliferation of phages requires a substantial reprogramming of the biosynthetic machinery of the host cell and the subsequent folding of a large number of the phase-encoded proteins necessary for viral replication and virion assembly, it is thus not surprising that host- and/or phase-encoded molecular chaperones are central to several key stages of the viral life cycle (21). Remarkably, both major molecular chaperone families, i.e. DnaK (Hsp70) and GroEL (Hsp60), were originally discovered as essential host factors for phage development (22–26). The crucial role played by the stress-regulated multifunctional DnaK/DnaJ/GrpE chaperone machine (27) during initiation of phase λ replication well illustrates the involvement of molecular chaperones in temperate phage development. Indeed, DnaK, assisted by its cochaperones DnaJ and GrpE, orchestrates several key steps, leading to the activation of the DnaB helicase in the prernimosomal complex and the subsequent initiation of λ replication (28). In this case, (i) the DnaJ chaperone first binds to λP and DnaB proteins at the prernimosome and stabilizes the complex. (ii) DnaJ binding facilitates the recruitment of its DnaK chaperone partner to the complex and stimulates the interaction of DnaK with λP. (iii) The formation of a stable DnaK-λP complex induces the dissociation of λP from DnaB, thus initiating DNA replication. (iv) DnaK is then recycled with the help of its GrpE cochaperone, which accelerates nucleotide exchange and indirectly AP release (29).

In this work, we present a novel role for host-encoded molecular chaperones during early stages of phage development. Indeed, we have isolated the DnaJ chaperone as a major protein partner of the Torl RDF and demonstrated that such interaction was critical for the excision process to occur both in vivo and in vitro. Remarkably, stimulation by DnaJ did not require its DnaK chaperone partner. Our discovery that DnaJ may act as a solitary chaperone assisting Torl excisionase during KplE1 phage prophage excision sheds light on different levels of regulation of the temperate phage life cycle by molecular chaperones from the heat shock response.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Media**—Bacterial strains and plasmids are listed in Table 1. Strains were grown in LB medium supplemented when necessary with ampicillin (50 μg/ml), chloramphenicol (25 μg/ml), kanamycin (25 μg/ml), L-arabinose (0.2%, w/v), or isopropyl β-D-galactopyranoside (1 mm). Torl Magnetic Pulldown—The Torl pulldown assay was performed using carboxyl Adembeads (Ademtech). These beads are 300-nm superparamagnetic nanoparticles presenting a polymer core shell structure and a high density of carboxyl groups (>350 mol·g⁻¹). Activation and coating of the beads were performed as follows. 20 μl (0.6 mg) of carboxyl beads were washed twice in activation buffer, resuspended in 500 μl of activation buffer before incubation with 48 μl of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide cross-linker (4 mg/ml) for 10 min at 40 °C under agitation. Purified Torl (15 μl at 5 mg/ml) in activation buffer was then added before incubating the beads for a further 2 h at 40 °C with shaking (800 rpm in an Eppendorf Thermomixer). Control beads were incubated with 5 mg/ml BSA in activation buffer. Saturation was performed by adding 120 μl of 0.5 mg·ml⁻¹ BSA in activation buffer. Coated beads were then washed twice and resuspended in 250 μl of storage buffer.

Torl-coated and control beads were then incubated with a soluble fraction of MC4100 cells collected in midexponential growth. The cell culture (100 ml) arrested at an A₆₀₀ of 0.9 was centrifuged for 10 min at 8000 rpm (Eppendorf A-4-44 rotor) and resuspended in 1.5 ml of 40 mM Tris. Cells were lysed with a French press (cell pressure, 14,000 p.s.i.), and cell debris was removed by centrifugation for 20 min at 20,000 rpm (Sorvall SS-34 rotor). The soluble fraction was obtained after ultracentrifugation for 60 min at 45,000 rpm (Beckman 70.1 Ti rotor). Coated beads (150 μl) were incubated with 350 μl of the soluble fraction and incubated for 90 min at 20 °C with shaking (800 rpm). Beads were washed three times in 40 ml Tris buffer, pH 7.4 until no coloration was obtained with Bradford solution. Elution from the beads was performed by addition of 40 μl of denaturing loading buffer and incubation for 5 min at 95 °C.
| Strains          | Genotype/sequence | Description                                   | Source/Ref. |
|-----------------|-------------------|-----------------------------------------------|-------------|
| MC4100          |                   |                                               | M. C. Casadaban |
| C600 dnaJ259, Tet<sup>+</sup> |                   |                                               | 36          |
| LCB6094         | MC4100 dnaJ259, Tet<sup>+</sup> |                                               | This work   |
| A137            | MC4100 dnaJ<sup>+</sup>  |                                               | 57          |
| Δ<sup>+</sup>   | MC4100 dnaJ<sup>+</sup>  |                                               | 57          |

| Plasmids        | Description                                          | Source/Ref. |
|-----------------|------------------------------------------------------|-------------|
| pGPJ-His        | pBAD22 vector containing dnaJ coding sequence with a His<sub>6</sub> tag | 30          |
| pH33Q           | pGPJ-His derivative containing His-33 to Gln mutation | 30          |
| pldelH2-His     | pGPJ-His derivative containing DnaJ helix 2 deletion (residues 18–32) | This work   |

| Primers         | Description                                          |
|-----------------|------------------------------------------------------|
| jdelH2<sub>dir</sub> | CCGGACCCTAACCAGGGTGACAAA<sup>a</sup>                      |
| jdelH2           | TTTGTCACCCCTGGTTACGGTCCGGCGCTTTTGAAACGCTTA<sup>a</sup>      |
| pBAD-up          | TACCTGACGCTTTTTATCGCA                                      |
| pBAD-seq2        | GGGGACCAGCGGCTACTGGGCCAGCC                           |
| attL-pro         | AATGGATATAACGGAGCCCTCC                                    |
| attL-ter-CysS    | CYS-CATCGAGAACGCCGATATGGTTTTTC                           |
| pmupG-L-CysS     | CGGCTGAGAACGCCCAT                                        |
| pmupG-R          | AGGCAACTTCCCCACAGACAA                                      |
| attL-Spel        | GACTATGTTCAATCTGCTATACGGTGACAT                          |
| attL-KpnI        | GGGAATCCGGCTAAATTGGATCTGGATCC                            |
| attR-XbaI        | GCTCTAGAGGTTTTTAGGATAAACACAGATG                              |
| attR-HF2         | CTCTAAGCGCGCCAAAGTG                                     |
| mglC-L           | ATATTTCACCCAGCTCATG                                      |
| mglC-R           | TGGGCAGAAGAACATGAGAA                                     |
| lptG-L           | GAAAGGACCAAAACACTGATG                                    |
| lptG-R           | AGATGGTCAACTGACTGGTC                                     |
| gpt-L            | ATGAGCGAAGAACATGAGAA                                     |
| gpt-R            | TACAAACGCGATCGAGGAA                                      |

* Bold sequence indicates overlapping sequences.
Eluted proteins were then separated on a Tris-Tricine-SDS 10% acrylamide gel. Bands only appearing in the presence of TorI and in three independent experiments were identified with MALDI-TOF mass spectrometry.

**Mass Spectrometry Analysis**—Analysis was performed on a MALDI-TOF Microflex II mass spectrometer (Bruker) at the IFR88 Proteomic facility. Tryptic peptides were acquired with internal calibration on the theoretical tryptic peptides of the proteins of interest. We allowed up to four misinterpretations and a mass tolerance of 60 ppm for this tryptic peptide analysis.

**Immunoprecipitation**—The E. coli Δ^3 strain harboring the pGPJ-His plasmid (30) was transformed with pBTorI (15). Cells were grown until A_{600} reached 0.5 unit. Proteins expression was induced by the addition of 0.2% L-arabinose, and incubation continued for 4 h at 30 °C. Cells were pelleted, resuspended in 50 mM Tris-HCl, pH 7.6, 1 m NaCl, 20 mM imidazole, 20 mM β-mercaptoethanol, 0.5% Triton X-100, 0.6% Brij 58P (buffer A), DNase I, 1× protease inhibitor mixture set (EDTA-free; Calbiochem). Cells were lysed using a French press (cell pressure, 14,000 p.s.i.), and the supernatant was loaded onto a 1-ml nickel column (HisTrap™ FF, GE Healthcare) previously equilibrated with buffer A. The column was washed with 10 column volumes. To avoid precipitation of proteins, the detergent (Triton X-100 and Brij 58P) were gradually removed and compensated by a glycerol step gradient from 0 to 50%. To ensure total detergent removal, the column was washed with buffer B (Triton-HCl, pH 7.6, 1 mM NaCl, 20 mM imidazole, 20 mM β-mercaptoethanol, 50% glycerol). The His-tagged protein was eluted with 250 mM imidazole in buffer B and then dialyzed against 40 mM Tris-HCl buffer, pH 7.6, 100 mM KCl, 1 mM dithiothreitol (DTT), 50% glycerol using a PD-10 desalting column (GE Healthcare). Purified proteins were visualized by 12% SDS-PAGE followed by Coomassie staining. The protein concentrations were initially estimated by the Bradford methods, and then the purity was evaluated after SDS-PAGE using a Bio-Rad Experion system (IFR88 Transcriptional facility). The absence of DnaK in purified fractions of DnaJ mutants was assayed by Western blot using a DnaK-specific antisera (antibody collection of Genevexx) (data not shown).

**In Vitro Excision Assay**—Linear att sites were amplified by PCR with primer pairs attL–Spel/attR–KpnI for attL and attR–XbaI/attR–IHF2 for attR and then purified using the QIAquick PCR purification kit protocol (Qiagen). Reaction mixtures (50 µl) included linear att DNA sites (32 nM) in buffer containing 27 mM Tris-HCl, pH 7.6, 35 mM KCl, 10 mM spermidine, 1 mM EDTA, 1 mg/ml acetylated BSA, 15% glycerol. IHF (0.25 µM), Int5 (0.8 µM), TorI (1.6 µM), and DnaJ (3 µM) were added as indicated in the figures legends. TorI and DnaJ were initially preincubated at 37 °C for 30 min, and then Int5, IHF, and linear attL and attR DNA were simultaneously added. The reactions were carried out in optimized conditions at 37 °C for 1 h at an IHF:Int5:TorI:DnaJ protein ratio of 1:3:6:12. Reaction products were purified (QIAquick kit, Qiagen) and mixed with SYBR Green I (0.01%) before separation in a 2.5% agarose gel. The abundance of attP formed during in vitro excision assays was quantified by real time PCR as described (12) on a Mastercycler Ep Realplex (Eppendorf) using the SYBR Premix Ex Taq™ kit (TaKaRa) according to the manufacturer’s instructions.

**In Vivo Excision Assay and Real Time PCR Analysis**—Strains MC4100, dnak:Tn10 Δ42 (Tet^R^), and dnaJ259 were grown at 30 °C in LB medium until the A_{600} reached 0.1 unit, bicyclomycin (100 µg/ml) was added, and the growth resumed for 3 h at 30 °C under agitation. Control cells were grown without bicyclomycin. Appropriate culture dilutions were plated onto LB medium. The proportion of attL site in a colony was estimated by Q-PCR on colonies using the primer pair attL-ter and attL-pro. In parallel, the genes mgfC, htpG, and gpt were amplified by Q-PCR on the same colonies to evaluate the number of chromosomal copies using appropriate primer pairs (Table 1). Each colony was diluted in 20 µl of water, incubated for 10 min at 98 °C, and then transferred on ice. Q-PCRs were carried out as described above.

**Electrophoretic Mobility Shift Assays (EMSAs)—**EMSAs were carried out using purified proteins and fluorescently Cy5-la-
beled attL and pnuG DNA fragments that were PCR-amplified using MC4100 chromosomal DNA as a template with the primers pairs attL-pro/attL-ter-Cy5 and pnuG-L-Cy5/pnuG-R. DNA and purified proteins were mixed together at different concentrations (as indicated in the figure legends) in the presence of 4 mg/ml BSA and 0.5 mg/ml calf thymus DNA in buffer (40 mM Tris, pH 7.6, 50 mM KCl, 24% glycerol). Reactions were incubated for 30 min at room temperature. DNA-protein complexes were then separated using a 6% non-denaturing polyacrylamide gel (37:1 acrylamide:bisacrylamide ratio). A premigration step (1 h at 160 V) was carried out to reduce ionic charges, which may have destabilized the DNA-protein complex. Samples were then loaded and left to migrate at 90 V for 30 min and then at 160 V for an additional 2 h in 0.5× Tris borate-EDTA running buffer. The gel was scanned using an FLA5100 (Fuji) scanner using an excitation wavelength of 635 nm (800-V scanning intensity) and an emission wavelength of 665 nm. Data were analyzed using MultiGauge (version 2.3) software.

Luciferase Refolding Assay—Reactivation of firefly luciferase was performed essentially as described (30) with minor modifications. Briefly, luciferase (Sigma) at a concentration of 25 μM was denatured for 2 h at 22°C in a solution containing 30 mM Tris-HCl, pH 7.6, 6 M guanidinium chloride, 5 mM DTT. The denatured luciferase was diluted to a final concentration of 0.125 μM into a reaction mixture (50–μl final volume) containing 100 mM MOPS, 500 mM KCl, 50 mM MgCl2, 20 mM creatine phosphate, 0.1 mg/ml creatine kinase, 5 mM ATP, 0.015% bovine serum albumin, 0.5 μM DnaK, 0.125 μM GrpE. All components were incubated on ice. Renaturation was initiated by adding either DnaJ or DnaJ mutants (0.125 μM each). The luciferase activity was measured at different time points after incubation at 22°C by using 10 μl of the luciferase assay system from Promega (E1500) and a Berthold Centro LB960 luminometer. Average values of two independent experiments were plotted.

RESULTS

DnaJ Interacts with TorI RDF—To identify new molecular partners of the TorI excisionase, a pulldown experiment was performed with a soluble fraction of E. coli cells grown in rich medium and harvested in exponential phase. The extract was incubated together with magnetic beads coated with TorI under native conditions; the beads were magnetically separated from the supernatant and washed extensively before elution of the bound proteins. A control experiment was carried out using beads coated with BSA and showed that all proteins eluted with TorI-coated beads were due to a specific interaction. The proteins from the elution fractions were separated using a 10–16% Tricine SDS-gel and identified by MALDI mass spectrometry (Fig. 1A). The data shown are representative of three independent experiments. The most abundant protein found binding specifically to TorI is itself. This is a consequence of the propensity of TorI protein to oligomerize (11). Three other proteins proved to bind specifically to TorI under such conditions: the fused acetaldehyde-CoA dehydrogenase AdhE (96.1 kDa; P0A9Q7 UNIPROT), the cochaperone DnaJ (42 kDa; P08622), and the ADP-β-glycero-δ-mannoheptose 6-epimerase RfaD (34.9 kDa; P67910). As DnaJ together with DnaK and GrpE has been shown to be involved in phase development as part of the replicative machinery (32, 33), the hypothesis that DnaJ and perhaps the complete DnaK/DnaJ/GrpE chaperone machine could specifically be involved in TorI-mediated excision was thus intriguing and deserved further attention. Therefore, we decided to first focus on DnaJ-TorI interaction. To confirm that TorI and DnaJ interaction was possible in vivo with non-purified TorI, an immunoprecipitation assay was performed using purified anti-TorI IgG. Both DnaJ-His6 and TorI were co-expressed in the presence of 0.2% L-arabinose inducer in the Δ3 strain. Note that under such conditions the cellular level of plasmid-encoded DnaJ is about 15-fold higher than endogenous DnaJ. The cell lysates were then incubated overnight with anti-TorI antibodies before magnetic separation of the bound proteins (Fig. 1B). As a result, DnaJ was efficiently pulled down only in the presence of the TorI-producing plasmid and when the cell lysate was incubated with anti-TorI specific antibodies (Fig. 1B, compare lanes 1 and 2). Note that a very low background level of DnaJ was found under each condition most likely due to nonspecific binding of DnaJ to the superparamagnetic particles or to protein A. Control experi-
ments had shown that TorI and DnaJ antibodies did not cross-react (data not shown). Elution was performed under native conditions, and therefore some DnaJ-TorI complexes could be detected on the gel. Together, these results indicate a specific interaction between the TorI RDF protein and the cochaperone DnaJ.

**DnaJ-TorI Interaction Is Functional**—To pinpoint a possible role for DnaJ in KplE1 prophage site-specific recombination (Fig. 2A) in relation with the TorI excisionase, we included purified DnaJ protein in our in vitro specific recombination (12). To detect a significant increase in excision activity, this experiment was performed under suboptimal protein concentrations (see “Experimental Procedures”). Remarkably, the presence of purified DnaJ in the reaction dramatically increased the excision efficiency (Fig. 2C, compare lane 2 with lane 5). The reaction product attP was quantified by Q-PCR and was 30-fold more abundant in the presence of DnaJ compared with the control experiment performed in the absence of DnaJ (Fig. 2C, compare lanes 2 and 5), indicating that DnaJ increased the excision efficiency. These results demonstrate that the interaction between the TorI excisionase and the DnaJ chaperone is functional, thus revealing a critical role for DnaJ in KplE1 prophage excision.

As stated above, DnaJ generally acts in concert with its chaperone partner DnaK (Hsp70). As a *bona fide* cochaperone, it binds specific protein clients and delivers them to the DnaK chaperone, which then assists the folding reaction. However, our results suggested that DnaJ could efficiently perform in solo as a chaperone during prophage excision. To further prove that the effect of DnaJ on excision was related to its chaperone activity, we asked whether the DnaJ J-domain, which is absolutely required for its DnaK cochaperone function (34, 35), was also dispensable for site-specific recombination. Two different DnaJ mutants were thus assayed in vitro, i.e. DnaJH33Q with a His-33 to Gln amino acid substitution in the J-domain that abolishes all known DnaK cochaperone functions in vivo and in vitro (36) and DnaJΔh2 with a deletion of the second α-helix in the J-domain. As for DnaJH33Q, the DnaJΔh2 mutant lost its cochaperone activity (Fig. 2D). In contrast, both DnaJ mutants stimulated the in vitro excision in a similar manner to that of the DnaJ wild-type protein (Fig. 2C, compare lanes 3 and 4 with lane 2).

To make sure that DnaK was not co-purified with the mutant forms of DnaJ, we tested the DnaJ fractions by Western blot, which confirmed the absence of DnaK (data not shown). Taken together, these results indicate that DnaJ stimulates KplE1 excision in vitro independently of DnaK and that its J-domain is not involved in this process. However, at this stage, we cannot

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**FIGURE 2.** DnaJ stimulates excision in vitro and does not require functional J-domain. A, schematic representation of the site-specific recombination reaction. The KplE1 prophage is represented in the chromosomal context, and its 16 open reading frames are mentioned with arrows indicating the direction of transcription. chr., chromosome. B, DnaJ domain representation. Domains of DnaJ are shown from the N to C terminus: J-domain, glycine-phenylalanine-rich (G/F), zinc finger domain (Zn), C-terminal domains I and II (CTDs), and * dimerization domain. The J-domain sequence and the four α-helices that compose this domain are indicated. The H33Q mutation (dnaJ259) is indicated by an arrow. C, in vitro excision assay in the presence of DnaJ wild type and mutants. In vitro excision assays were performed with suboptimal concentrations of SSR proteins (0.5 μM TorI, 0.2 μM IntS, and 0.2 μM IHF). DnaJ wild type and mutants (3 μM) were added as follows: lane 1, no protein; lane 2, SSR + DnaJ; lane 3, SSR + DnaJΔh2; lane 4, SSR + DnaJH33Q; lane 5, SSR. After incubation for 1 h at 37 °C, reactions were loaded on a 2.5% agarose gel and quantified by real time PCR (see text). D, luciferase folding assay. DnaJ variant activity was assayed in an in vitro luciferase folding assay.
Involvement of DnaJ (Hsp40) in Prophage Excision

exclude further involvement of DnaK in this process because we could not assay DnaK in vitro. Indeed, despite extensive trials, the experimental conditions for DnaK chaperone activity in vitro proved to be incompatible with the SSR reaction (see “Experimental Procedures”). In particular, the high KCl concentration required for DnaK function totally inhibited the recombination reaction.

KpE1 Excision Is Impaired in Absence of DnaJ in Vivo—KpE1 is a defective prophage that lacks both the lytic and the Δ repressor modules. As a result, KpE1 is not inducible by the KpE1 is a defective prophage that lacks both the lytic and the recombination reaction.

In Fig. 3, the addition of 100 μg/ml bicineycin to induce KpE1 excision. Amplification of the attL recombination region is possible only when KpE1 is not excised. A total of 240 (WT), 180 (ΔdnaJ), and 100 (dnaJ259) colonies were assayed, and typical results are shown for 20 colonies (1–20). The control lane (C+) corresponds to the amplification of an untreated wild-type colony. Percentages of excision were calculated according to the Q-PCR results and were as follow: 98% (WT), 51% (ΔdnaJ), and 95% (dnaJ259).

FIGURE 3. DnaJ stimulates KpE1 excision in vivo. Colony PCR was performed on wild-type, dnaJ::Tn10–42, and dnaJ259 cells treated with bicineycin to induce KpE1 excision. Amplification of the attL recombination region is possible only when KpE1 is not excised. A total of 240 (WT), 180 (ΔdnaJ), and 100 (dnaJ259) colonies were assayed, and typical results are shown for 20 colonies (1–20). The control lane (C+) corresponds to the amplification of an untreated wild-type colony. Percentages of excision were calculated according to the Q-PCR results and were as follow: 98% (WT), 51% (ΔdnaJ), and 95% (dnaJ259).

amplified in 50% of the cells, revealing at least a 20-fold decrease in in vivo excision. To investigate whether a functional interaction of DnaJ with DnaK was dispensable in vivo, we took advantage of the well characterized dnaJ259 mutant allele, which encodes a DnaJ protein with the His-33 to Gln amino acid substitution. As described above, such DnaJH33Q mutant is unable to stimulate DnaK but retains all its known substrate binding properties. In this case, the dnaJ259 allele (DnaJH33Q) should support excision as well as the wild-type allele, although it does not stimulate DnaK activity. Indeed, in such a genetic background, excision was as efficient as in the presence of wild-type dnaJ (Fig. 3). These data demonstrate that DnaJ is capable of stimulating KpE1 excision in vivo independently of DnaK, a result that is in full agreement with the data obtained in vitro.

DnaJ Stimulates TorI DNA Binding Activity on attL—The TorI protein is a bifunctional protein that has protein and DNA binding activities (10, 11, 15). Because TorI was shown to bind DnaJ, an attractive hypothesis was that DnaJ was recruited to the recombination region by TorI. Accordingly, EMSA was used to characterize the possible role of DnaJ in TorI binding to attL DNA. All reaction conditions were carried out in the presence of calf thymus DNA and BSA to reduce the occurrence of nonspecific DNA-protein interactions and Cy5-labeled DNA. In these conditions, there was no direct interaction between attL and DnaJ at different DnaJ protein concentrations (5 and 10 μM) as there was no change in the distance of migration of attL (Fig. 4A, lanes 14 and 15 compared with lane 13). As expected, low concentrations of TorI (0.5 μM) were insufficient to result in a significant binding of attL (Fig. 4A, lane 10). However, the effect of DnaJ was clearly marked at 1 μM TorI (Fig. 4A, lane 7): remarkably, in this case, the addition of DnaJ triggered TorI binding to attL, which resulted in a shift in the migration distance of the target DNA (Fig. 4A, lanes 8 and 9). Similar results were found with higher concentrations of TorI where TorI was able to bind and shift more attL in the presence of DnaJ (Fig. 4A, lanes 5 and 6). This increased shift of DNA was still evident although subtle when high concentrations of TorI were used (Fig. 4A, lanes 2 and 3 compared with lane 1). Additional control experiments showed that when using a nonspecific DNA probe, such as the phupG promoter, which is not bound by TorI, DnaJ did not provoke TorI-DNA binding (Fig. 4A, lanes 16–21). This result demonstrates that DnaJ specifically stimulates TorI binding to its DNA target and is in full agreement with the increased excision recombination activity found in the in vitro recombination assays (Fig. 2C). Furthermore, no additional shift of the attL target DNA could be observed in the presence of DnaJ compared with the shift observed in the presence of TorI alone, and no DnaJ could be detected at the level of the TorI-attL complex by Western blot using a DnaJ antiserum because DnaJ barely entered the gel under native conditions (Fig. 4B). This suggests that DnaJ is not part of the final TorI-attL complex and rather has a transient role in agreement with a bona fide chaperone activity.

DISCUSSION

Viruses are reliant on host cell machineries for productive infection, and molecular chaperones have long been described as important elements of viral infection (for reviews, see Refs.

4 R. Menouni and M. Ansaldi, unpublished results.
38 and 39). As an example, Hsp70 proteins are involved in multiple steps of virus development, such as cell entry, nuclear import for eukaryotic viruses, replication, and assembly. It is interesting to note that some viruses, including T4 phage and simian virus 40 (SV40), encode their own chaperones or cochaperones that are involved in DNA replication and/or virion assembly (40–42).

The E. coli chaperonins (GroEL/GroES) and the DnaJ/DnaK/GrpE chaperone machine have been identified through a genetic screen that focused on phage development (22, 26, 43). It was initially discovered that the DnaK/DnaJ/GrpE machine plays a key role in phage replication through disassembly of the ΛO-ΛP-DnaB complex at oriA. This results in the release of ΛP and the initiation of DNA replication by the functional ΛO-DnaB complex (44, 45). However, no roles have ever been described for these chaperones in lysogeny escape. Thus, the finding that the stress chaperone DnaJ is involved in prophage excision constitutes a new link between an early step of the switch from lysogeny to lytic development and the heat shock response.

The newly identified interaction between Torl and DnaJ (Fig. 1) suggested that such an interaction could have an impact on the excissive recombination of the KplE1 prophage. Indeed, when added to the in vitro excision assay, DnaJ provoked a dramatic increase in recombination efficiency. Remarkably, such stimulatory effect of DnaJ did not require the presence of either its DnaK and GrpE partners or a functional J-domain (Fig. 2). This effect was further supported in vivo because in a dnaJ-null background the efficiency of the excisive recombination was significantly impaired when compared with both wild-type and dnaJ259 strains (Fig. 4). Taken together, these data demonstrate that solitary DnaJ stress chaperone is capable of stimulating prophage excision and is thus involved in a prereplicative step of lytic development.

All the known DnaJ activities in vivo are linked to its DnaK cochaperone functions. However, DnaJ can bind substrates in vitro and protect them from aggregation independently of DnaK. Such chaperone function of DnaJ is carried out by its central zinc-binding domain, its C-terminal domains, and most likely its glycine-phenylalanine-rich region (46) and is thus unrelated to a functional J-domain (47–49). Although we cannot firmly exclude an involvement of DnaK, our results strongly suggest that stimulation of Torl-mediated excision by DnaJ solely relies on such chaperone functions. Indeed, the dnaJ259 allele, which is thermosensitive and does not support phage replication (50), behaves in vivo like a wild-type dnaJ (Fig. 4).

What is remarkable in Torl-DnaJ interaction is that upon binding to its specific DNA target Torl seems to be efficiently released from DnaJ without the need for downstream DnaK and GrpE, thus further supporting a DnaK-independent func-

FIGURE 4. DnaJ enhances Torl DNA binding activity in vitro. A, EMSA experiments were performed in the presence of various Torl and DnaJ protein concentrations as described under “Experimental Procedures.” Arrows indicate the different forms of Cy5-labeled DNA: ds-attL, unbound double-stranded DNA; ss-attL, single-stranded unbound attL DNA; Torl-attL, double-stranded Torl-bound DNA; and pnu8G. Note that the presence of single-stranded unbound attL DNA is an artifact form that does not shift in the presence of any of the proteins. B, EMSA experiments were performed in the presence of 1 mM Torl and 5–10 mM DnaJ, the gel was then transferred, and the blot was immunostained with a DnaJ antibody.
It has been observed that the affinity of TorI for the recombinative manner (11). However, our data unambiguously demonstrate that the affinity of TorI for the attL recombinational region of the KpIE1 prophage in a highly cooperative manner (11). Furthermore, the overall shape of the proteins does not provide sufficient similarity to propose a particular structural motif that could be involved in DnaJ binding. In the case of RepE, DnaK and GrpE are also involved in this process because the mini-F plasmid does not replicate in any individual mutant of the DnaK/DnaJ/GrpE machine (54). Such a scenario could also be applied to the original KpIE1 phage genome, which integrated at the argW tRNA site. However, because KpIE1 became non-replicative, this dependence could no longer be observed, and exclusive recombination likely relies solely on DnaJ.

We show that DnaJ enhances the binding of TorI to its specific DNA substrate. TorI binds to five distinct sites on the attL recombinational region of the KpIE1 prophage in a highly cooperative manner (11). However, our data unambiguously demonstrate that the affinity of TorI for the attL substrate increases in the presence of DnaJ, which does not bind itself to this DNA substrate. The E. coli J-domain-containing proteins, such as DnaJ, have been described as able to bind DNA (55); however, using the attL and pmapG substrates and stringent conditions (competitor DNA in 100-fold excess), we did not observe any DnaJ binding to DNA (Fig. 3). Moreover, in the presence of both TorI and DnaJ, the nucleoprotein complex formed with attL migrated similarly to that in the presence of TorI alone, suggesting that DnaJ is not present in the final complex. An attractive hypothesis is that DnaJ remodels TorI in a conformation that increases its affinity to its DNA target, and upon binding, the chaperone would subsequently be released from TorI. We can also predict that other recombination systems, in particular those of the infectious phages HK620 and Sf6 that contain highly similar recombination modules (12), will be enhanced by DnaJ.

With respect to temperate phage development, it is tempting to link stress induction of lysogeny escape to chaperone-mediated exclusive recombination. Indeed, heat shock has been shown to induce the λ prophage through a slightly different regulatory path than the DNA damage pathway (56). However, to date, no apparent link has been identified between exclusive recombination and the heat shock response, although excision of the prophage genome is a crucial step following the decision to switch from lysogeny to the lytic mode.

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