JB Accepted Manuscript Posted Online 10 April 2017
J. Bacteriol. doi:10.1128/JB.00058-17

MS2 lysis of *Escherichia coli* depends on host chaperone DnaJ

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Running Title: MS2 lysis depends on DnaJ.

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Abstract:

The L protein of the ssRNA phage MS2 causes lysis of *E. coli* without inducing a bacteriolytic activity or inhibiting net peptidoglycan (PG) synthesis. To find host genes required for L-mediated lysis, spontaneous Ill (*insensitivity to L lysis*) mutants were selected as survivors of L expression and shown to have a missense change of the highly-conserved proline (P330Q) in the C-terminal domain of DnaJ. In the $dnaJ_{P330Q}$ host, L-mediated lysis is completely blocked at 30°C without affecting the intracellular levels of L. At higher temperatures (37°C and 42°C) both lysis and L accumulation are delayed. The lysis block at 30°C in the $dnaJ_{P330Q}$ mutant was recessive and could be suppressed by *L* overcomes *dnaJ* (*Lodj*) alleles selected for restoration of lysis. All three *Lodj* alleles lack the highly basic N-terminal half of the lysis protein and cause lysis ~20 min earlier than the full-length L. DnaJ was found to form a complex with full-length L. This complex was abrogated by the P330Q mutation and was absent with the Lodj truncations. These results suggest that, in the absence of interaction with DnaJ, the N-terminal domain of L interferes with its ability to bind to its unknown target. The lysis retardation and DnaJ chaperone-dependency conferred by the non-essential, highly basic N-terminal domain of L resembles the SlyD-chaperone dependency conferred by the highly basic C-terminal domain of the E lysis protein of ϕX174, suggesting a common theme where single-gene lysis can be modulated by host factors influenced by physiological conditions.

Importance:

Small single-stranded nucleic acid lytic phages (*Microviridae* and *Leviviridae*) lyse their host by expressing a single "protein antibiotic". The protein antibiotics from two out of three prototypic small lytic viruses have been shown to inhibit two different steps in the conserved PG

biosynthesis pathway. However, the molecular basis of lysis caused by L, lysis protein of the third prototypic virus, MS2, is unknown. The significance of our research is in identifying DnaJ as a chaperone in the MS2 L lysis pathway and the identification of the minimal lytic domain of MS2 L. Additionally, our research highlights the importance of the highly-conserved P330 residue in the C-terminal domain of DnaJ for specific protein interactions.

Introduction:

The single-stranded RNA phage MS2 is one of the simplest viruses, encoding just four proteins. Of the four proteins, three of them, RNA-dependent RNA replicase (Rep), major capsid protein (Coat), and maturation protein (A) are involved in viral replication and assembly. The fourth protein is the lysis protein (L), which causes lysis of the host and thus controls the length of infection cycle (1). *L* was the first gene shown to be embedded in two different genes, coat and replicase, in this case in the +1 reading frame of each gene (2) (Fig. 1). Expression of *L* from a plasmid is necessary and sufficient to elicit lysis (3). *L* is one of the three canonical "single gene lysis" systems (SGL) used by small phages to effect lysis, the other two being *E* from the ssDNA phage ϕX174, representing the ubiquitous *Microviridae*, and A_2 from ssRNA phage Qβ, representing the *Allolevivirida*e (1). Genetic and molecular analysis revealed that both E and A_2 inhibit specific steps in the PG biosynthesis pathway: A_2 inhibits MurA, which catalyzes the first committed step, and E inhibits MraY, which catalyzes the formation of the first lipid-linked intermediate (4-6). In both cases, the isolation of dominant mutations conferring resistance to the lethal function of the lysis protein and the mapping of these mutations to the gene encoding the biosynthetic enzyme was the key to deciphering the lytic mechanism. In the case of *E*, the first and most common mutations conferring resistance to lysis mapped to a different gene, *slyD*,

encoding a cytoplasmic, FKBP-type cis-trans peptidyl-prolyl isomerase (7). These mutations were recessive, however, and subsequent investigation revealed that SlyD was required for the stability of E, which has 5 Pro residues in its 91 aa length. Suppressor mutations in *E* were all up-translation alleles, compensating for the instability of the E protein (8).

In contrast to E and A_2, which have been called "protein antibiotics" because of the functional resemblance to cell wall antibacterial agents (9), no clear conceptual framework exists for the lytic function of the 75 aa L protein (Fig. 1). L has a hydrophilic N-terminal domain dominated by multiple basic residues and a hydrophobic C-terminal domain, presenting an interesting comparison with ϕX174 E (3). Genetic analysis had shown that the lytic function of E requires only the N-terminal hydrophobic domain and that the highly-charged, basic C-terminal domain could be replaced by unrelated sequences including β-galactosidase and GFP (4, 10). Drawing on this comparison, the van Duin group showed that expression of N-terminal deletions of L retaining as little as 42 C-terminal residues were fully lytic and, indeed, truncations retaining only the last 27 residues had partial function (3). Thus E and L, although lacking any sequence similarity, seem to have mirror-image organization of functional domains. However, unlike E, induction of L did not lead to a block in PG synthesis, as assessed by incorporation of [^{3}H]-diaminopimelate (11). Moreover, a synthetic peptide corresponding to the C-terminal 25 aa was reported to dissipate proton motive force (pmf) of *E. coli* inverted membrane vesicles and cause fluorescent dye leakage in reconstituted liposomes (12). Interestingly, despite the non-essential character of the N-terminal domain and the absence of any other secretory or membrane localization signals, it was reported that L was primarily localized in the periplasmic zones of adhesion between inner membrane (IM) and outer membrane (OM), also known as Bayer's patches (13). Moreover, biochemical analysis of the murein from cells that had undergone L-

mediated lysis was reported to have a slightly decreased average chain length of glycan strands and slightly altered cross-linking between them. In addition, L-mediated lysis was blocked in cells grown at low pH, where penicillin-induced autolysis was also inhibited. Taken together, these results were interpreted as support for a general model where L somehow activates host autolytic enzymes, such as lytic transglycoslyases and D-D endopeptidases (14). Unfortunately, in the nearly three decades since these studies, no molecular link between the putative autolytic response, or indeed any host protein, and L has been established.

With the aim of identifying host factors involved in L-mediated lysis a genetic approach was undertaken. Here, we report the identification of one such factor, the host chaperone DnaJ, and the results are discussed in terms of a model for L function and regulation.

Results

Selection of Ill mutants

As our first attempt to identify host factors involved in L-mediated lysis, we cloned the *L* gene into a medium copy vector under the control of the lambda late promoter, pR', which in turn is driven by the inducible supply of the lambda late activator, Q, from a low copy lac-ara vector. Induction of cells carrying these two plasmids (pQ and pRE-*L*) results in lysis in approximately the same time as infection by phage MS2, with comparable levels of L synthesis (Fig. 2A, 3A). HfrH cells carrying these plasmids were mutagenized with EMS and subjected to two rounds of induced lysis before plating for survivor colonies. Of 3,300 colonies screened, 5 were found to be Lac+, Q+, $MS2^R$ and $M13^S$ (15). However, Hfr mapping and P1 transduction revealed that all five were recessive missense alleles of *pcnB*, encoding the polyA polymerase of *E. coli* (Fig. 2B). As expected, the *pcnB* defect reduced the copy number of the pRE-*L* plasmid to

106 approximately single copy (not shown;(16)), which accounted for the survival of these mutants
107 in our inducible plasmid system. Interestingly, these *pcnB* missense mutants were clustered in
108 the polyA polymerase active site (Fig. 3B) and conferred dominant resistance to MS2 (Fig. 2C),
109 suggesting a heretofore unsuspected role of PcnB in the MS2 infection cycle.
110 To avoid host mutants like *pcnB* that reduce either the plasmid copy number or
111 expression of the L gene, we constructed a modified *L* expression vector, pKC11, with *L* and the
112 *lacZ*α gene in tandem under an *ara* promoter. This allowed screening for blue survivor colonies
113 after *L* induction. Expression of *L* from pKC11 was found to cause lysis at ~45 min after
114 induction. From approximately 200 survivor colonies screened, 2 blue colonies with irregular
115 morphology were isolated on X-gal/arabinose plates. Both isolates exhibited an absolute plating
116 defect for MS2 (Fig. S1). We next tested the kinetics of lysis in liquid culture in comparison with
117 the parental host and found that the mutants displayed a 40-50 min delay in the onset of lysis
118 (Fig. 4A). To eliminate the possibility that the mutants confer a non-specific resistance to lysis,
119 we tested lysis by E of ϕX174 and A_2 of Qβ in the mutant hosts and found no significant delay in
120 either case (not shown). Thus, the mutants confer partial resistance specific to L-mediated lysis,
121 enough to delay its onset, and were designated Ill mutants (*insensitivity to L lysis*).
122
123 **The Ill phenotype is due to a dnaJ mutation**
124 In order to assign the Ill phenotype to a specific host gene, genomic DNA from two Ill
125 mutants was purified and subjected to whole genome sequencing. An analysis of the genomic
126 data revealed that both the Ill mutants, which may have been siblings, had a P330Q missense
127 mutation in *dnaJ*. To determine whether the *dnaJ* mutation was the sole factor responsible for the
128 lysis phenotype, the mutation was transduced to a new background. The newly constructed

129 *dnaJ*$_{P330Q}$ mutant retained the observed blue-colony survivor phenotype of the parental mutant
130 (not shown). Moreover, the *dnaJ*$_{P330Q}$ mutation exhibited strictly recessive behavior, with L-
131 mediated lysis fully restored in a merodiploid (Fig. 4BC).

132 Since DnaJ is a heat shock protein (17), we examined the effect of temperature on the
133 kinetics of L-mediated lysis in wild type and mutant *dnaJ* backgrounds. As shown in Fig. 5A,
134 the *dnaJ*$_{P330Q}$ allele exhibited an absolute lysis defect at 30°C, as well as significant lysis delays
135 at higher temperatures (Fig. 5B). In addition, the Δ*dnaJ* allele exhibited a more modest delay in
136 lysis when compared with the lysis profile in the Ill mutant (Fig. 5C). Immunoblot analysis
137 showed that L accumulation paralleled the lysis defect at 37°C and 42°C, but was normal at 30°C
138 in the wild-type and *dnaJ*$_{P330Q}$ backgrounds (Fig. 6). Irrespective of the temperature-dependence
139 of the phenotype, the lysis defect of the P330Q allele is not due to an appreciable defect in the
140 DnaJ/DnaK folding pathway, as assessed by the ability of phage lambda to propagate (Fig. S2).
141

142 **The Lodj mutants by-pass DnaJ**

143 To examine L dependence on DnaJ, we sought suppressors in *L* that could overcome the
144 absolute lysis defect at 30°C in the *dnaJ*$_{P330Q}$ host. To achieve this, we used the plasmid release
145 technique (see Methods) to enrich for *L* alleles with restored lytic competence in the mutant
146 background. Plasmid DNAs prepared from individual lytic transformants, designated as *Lodj* (*L*
147 *overcomes dnaJ*) alleles, were sequenced. Each *Lodj* mutant plasmid was found to have a single
148 nucleotide deletion that created an *L* gene encoding a protein in which the entire N-terminal half
149 of L was replaced by a few N-terminal residues of the Rep protein (Fig. 7A). To verify that *Lodj*
150 alleles were indeed lytically functional, we cloned the new truncated *L* genes into an inducible
151 plasmid and showed that the lysis timing supported by the *Lodj* alleles was comparable and early

152 in both parental and *dnaJ*$_{P330Q}$ mutants hosts at 30°C as well as 37°C (Fig. 7B). To rule out the
153 possibility that it is the presence of the first few amino acid residues of Rep that confers the
154 ability to cause lysis in the *dnaJ*$_{P330Q}$ background, we used site-directed mutagenesis to replace
155 the first 36 codons of L with His-tag (Fig. 7A), and showed that this construct also functions as a
156 *Lodj* allele (not shown). Taken together, these results indicate that it is the presence of the
157 dispensable, N-terminal, highly basic domain of L that confers the requirement for DnaJ and that
158 the N-terminal truncations supported significantly early lysis compared to the full-length allele.
159

160 **DnaJ interacts with MS2 L**

161 To further investigate the role of DnaJ in L-mediated lysis, we asked if DnaJ and L
162 formed a complex. We constructed both *H*$_6$*-L* and *H*$_6$*-Lodj*, encoding L and Lodj proteins tagged
163 with an N-terminal hexahistidine sequence, and showed that both were fully functional (not
164 shown). Cultures carrying these plasmid-borne alleles were induced and membrane extracts
165 prepared from samples taken immediately before lysis. Pull-down assays using Dynabeads® (see
166 Methods) showed that DnaJ was associated with L in the parental cells but not in the *dnaJ*$_{P330Q}$
167 Ill mutant (Fig. 8); this association was abrogated for the LodJ mutant in both parental and Ill
168 mutant backgrounds. Taken together, these results indicate that L and DnaJ form a membrane-
169 associated complex in vivo, and that the complex depends on the non-essential N-terminal
170 segment of L interacting with the C-terminal domain of DnaJ.
171

172 **Discussion**

173 The *Leviviridae*, among the most ancient and possibly the simplest known viruses, effect
174 rapid and efficient lysis of the host without inhibiting cell wall synthesis through the action of the

L protein (11). For Gram-negative bacteria, this is the only known lysis phenomenon mediated by antibiotics or by phage that does not involve either inhibition of PG synthesis or elaboration of a muralytic enzyme by the phage. Understanding of how L triggers an autolytic response and identifying the host factors involved in the response might reveal an entirely new perspective for novel antibiotics.

Prior perspectives on L function

Most of the extant work on L-mediated lysis involved a combination of biochemical and electron microscopy analysis of the murein structure in both intact and L-lysed bacteria. A model was proposed in which L protein localized to the periplasmic zones of adhesion and caused the inappropriate activation of cellular autolytic functions such as lytic transglycosylases and D-D endopeptidases (13, 14). Moreover, it was shown that a defect in membrane-derived oligosaccharide (MDO) biosynthesis provided resistance to L-lysis, possibly by impeding appropriate localization of the lysis protein (18). However, this model lacked genetic evidence and afforded no clear molecular framework that could lead to mechanistic understanding. In addition, the very existence of zones of adhesion in growing cells has become controversial, since they have not been observed in cryo-electron microscopic images but only in cells that have undergone dehydration and fixation for transmission EM (19). Importantly, the MDO-dependency was not specific to L-mediated lysis, as the lysis protein E of the ssDNA phage ϕX174 displayed a similar dependency (18). At the time, E was also thought to induce autolysis (20). However, we have since shown unambiguously that E is a specific inhibitor of MraY and causes lysis by inhibiting the biosynthesis of Lipid II (4, 6). Thus, it seems likely that the MDO

197 sensitivity of these lysis pathways, one of which involves a blockage of PG synthesis and the
198 other does not, is indirect and non-specific.

199 **The role of DnaJ in L-mediated lysis**

200 Here we have taken a genetic approach to identify host factors required for L lysis, by
201 the conceptually simple approach of selecting for host mutants resistant to L function. The
202 results show that the host chaperone DnaJ is one such factor. We have shown that the P330Q
203 missense change in DnaJ, although preserving the essential heat shock function of DnaJ, confers
204 a defect in L-mediated lysis, absolute at 30ºC and partial at 37ºC. Proline 330 is the most
205 conserved residue in the C-terminal domain of DnaJ, present in 689 of the 690 full-length DnaJ
206 sequences from Proteobacteria (http://eggnog.embl.de/version_3.0/index.html). The one
207 exception, from a 2003 genomic sequence of *S. flexneri*, is probably a sequencing error, since
208 more recent *S. flexneri* genomes do not show this variance. Little is known about the biological
209 function of the C-terminus. Proline 330 was chosen as the end of the last DnaJ subdomain before
210 a putative extreme C-terminal domain that was shown to be required for dimerization of DnaJ
211 (21). Although the block in lysis at 30°C is sufficiently strict to allow the isolation of the Lodj
212 intragenic suppressor mutations, it is unclear why the lysis-defect phenotype is leakier at 37ºC
213 and 42ºC. It has been estimated that at 37°C there are fewer than 500 copies of DnaJ/cell and
214 the rate of synthesis of new DnaJ molecules at 30°C is ~10-fold lower than at 37°C. (Bardwell
215 et al 1986). It is possible that at higher temperatures (37°C and 42°C) higher levels of $DnaJ_{P330Q}$
216 and/or the induction of other heat-shock proteins contribute to partial rescue of the lysis defect

217 The lysis-defective phenotype of the Ill mutant, $dnaJ_{P330Q}$, is L-specific, with no effect on
218 lysis by E or A_2, the other two prototypic SGL proteins. Since the delayed lysis phenotype is
219 specific for L-mediated lysis and not a general defect, the simplest notion is that the missense

change in DnaJ abrogates an important interaction between DnaJ and L. This idea is supported by the finding that DnaJ, but not $DnaJ_{P330Q}$, co-purifies with his-tagged L.

What role does DnaJ play in L-lysis? It is clearly not the target of L, since deletions of the N-terminal domain of L relieve the dependency on DnaJ and restore normal L-mediated lysis, indeed the Lodj derivatives, expressed from isogenic plasmid environments, evoke lysis much faster than the parental full-length proteins. The simplest idea is that DnaJ is required for proper folding of full-length L but not of the truncated Lodj proteins. Figure 9 shows a cartoon rendition of the model. In the WT host, DnaJ interacts with the improperly-folded N-terminal hydrophilic domain, thus avoiding a steric clash with a putative cytoplasmic domain of the (still-unknown) target protein. Once the complex is formed, DnaJ can dissociate and catalyze further L-target events. In support of this notion, there is precedent for DnaJ acting as a chaperone independent of the DnaK-ATPase heat shock activity, in stabilizing a Lys/Arg rich C-terminal domain of TorI, the recombination directionality factor of the KplE1 cryptic prophage of *E. coli* (22). In the Δ*dnaJ* host, compensatory elevations in other, less-specific chaperones, would lead to similar folding events, although with slower kinetics in the absence of the specific interaction. By contrast, the Lodj truncations lack the N-terminal domain and thus do not suffer from the potential steric clash with the target. This model for DnaJ-mediated activation of L assumes that L has a protein target (Fig. 9). However, the same arguments could be used for the role of DnaJ activating L by converting it to a conformation allowing its oligomerization, a notion more compatible with the model mentioned above, in which Goessens et al. (12) implicated the C-terminal 25 residues of L in the formation of oligomeric pores in the cytoplasmic membrane. While this scenario cannot be ruled out, we favor a general model for L having protein target based on comparisons to the other lysis proteins of single-strand nucleic acid phages (9) and to

the fact that to date no association between membrane depolarization and autolysis in *E. coli* has been reported.

From this perspective, the parallel with the lysis protein E and the cytoplasmic chaperone SlyD is striking. SlyD is absolutely required for the proteolytic stability of E, which like L, has a large dispensable domain rich in charged and hydrophilic residues. Like L, removal of this dispensable domain also abrogates the chaperone dependence of E, although the position of the dispensable domain, at the C-terminus in E and the N-terminus in L, is opposite in the two lysis proteins. We suggest these dispensable domains, evidently requiring chaperone activity for proper folding, have evolved as regulatory "damping" features of the two lysis proteins. Among general phage functions like genome replication and virion morphogenesis, lysis is distinct in that maximum efficiency and speed is undesirable. Moreover, in these simple phages with highly constrained genome sizes, both the *E* and *L* lysis genes were forced to evolve in alternate reading frames of essential genes. It is logical that the smallest possible lytic domain would emerge from such evolutionary constraint, and, indeed, the essential segments of E and L are the N-terminal 32 and C-terminal 30 residues, respectively. In these severely restricted out-of-frame contexts, it would be much less challenging to evolve a highly-charged region lacking secondary structure and thus compromised in terms of unassisted folding. The acquisition of the crippled domain conferred a requirement for interaction with a cytoplasmic chaperone and thus provided a context for retarding lysis to allow time for assembly of progeny virions. Indeed, this scenario would also confer potential for physiological regulation of lysis timing in the E and L systems via the level or activity of the respective chaperone. In passing, we note that the distinctive domain structure of MS2 L and its homologs is not common to all ssRNA phage lysis proteins. For example, in the coliphage M and the *Caulobacter* phage Cb5, the predicted membrane

domains are at the N-terminus, more akin to the arrangement in ϕX174 E. In these cases, similar regulatory interaction with a chaperone protein might be involved in lysis timing, as proposed for the FKBP-like cytoplasmic chaperone SlyD and E (8).

The results reported here thus complement the extensive studies by van Duin and colleagues on the regulation of the *L* gene at the level of translation (23, 24). These studies revealed that sequestration of the Shine-Dalgarno and start codon of *L* by the formation of a stable RNA hairpin secondary structure effectively represses *L* expression. *L* translation is thought to require that ribosomes that terminate at the *coat* gene stop codon randomly backtrack and reinitiate translation at the *L* start codon. Based on the relative levels of coat monomers, levels of L protein, and random probability of drifting ribosomes, it is estimated that only 5% of the ribosomes backtrack to reinitiate at *L* (25). This was proposed to ensure that L accumulates gradually in the cell and thus provides time for progeny maturation. Our results suggest that the function of L is further regulated by a post-translational regulator, DnaJ. It may be interesting to explore whether levels of DnaJ activity can be correlated with the length and fecundity of MS2 infection cycles under different physiological conditions.

Materials and Methods

Culture growth, antibodies, and chemicals.

Unless indicated, LB broth and agar were used as growth medium. When indicated, media was supplemented with ampicillin (Amp), kanamycin (Kan), chloramphenicol (Cam), and tetracycline (Tet) at concentrations of 100, 40, 10, and 10 μg ml^{-1} respectively. Growth in liquid cultures and lysis was monitored by measuring at A_{550} as previously described (26). When

indicated, isopropyl β-D-thiogalactopyranoside (IPTG) (Research Products International), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), and arabinose were added at a final concentration of 1 mM, 10 µg/ml and 0.4%, respectively. Primary antibodies against MS2 L peptide "TPASTNRRRPFKHEDC" were raised in rabbit (Sigma Genosys), mouse anti-His antibodies were purchased from Sigma, and rabbit anti-DnaJ antibodies were purchased from Enzo Life Sciences. Secondary Goat anti-mouse-HRP and goat anti-rabbit-HRP antibodies were purchased from Thermo Scientific. Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich.

Bacterial strains and bacteriophages

The bacterial strains and bacteriophages used in this study are described in Table 1 and primers are described in Supplementary Table S1. The $dnaJ_{P330Q}$ allele was moved from the Ill1 mutant into the threonine-auxotroph RY34314 (TB28 Δ*thrC*::*kan*) by P1 transduction (27), selecting for growth on M9 minimal agar supplemented with 0.2% glucose (28). The *dnaJ* locus was amplified from Thr^+ transductants using primers KC130 and KC131and sequenced to confirm the mutation. The *dnaJ* merodiploids were constructed by mating RY15784 [F'104 *thr-leu* Δ*leuA*::*cat*] strain with either TB28 or RY34356 (TB28 $thrC^+$ $dnaJ_{P330Q}$) and selecting exconjugants on LB supplemented with both Kan and Cam. Similarly, RY34155 was constructed by mating RY34154 with XL1-Blue and selecting exconjugants on LB supplemented with both Amp and Tet. RY34179 strain was constructed by P1 transduction of the Δ*dnaJ*::*kan* marker from JW0014.

311 **Plasmids.**

312 The plasmids used in this study are in Table 1. A DNA fragment containing synthetic
313 tandem *L* (L^{syn}) and *lacZα* genes was cloned under the pBAD promoter in the plasmid pBAD24,
314 resulting in the plasmid construct pKC11. In this construct, both L^{syn} and *lacZα* genes were
315 codon-optimized for *E. coli* expression (http://www.idtdna.com/CodonOpt) and the synthetic
316 DNA with flanking EcoRI and HindIII sites at the 5' end and 3' was cloned into pUC57 at
317 GenScript®. Using the same restriction sites, the synthetic fragment was moved into pBAD24 by
318 standard techniques. The plasmid pKC12 was constructed by sub-cloning *his6-L* from pRE-His6-
319 L (15). The DNA of *his6-L* was PCR-amplified with primers KC18 and KC19, gel purified,
320 digested with EcoR1 and HindIII, and cloned into pBAD24. The plasmids pKC13, pKC14, and
321 pKC15 were obtained through selection for *Lodj* alleles (see below). pKC16 was constructed by
322 site-directed mutagenesis (SDM) of pKC12 with primer KC149.
323
324 **MS2 infection**
325 Cultures of male strains were grown to A_{550} ~0.4 and then diluted to 0.1 in prewarmed
326 LB medium. Then MS2 lysate was added to the freshly diluted cultures at indicated MOIs
327 (multiplicity of infection).
328
329 **Ill mutant selection**
330 Cultures (25 ml) of RY34155 were grown to A_{550} of 0.2 and induced with arabinose.
331 After ~2 h the lysate was harvested by centrifugation at 10,000 x g for 10 min. The pellet was
332 washed once with PBS (phosphate buffered saline pH 7.2), and the survivors were allowed to
333 recover overnight in 5 ml LB with appropriate antibiotics. The induction procedure was repeated

and the survivors from the second round of induction were serially diluted in PBS and plated on LB agar plates supplemented with appropriate antibiotics, IPTG, X-Gal, and arabinose. A total of 6 blue colonies were isolated and screened for MS2 phage resistance by using cross-streaks as previously described (4). Phage-insensitive isolates were studied further by monitoring lysis phenotypes in induced cultures as above. To quantify the expression of L in the mutants, 1 ml sample was mixed with 111 µl of cold 100% trichloroacetic acid (TCA) as previously described (29). The precipitates were collected by centrifugation at 13,000 rpm for 10 min in a microcentrifuge. The pellets were washed with 1 ml cold acetone and air-dried. The dried pellets were resuspended in 2X sample loading buffer with β-mercaptoethanol and boiled for 10 min. Equivalent amounts of protein were analyzed by SDS-PAGE and Western blotting as previously described (30). Both primary and secondary antibodies were used at 1:3000 dilution.

DNA sequencing and analysis

Sequencing for checking cloning and strain construction was done by Eton Biosciences (San Diego). For whole genome sequencing, genomic DNA extracted with the Qiagen gDNA kit was used to prepare 250 bp paired-end libraries using the Illumina TruSeq Nano DNA LT Library kit (Set A) according to the manufacturer's instructions. Whole genome sequencing was done at the DNA Sequencing Facility of the Institute for Cellular and Molecular Biology at UT Austin. The raw sequencing data was processed on Mutation Analysis Beta 1 (2014-06-27) available at www.cpt.tamu.edu/galaxy/workflow. Briefly, Bowtie2 was used to align the trimmed reads to the MG1655 reference sequence (accession number: NC_000913.3). To facilitate visual display of single nucleotide polymorphisms (SNP) or insertions/deletions (indel), the BAM output from Bowtie2 was processed through Mpileup and BCFtools to generate

Variant Call Format (VCF), a standardized text file format. The SNP/indel variants with QUAL scores >100 in both parental (RY34155) and Ill mutants were scored as positive. The positive variants present in Ill mutants but absent in parental strain were further characterized.

Error-prone PCR mutagenesis and selection for *L* overcomes *dnaJ* (LodJ) mutants.

Error-prone PCR mutagenesis of the *L* gene was done using GeneMorph II Random Mutagenesis kit (Agilent Technologies) per the instructions provided in the kit. Briefly, ~900 ng of the *L* gene template (amplified from pRE-L) was used in the reaction with primers KC30 and KC31 to provide ~1 bp change/dsDNA molecule. The randomly mutagenized PCR product was gel-purified, digested with EcoRI and HindIII, ligated into pBAD24 digested with the same enzymes and electroporated into XL1-Blue. The transformants were pooled and the plasmid DNA was extracted using a Qiagen mini-prep kit to generate a library of *L* mutants. Plasmids carrying *Lodj* alleles that restored lysis in the $dnaJ_{P330Q}$ background were obtained by plasmid release. Briefly, ~100 ng of the mutant library was electroporated into RY34356.The transformants were pooled, diluted 1: 20,000, grown in 25 ml of LB supplemented with appropriate antibiotics to A_{550} ~0.2 and induced with arabinose. After 50 min, the culture medium was collected by centrifugation at 10,000 x g. A volume of 20 ml of the supernatant medium was filtered through a 0.22 µm syringe filter (VWR) and then passed through a QIAprep 2.0 Spin Column (Qiagen). The bound DNA was eluted in 20 µl of sterile water. Ten µl of the released plasmids were retransformed into the same strain and the procedure was repeated for another two rounds. At the end of two rounds of amplification, the plasmid DNA was extracted and sequenced.

Pull-down of L and Lodj proteins

LB cultures (500 ml) of TB28 with appropriate plasmids were grown to A_{550} ~0.4, induced with arabinose at t=0 and harvested at allele-specific times by rapid cooling in ice and centrifugation at 10,000 x g for 10 min: 20 min (*his*$_6$*Lodj*1 allele), 40 min (*L* in TB28), and 60 min (*L* in *dnaJ*^{P330Q}). The cell pellets were resuspended in ~3 ml PBS supplemented with the P8849 Protease Inhibitor Cocktail (Sigma; 1 µl/35 A_{550} units original culture) and passed through an Aminco French Pressure cell at 16,000 psi three times to lyse the cells. The lysate was centrifuged at 10,000 x g for 10 min to remove intact cells. The cleared supernatant was centrifuged at 100,000 x g in TLA 100.3 rotor (Beckman TL100 centrifuge) to collect membrane fractions. The membrane fraction was then resuspended in 1 ml of STE (50 mM Tris pH 8.0, 300 mM NaCl, 1% Empigen BB (Fluka), pH 8.0) and incubated overnight at 4°C with gentle mixing. The detergent extract was centrifuged at 100,000 x g to separate detergent solubilized proteins from detergent-insoluble components. The supernatant (~900 µl) was collected, mixed with 50 µl of Dynabeads® His-tag beads and incubated 5 min at room temperature on a roller drum. The binding and elution protocol was followed per manufacturer's instructions except that beads were washed 5 times in STE. A 20 µl volume from each elution was mixed with 20 µl of 2X sample loading buffer, heated at 100ºC for 10 min, and then analyzed by Western blot.

Acknowledgements

This work was supported by Public Health Service grant GM27099 and by the Center for Phage Technology at Texas A&M University, jointly sponsored by Texas A&M AgriLife. Additional funding for this project and support to Tran J.S., was provided by the Beckman Scholars Program administered by the Arnold and Mabel Beckman Foundation. We thank Dr.

403 Catrina Reed for important constructs and advice. Methods, results and analysis pertaining to the
404 role of *pcnB* in resistance to plasmid-borne *L* expression have been previously reported in the
405 dissertation of Dr. Brenley McIntosh (15). The clerical assistance of Daisy Wilbert is highly
406 appreciated.
407

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492 **Figure Legends**

493 **Figure 1.**

494 **Genome organization of ϕX174 and MS2 phages and similarities between their lysis**

495 **proteins.** Shown are the maps of MS2 and linearized ϕX174 genomes, drawn to scale. The lysis

496 genes of the two phages are shaded blue. The lysis gene, *L*, of MS2 encodes a 75 aa lysis protein

497 and the lysis gene *E*, of ϕX174 encodes a 91 aa protein. The residues spanning the TM domain

498 are highlighted in blue. Basic and acidic residues are indicated above the primary structure of the

499 lysis proteins.

500

501 **Figure 2.**

502 **Expression of L from the dual plasmid system, pQ pRE-L, is sufficient for lysis** (A) Lysis

503 profile from induction of the dual plasmid system with MS2 L under pR' promoter using 1mM

504 IPTG or 1mM IPTG and 0.2% arabinose (ara) in RY15177 compared to lysis by MS2 infections

505 of RY15177 at MOI of 5. Cultures were grown at 37°C. ●, pQ + IPTG; ■, pRE-*L* + IPTG; ♦, pQ

506 pRE, + IPTG; ▼, pQ pRE-*L*, + IPTG; ▲, pQ pRE-*L*, +IPTG, +ara; □, pQ pRE-*L*, uninduced; ○,

507 MS2. (B) *pcnB* alleles are recessive. Induction of plasmid-borne *L* using IPTG (final

508 concentration of 1mM) at time 0. Host and plasmid: ●, *pcnB*$^{+}$, vector; ○, *pcnB*$^{+}$, pCA24N-*pcnB*;

509 ■, BMC1, vector; □, BMC1, pCA24N-*pcnB*; ♦, BMC2, vector; ◊, BMC2, pCA24N-*pcnB*; (C)

510 *pcnB* alleles confer dominant resistance to MS2 infection. Infection of *pcnB* alleles with MS2 at

511 MOI 5 at time 0. ●, *pcnB*$^{+}$, vector; ■, *pcnB*$^{+}$, pCA24N-*pcnB*; ♦, *pcnB*$^{-}$, vector; ▲, *pcnB*$^{-}$,

512 pCA24N-*pcnB*; □, BMC1, vector; ○, BMC1, pCA24N-*pcnB*; Δ, BMC2, vector; x, BMC2,

513 pCA24N-*pcnB*. The BMC1 and BMC2 *pcnB* mutations are defined in the legend to **Fig. 3.** This

514 figure was adapted from (15).

Figure 3.

Plasmid-borne L is expressed at a level comparable to that attained in MS2 infected cells. MM represents the molecular mass marker, with sizes in kDa indicated on the left. Samples were collected at the indicated times after induction of the plasmids with IPTG (p) or infection with MS2 at an MOI of 5 (MS2). (B) The mutations of *pcnB* that confer the dominant MS2-insensitivity are shown as missense changes in the region defined by residues 72 through 93. The strains BMC1 and BMC2 in **Fig. 2** have the allelic changes marked 1 and 2 in panel B. This figure was adapted from (15).

Figure 4.

L-mediated lysis in *dnaJ*$_{P330Q}$ background is delayed and the allele is recessive to wild-type. (A) The lysis profile of L in wild-type and *dnaJ*$_{P330Q}$ background at 37°C. Cultures were grown to A_{550} ~0.2 and induced with arabinose 0.4% (w/v) final. Symbols: ○, TB28 and empty vector; ◊, *dnaJ*$_{P330Q}$ and empty vector; □, TB28 and *L*; X, *dnaJ*$_{P330Q}$ and *L*. (B and C) Same as (A) except the strains are merodiploid for *dnaJ* and the lysis profiles were taken at 30°C (B) and 42°C (C).

Figure 5.

L-mediated lysis in *dnaJ*$_{P330Q}$ and Δ*dnaJ* backgrounds. (A) The lysis profiles of L in wild-type and *dnaJ*$_{P330Q}$ backgrounds at 30°C. Cultures were grown to A_{550} ~0.2 and induced with arabinose 0.4% (w/v) final. (B) Same as (A) except the lysis profiles were taken at 42°C. (C) The lysis profile of L in wild-type and Δ*dnaJ* backgrounds at 37°C. ○, TB28 and empty vector; ◊, *dnaJ*$_{P330Q}$ or Δ*dnaJ* and empty vector; □, TB28 and *L*; X, *dnaJ*$_{P330Q}$ or Δ*dnaJ* and *L*.

538 **Figure 6.**

539 **L accumulation in *dnaJ* mutants.** (A) L accumulation is delayed in *dnaJ*$_{P330Q}$
540 background when compared to wild-type at 37°C and 42°C but not at 30°C. (B) L accumulation
541 in wild-type and Δ*dnaJ* at 37°C (bottom). The absence of DnaJ in Δ*dnaJ* is shown by blotting
542 with anti-DnaJ antibody (top). The bands corresponding to L and DnaJ are indicated with an
543 arrow on the right. The molecular mass standard in kDa is represented on the left. The time
544 points of sample collection are shown directly above the blot.

545

546 **Figure 7.**

547 **The *Lodj* alleles suppress lysis block in *dnaJ*$_{P330Q}$ mutants.** (A) Primary structure of L
548 aligned with three Lodj proteins and His$_6$-L$_{37\text{-}75}$. Amino acids that are different from wild type L
549 are highlighted. (B and C) The lysis profiles of L and Lodj1-3 in wild-type and *dnaJ*$_{P330Q}$
550 backgrounds at 30°C (B) and 37°C (C). ○, TB28 and empty vector; □, TB28 and *L*; ⊞, *dnaJ*$_{P330Q}$
551 and *L*; ◊, TB28 and *Lodj1*; X, TB28 and *Lodj2*; ♦, TB28 and *LodJ3*; ▲, *dnaJ*$_{P330Q}$ and *Lodj1*; Δ,
552 *dnaJ*$_{P330Q}$ and *Lodj2*; ■, *dnaJ*$_{P330Q}$ and *Lodj3*.

553

554 **Figure 8.**

555 **DnaJ interacts with L but not with Lodj.** Membrane extracts containing H$_6$-L or H$_6$-Lodj1
556 were solubilized in detergent were bound to anti-his Dynabeads® and the proteins bound to the
557 beads were analyzed by Western blotting with anti-DnaJ (top) and anti-His antibodies (bottom).
558 The bands corresponding to DnaJ, H$_6$-L, and H$_6$-Lodj1 are indicated by an arrow on the right.
559 The molecular mass standards are represented on the left.

560

561 **Figure 9.**

562 **Model for the role of DnaJ in L-mediated lysis.** DnaJ interacts with highly basic N-
563 terminal domain of L at the membrane, possibly to remove steric constraints inhibiting the
564 interaction of L (red) with its target (green). The $DnaJ_{P330Q}$ variant loses its ability to interact
565 with L, leading to a less stable interaction between L and its target, resulting in a delayed and
566 gradual onset of lysis. However, in the complete absence of DnaJ, compensatory cellular
567 chaperone activities stabilize the L-target interaction, resulting in a modest delay in lysis. The
568 products of the Lodj alleles lack the N-terminal domain and thus bypass the requirement for
569 DnaJ. The thickness of the arrows indicates favorability for complex formation: the thicker the
570 arrow, the more favorable the interaction between L and its target.
571

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TABLE 1 Strains, phages, and plasmids

Strain, phage, or plasmid	Relevant genotype or description	Reference
Strains		
XL1-Blue	*recA endA1 gyrA96 thi hsdR17 supE44 relA1 lac* [F'::Tn*10 proA*$^{+}$*B*$^{+}$*lacI*^{q} *Δ(lacZ)*$_{M15}$]	Stratagene
MG1655	*ilvG- rfb*-50 *rph*-1	(27)
TB28	MG1655 *lacIZYA <>frt*	(28)
JW0003	Δ*thrC*::*kan*	(29)
JW0014	Δ*dnaJ*::*kan*	(29)
RY15784	F'104 thr-leu Δ*leuA*::*cat*	Laboratory strain
RY15177	HfrH lacI^{q} *fhuA*::Tn*10*	(14)
RY34179	TB28 Δ*dnaJ*::*kan*	This study
RY34314	TB28 Δ*thrC*::*kan*	This study
RY34356	RY34314 *thrC*$^{+}$ *dnaJ*$_{P330Q}$	This study
RY34154	TB28 pKC11	This study
RY34155	RY34154 [F'::Tn*10 proA*$^{+}$*B*$^{+}$*lacI*^{q} *Δ(lacZ)*$_{M15}$]	This study
Phages		
MS2	ssRNA phage MS2	Laboratory stock
λCI857 *bor*::*kan*	dsDNA phage lambda with thermosensitive CI repressor. Non-essential *bor* gene replaced with kanamycin-resistance gene	Laboratory stock
Plasmids		
pBAD24	*bla araC P*$_{ara}$	(30)
pRE-L	*L* gene from MS2 cloned under the lambda late promoter pR'	(14)
pUC57-*L*^{syn} *lacZα*	Synthetic tandem *L* (*L*^{syn}) and *lacZ*$_{α}$ genes cloned between EcoRI and HindIII	GenScript®
pKC11	*bla araC P*$_{ara}$*::L*^{syn} *lacZα*	This study
pKC12	*bla araC P*$_{ara}$*::his*$_{6}$ *-L*^{syn}	This study
pKC13	*bla araC P*$_{ara}$*::Lodj1*	This study
pKC14	*bla araC P*$_{ara}$*::Lodj2*	This study
pKC15	*bla araC P*$_{ara}$*::Lodj3*	This study
pKC16	*bla araC P*$_{ara}$*::his*$_{6}$ *-Lodj1*	This study

572

573

574

575

Figure 1.

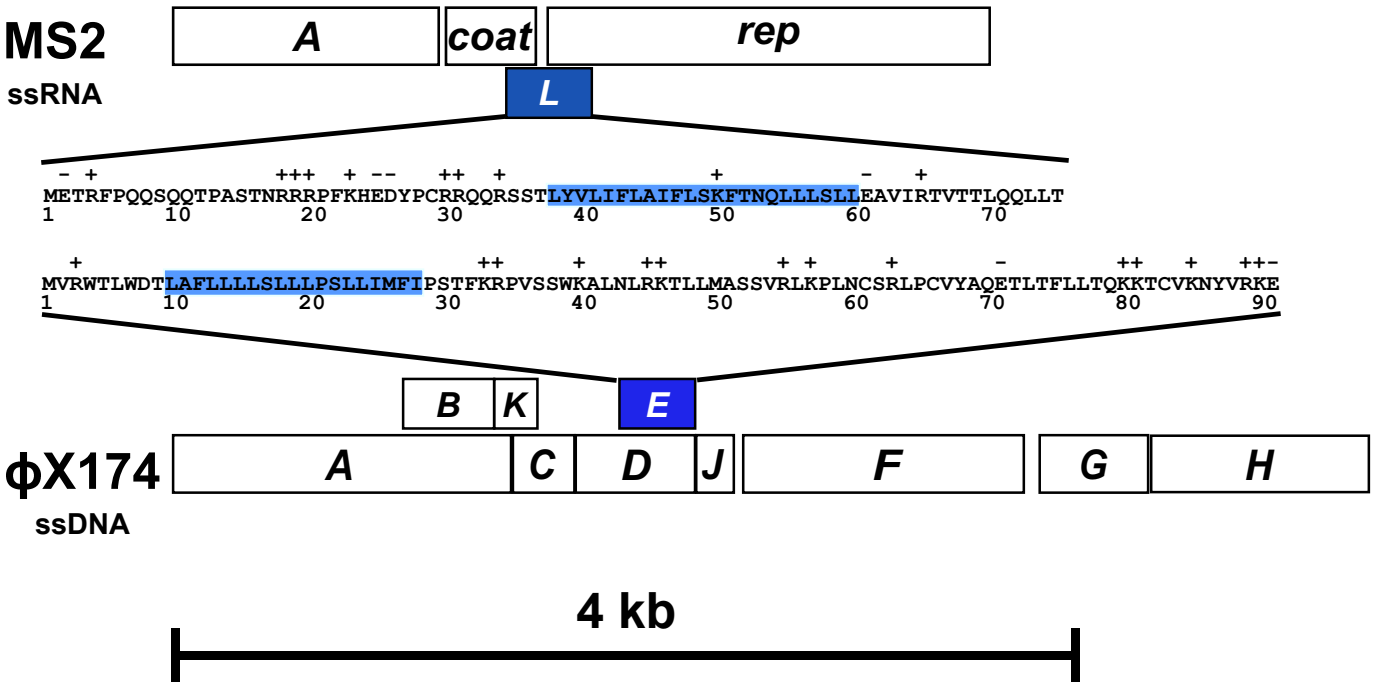


Figure 2.

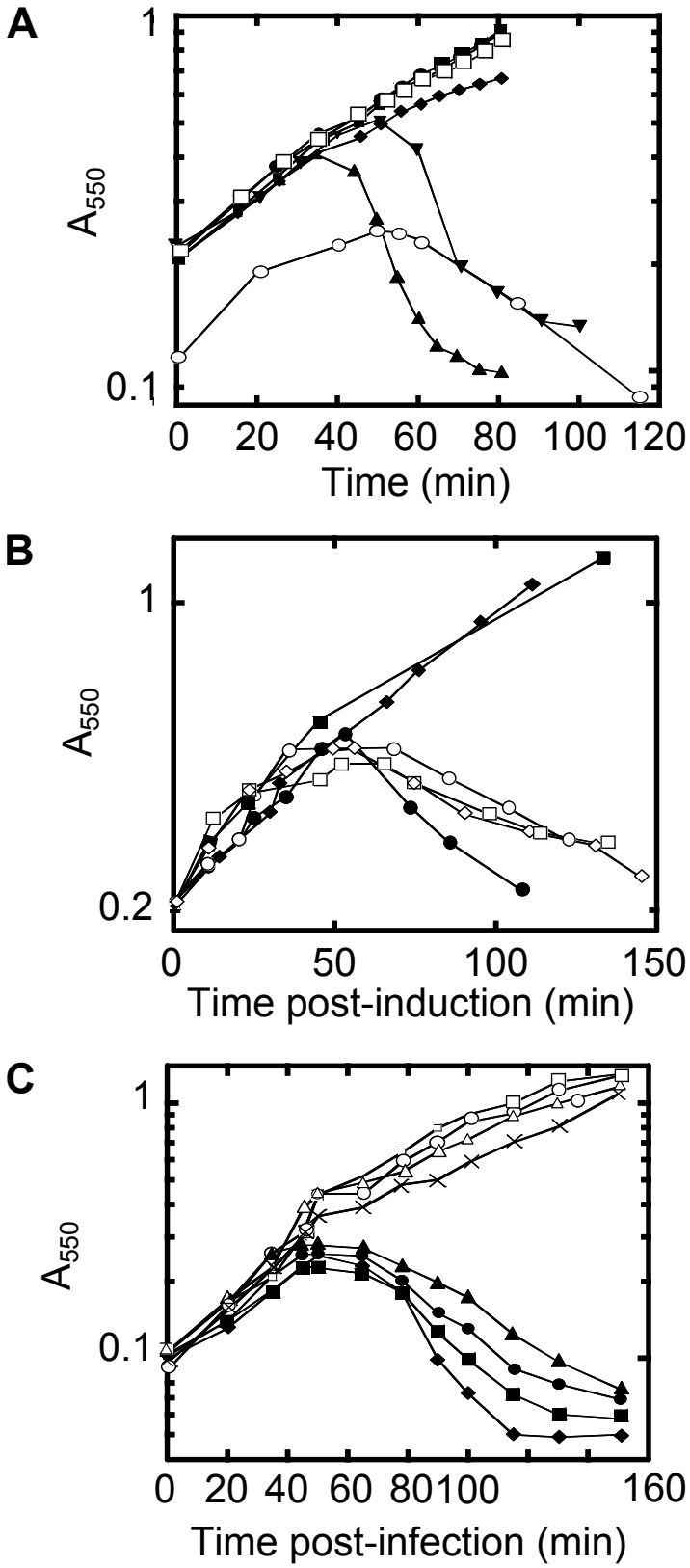
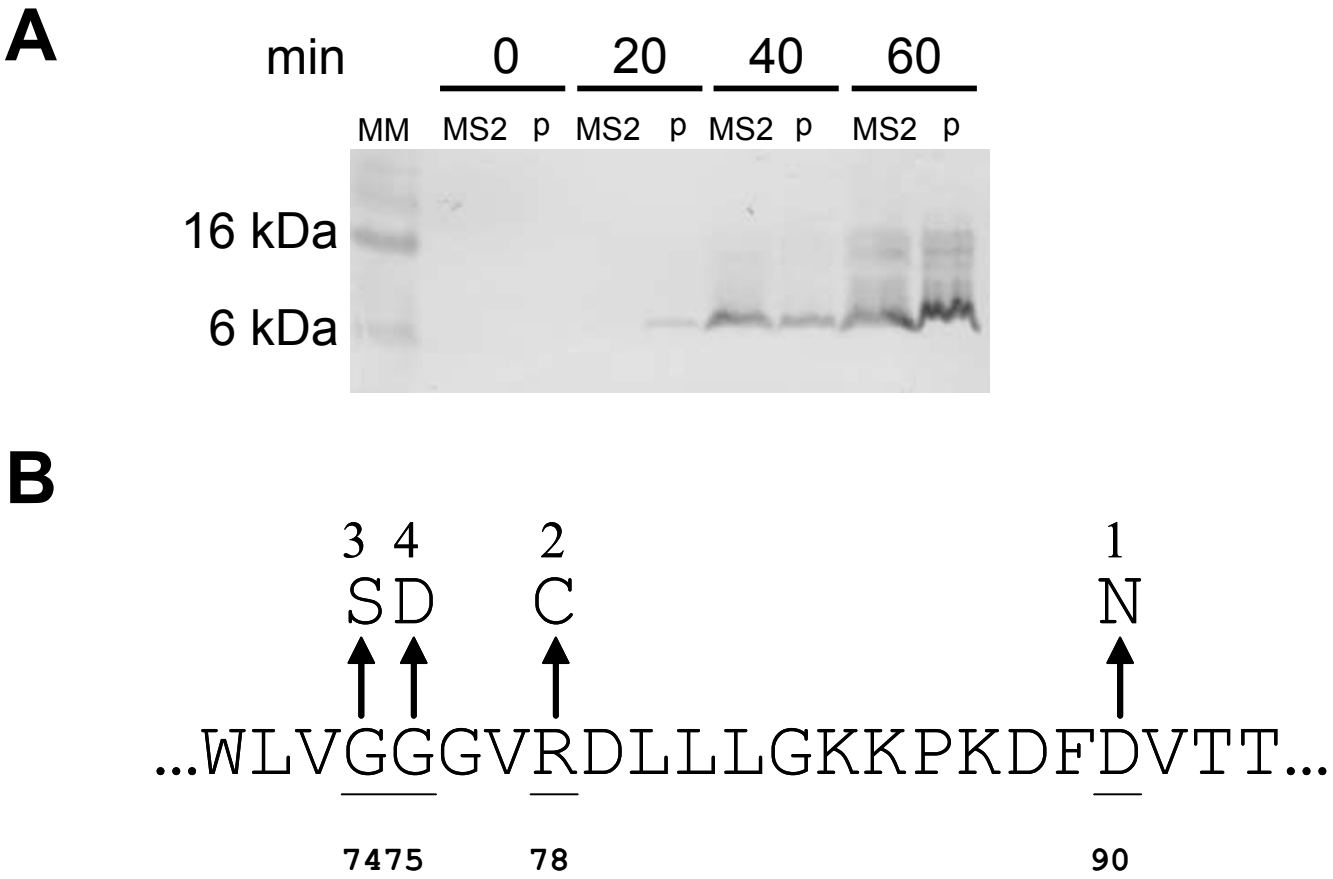


Figure 3.



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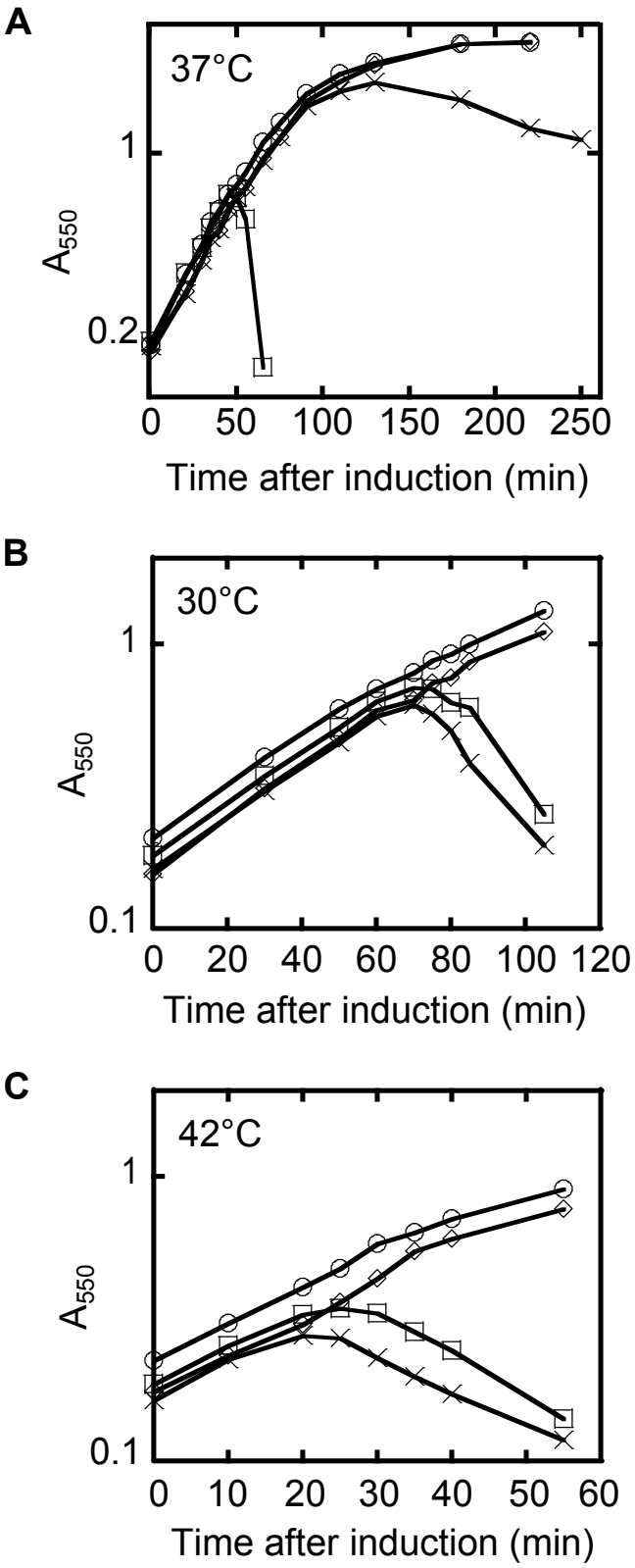

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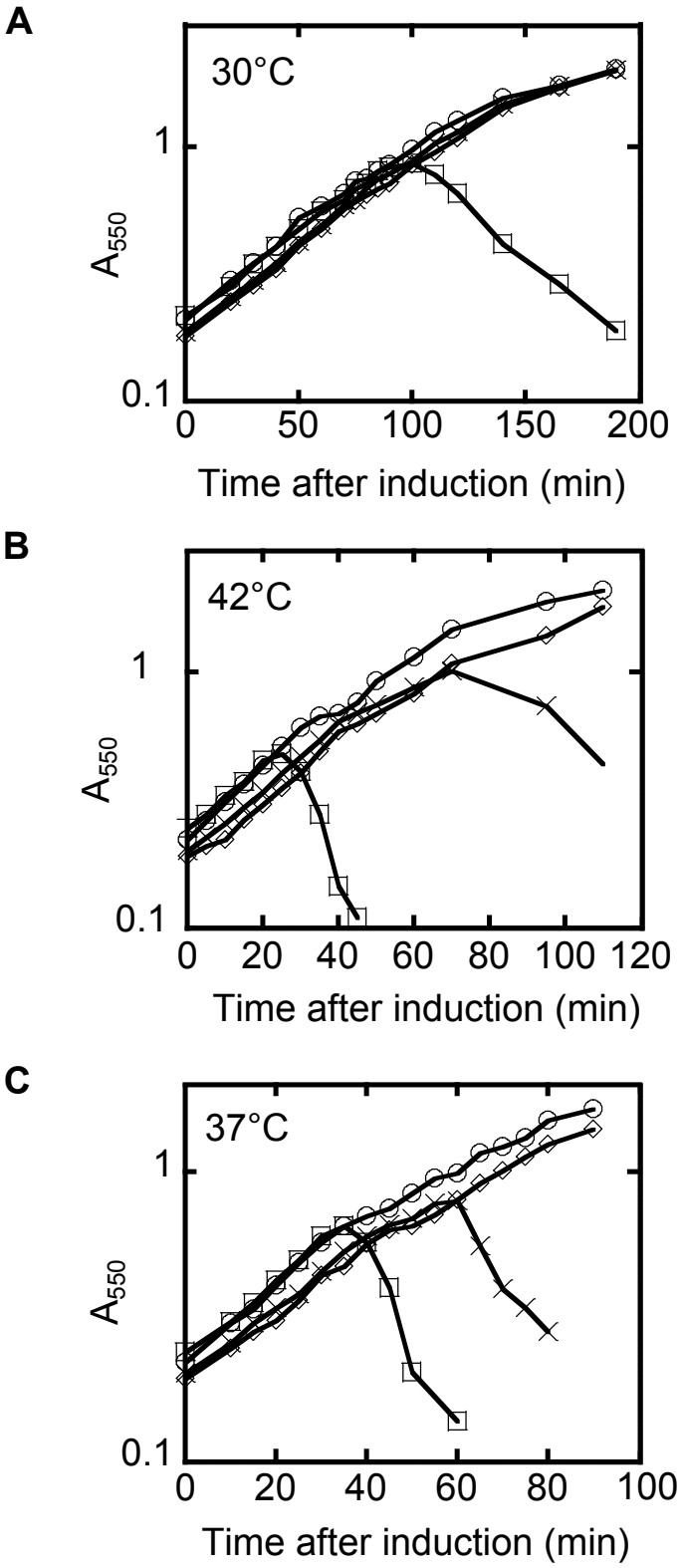

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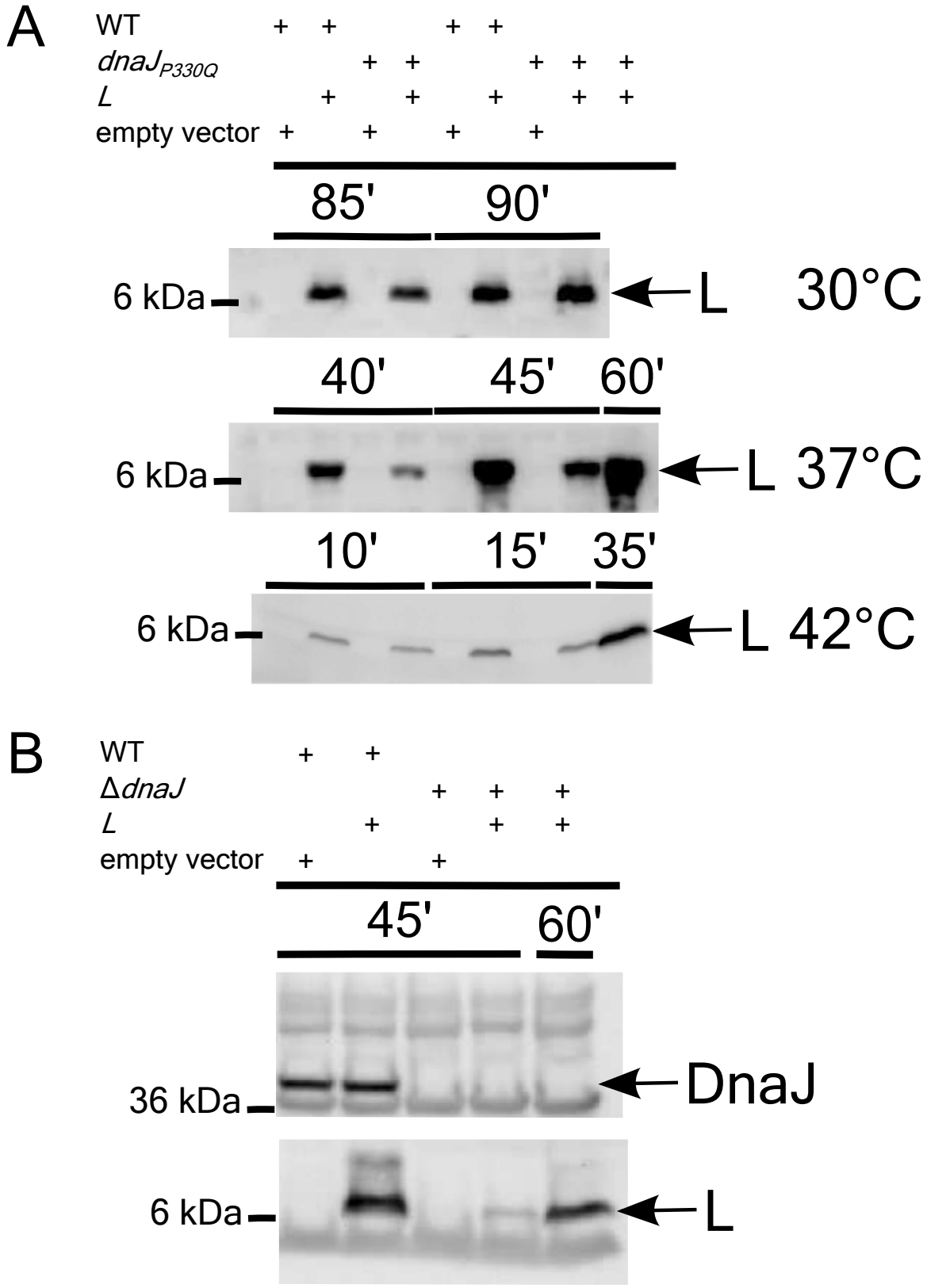
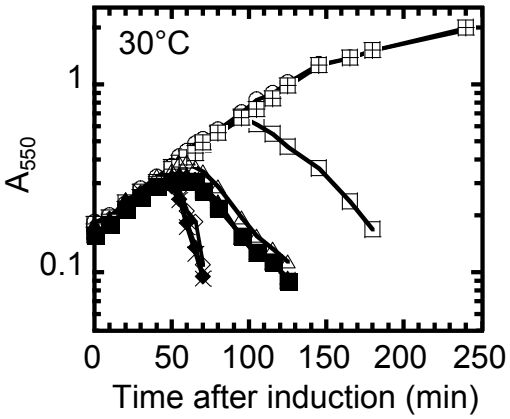


Figure 7.

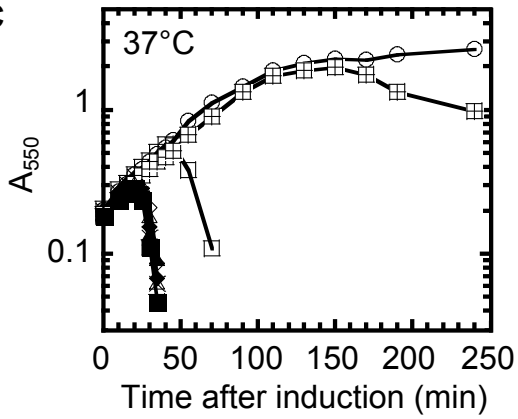
A

```
1       10        20        30        40        50        60        70
  - +             +++  + --   ++  +               +          -   +
METRFPQQSQQTPASTNRRRPFKHEDYPCRRQQRSSTLYVLIFLAIFLSKFTNQLLLSLLEAVIRTVTTLQQLLT   WT
                           MSKTKRSSTLYVLIFLAIFLSKFTNQLLLSLLEAVIRTVTTLQQLLT   Lodj1
                           MSKTTKKFNSYVLIFLAIFLSKFTNQLLLSLLEAVIRTVTTLQQLLT   Lodj2
                           MSKTTKKFNSLCMIFLAIFLSKFTNQLLLSLLEAVIRTVTTLQQLLT   Lodj3
                  MGSHHHHHHSQDPRTLYVLIFLAIFLSKFTNQLLLSLLEAVIRTVTTLQQLLT   His6-L37-75
```

B



C



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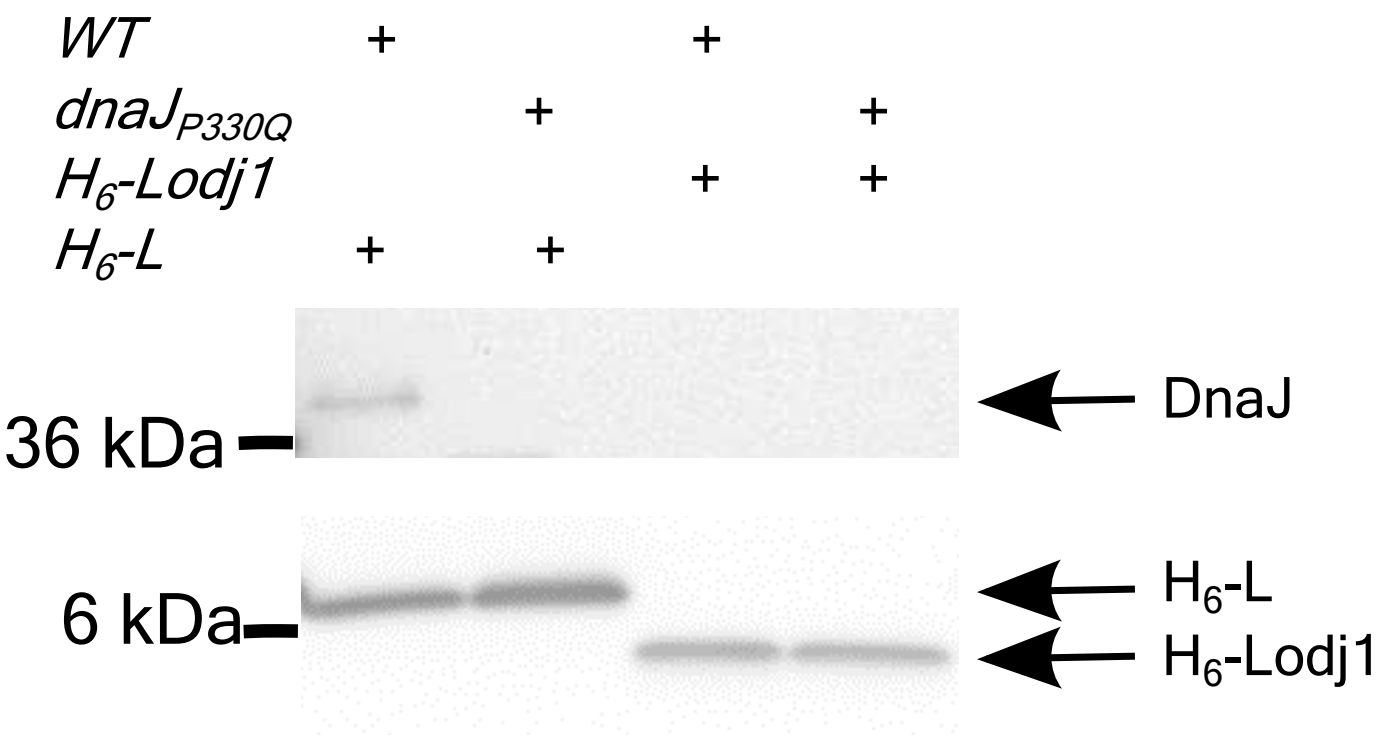

Figure 9.