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Dominant Lethal Mutations in the *dnaB* Helicase Gene of *Salmonella typhimurium*

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A class of dominant lethal mutations in the *dnaB* (replicative helicase) gene of *Salmonella typhimurium* is described. The mutated genes, when present on multicopy plasmids, interfered with colony formation by *Escherichia coli* host strains with a functional chromosomal *dnaB* gene. The lethal phenotype was expressed specifically in *supE* (glutamine-inserting) host strains and not in Sup$^+$ strains, because the mutant genes, by design, also possessed an amber mutation derived from a glutamine codon. Mutations located at 11 sites by deletion mapping and DNA sequence analysis varied in the temperature dependence and severity of their lethal effects. None of the mutations complemented a *dnaB*(Ts) host strain at high temperature (42°C). Therefore, these nonfunctional DnaB proteins must engage some component(s) of the DNA replication machinery and inhibit replication. These mutations are predicted to confer limited, specific defects in either the catalytic activity of DnaB or the ability of DnaB to interact with one of its ligands such as DNA, nucleotide, or another replication protein. The variety of mutant sites and detailed phenotypes represented in this group of mutations may indicate the operation of more than one specific mechanism of lethality.

The DnaB protein of *Escherichia coli* and a structurally similar (38) and functionally interchangeable (25) protein from *Salmonella typhimurium* are helicases (22) whose actions at replication origins and replication forks involve multiple overlapping functions. These functions include assembly into a replication complex at a bacterial origin that is dependent on DnaA and DnaC proteins (10); ATP binding and DNA-dependent ATP hydrolysis (2, 29, 33); helicase action associated with nucleotide hydrolysis, 5′ to 3′ migration on single-stranded DNA, and movement of a replication fork (9, 22); and interaction with primase (1, 4, 22). In addition, DnaB monomers associate to form hexamers, presumably the active form in vivo (7, 32), and DnaB interacts with other proteins during replication of bacteriophages λ and φX174 and plasmid ColE1 (5, 6, 13, 14, 23, 26).

How is the DnaB protein organized to carry out these functions? Some insight into this question has been gained from analysis of fragments generated by limited trypsin digestion of wild-type DnaB of *E. coli* (29). In that study (29) two protease-stable domains (residues 15 to 125/127 and 172 to 470) were identified in the 470-residue protein. The carboxyl-terminal domain exhibited nucleotide binding and single-stranded DNA-dependent nucleotide hydrolysis and was able to self-oligomerize. A conformational change in DnaB associated with the hydrolysis of ATP to ADP (3) was inferred from the much greater sensitivity of the internal trypsin cleavage sites in the presence of ADP than in the presence of the ATP analog adenosine-5′-*O*-(3′-thiotriphosphate) (ATPγS) (2). The isolated domains, either alone or in combination, exhibited no replication activity.

Another clue about the functional organization of DnaB comes from the similarity at the amino acid primary sequence level between DnaB and two other DNA replication proteins (8, 38): the gene 4 helicase primase of coliphage T7 and the gene 12 protein of *Salmonella* phage P22 (its presumed helicase) (37). Common identities among these three proteins are concentrated in the carboxyl portion of the proteins. One region of similarity probably corresponds to the ATP-binding site (15, 38), in accord with the assignment of ATP binding to the carboxyl domain in the proteolysis study. Another region of similarity could correspond to a helicase core sequence (38), but there is no independent verification for this conjecture.

A different, potentially more sensitive way to determine structure-function relationships in multifunctional proteins is to study mutations that alter one function of the protein while leaving other functions unchanged. The rewards of such an approach can be realized if suitable mutations can be identified and if suitable biochemical tests can be carried out to determine the specific nature of the alteration in mutant proteins. DnaB appears to meet the requirement for suitable biochemical assays (29), but there have been few suitable mutants (17, 20). We have previously described (24) one class of function-specific *dnaB* mutations, those that allow growth of *dnaC*(Ts) strains at a nonpermissive temperature. In this report we describe another such class of *dnaB* mutations. These mutant proteins are unable to promote replication, but they retain partial function, as indicated by the dominance of their phenotypes. The mutations are found in both of the domains defined by proteolysis and in the protease-sensitive linker region connecting the domains. This scattering of mutation sites suggests the operation of several different specific mechanisms of dominant lethality. For at least six of the mutations, which lie outside the oligomerization region of *dnaB*, the dominance could reflect the formation of nonfunctional DnaB oligomers containing a mixture of mutant and wild-type subunits.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this study are listed in Table 1. All plasmids were derivatives of pUC8 (36) which contain a *dnaB* fragment from *S. typhimurium* cloned in the *Hin*dIII-*Eco*RI site (38). In addition, in plasmid pFF157 the intergenic region from phage M13 was inserted near the 3′ end of the plasmid *bla* gene. The source of this M13 fragment was plasmid pZ152 (39),

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TABLE 1. Bacterial strains used in this study[a]

Strain	Genotype	Construction or source
RM990 (KK2186)	(F′ *traD36 proAB*$^+$ *lacI*^{q} Δ*lacZM15*) Δ(*lac pro*) *thi rpsL supE endA sbcB*	39
RM1031	Like RM990, but *dnaB22*(Ts) *zij-2354*::Tn*10*1617	P1 transduction (28)
RM1036	Like RM990, but *mutD5* *zaf-13*::Tn*10*	P1 transduction with strain KH1214 (11)
GE429	HfrH Δ*lac*U169	G. Weinstock
RM1094	HfrH (*dnaB*$^+$ *lac*$^+$ *sup*$^+$)	Transduction of GE429 to *lac*$^+$
RM1121	(F::Tn*10*) *recB21 recC22 sbcB15 his-4 arg-3 leu-6 proA2 thr-1 thi-1 rpsL31 galK2 lacY1 ara-14 xyl-15 kdgK51 tsx-33 sup*$^+$	R. Kolodner

[a] All strains were *E. coli* K-12. The designation *sup*$^+$ indicates the absence of any amber suppressor.

and its site of insertion in pFF157 was the same as that in pZ152. The *dnaB* insert in pFF157 was 2,007 base pairs, as determined by DNA sequence analysis (38), and contained the *dnaB34*(Am) allele. Plasmid pFF157 did not direct the synthesis of any α-complementing β-galactosidase fragment. Various *dnaB* deletion plasmids were derived from pFF17 as described previously (38).

Plasmid mutagenesis. A transducing lysate of plasmid pFF157, which was prepared by infection of a plasmid-bearing strain with helper M13, was propagated through the *mutD5* mutator strain RM1036 by infection at a multiplicity of approximately 1 (considering both transducing particles and helper M13), followed by overnight growth at 37°C in LB-thymidine (12). The mutagenized lysate was sterilized by heating it to 70°C.

Primary screen for dominant mutations. A log-phase culture of the Lac$^+$ strain RM1094 concentrated to 10^{10} cells per ml in LB medium was mixed with the mutagenized pFF157 lysate at a ratio of no more than 1 ampicillin-transducing unit per 10 cells. Infection was allowed to proceed at room temperature for 30 min, after which 0.1 ml of the mixture was added to 5 ml of LB medium containing 4×10^{11} M13 per ml. This mixture was incubated on a roller drum at 37°C for 45 min. Next, a portion of the helper-infected cells was washed 3 times by microcentrifugation to remove free M13 and transducing particles. The washed cells were diluted to give a calculated ~200 ampicillin-resistant cells per 0.1 ml, and this amount of the dilution was spread, together with 0.1 ml of a fresh culture of the Lac$^-$ strain RM990 on a 2,3,5-triphenyltetrazolium chloride (TTC)–lactose–ampicillin plate. These plates were incubated overnight at 37°C. Most colonies formed by this procedure contained multiple red and white sectors, reflecting side-by-side transduction of the two different strains; white colony mutants were sought. Transducing particles were recovered from white colonies with additional M13 helper, and their properties were evaluated.

The rationale for this procedure is the following. The primary recipient, strain RM1094, was *sup*$^+$ (nonsuppressing), and therefore, it could be transduced to ampicillin resistance regardless of the potential presence of a dominant lethal mutation in the plasmid *dnaB34*(Am) gene. The secondary recipient, strain RM990, was *supE*, and consequently it was a host in which the dominant lethality could be expressed. By virtue of the helper M13 included in the experiment, the *dnaB* plasmid could be transmitted from the primary transductants to other cells, resulting in ampicillin-resistant colonies that contained both white sectors derived from strain RM1094 and red sectors derived from strain RM990. If the plasmid carried a dominant lethal mutation, the secondary transduction of RM990 failed and the colony was pure white.

An important consideration in the design of the procedure was the necessity of maintaining the infectibility of the secondary recipient. One feature of M13 biology is that cells, once infected, become resistant to further infection by M13, including any plasmid packaged as M13 (31). Therefore, a seemingly simple alternative procedure, such as seeding the ampicillin plate with excess helper M13, completely blocks secondary transduction. The procedure that we used ensured that most primary transductants received helper phage, whereas the secondary recipients were free of helper, at least initially.

TTC-lactose-ampicillin agar was based on LB agar (containing the following, per liter: 10 g of tryptone, 5 g of yeast extract, 10 g of agar, 5 g of NaCl, and 1 ml of 1 N NaOH). After autoclaving, the agar was modified by the addition, per liter, of 50 ml of 20% (wt/vol) lactose, 100 mg of ampicillin, and 12.5 ml of filter-sterilized solution of TTC (4 mg/ml in water). The TTC solution was stable for at least 6 months at 4°C in the dark.

Characterization of candidate plasmids. Titers of transducing particles in lysates obtained from colonies that were white in the primary screen were estimated by spreading together a diluted portion of the lysate and ~2×10^8 freshly grown cells of a suitable host strain on a selective plate and incubating the plate overnight. For measurement of the total titer, strain RM1094 and LB-ampicillin (100 μg/ml) plates were used; incubation was at 37°C. For measuring *dnaB* complementation, strain RM1031 (*dnaB22*) and LB plates were used; incubation was at 42°C. For measurement of lethality, strains RM990 (*dnaB*$^+$) and RM1031, in combination with LB-ampicillin plates, were used; incubation temperatures were as indicated in the text. In most cases, lethality was expressed as the fraction of total transducing particles that were able to form ampicillin-resistant transductants in the lethality tests. In a few cases, lethality was indicated by a reduction in the size of transductant colonies rather than a reduction in number.

Deletion mapping. The mutations in the dominant lethal plasmids were mapped by marker rescue with the set of deletions of *dnaB*$^+$. For each marker rescue experiment, strain RM1121 harboring one of the deletion plasmids was infected with a transducing lysate of the dominant lethal plasmid. Since RM1121 was *sup*$^+$, the lethality of the plasmid was not expressed in this host. After overnight incubation, the supernatant of the culture was collected and sterilized by heating it to 70°C. This supernatant was then assayed for the presence of *dnaB*$^+$ transducing particles by spotting a drop of this supernatant onto a lawn of strain RM1031 and incubating the plate overnight at 42°C. The formation of temperature-resistant transductants within the spots was taken to signal the formation of *dnaB*$^+$ recombinant plasmids by marker rescue in strain RM1121. As a control for spontaneous reversion of the lethal plasmid, a sham marker rescue with strain RM1121(pUC8) was also carried out.

DNA sequencing. For each mutation, the identified map interval was sequenced by the dideoxy method with a

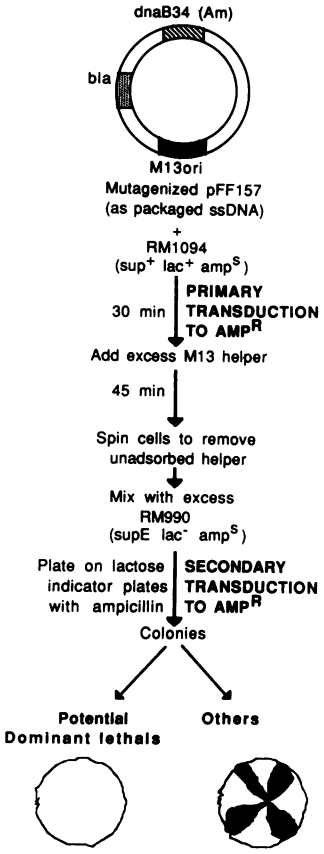

FIG. 1. Screen for dominant lethal *dnaB* mutations (see text).

subclone of the mutant gene in M13mp18 or M13mp19 (30) as the template and a suitable synthetic oligonucleotide as the primer.

RESULTS

Isolation of dominant lethal *dnaB* mutations. In these experiments we sought mutations in a plasmid-borne *dnaB* that interfered with the growth of strains with a functional chromosomal *dnaB*. The scheme used to detect such mutations is outlined in Fig. 1. The parental *dnaB* gene, carrying an amber mutation derived from glutamine codon 149, was carried in a pUC8 derivative that conferred ampicillin resistance and that possessed an origin of replication derived from phage M13. In the presence of M13 helper phage, the plasmid was packaged into infectious particles that transduced another cell to ampicillin resistance. Two types of bacterial cells were used. Strain RM1094 was Lac^+ and Sup^+ (nonsuppressing), whereas strain RM990 was Lac^- and *supE* (glutamine-inserting). During screening on lactose indicator plates containing ampicillin, the plasmids were introduced into strain RM1094 (primary transduction), where they were packaged and extruded as filamentous particles that then transduced strain RM990 to ampicillin resistance (secondary transduction). Therefore, a typical ampicillin-resistant colony formed by transduction with the parent plasmid contained red and white sectors corresponding to the Lac^- and Lac^+ cells, respectively. In contrast, plasmids with mutations of the desired type were able to transduce strain RM1094, but were unable to complete the secondary transduction of strain RM990 because the presence of the amber suppressor in RM990 enabled the synthesis of the interfering DnaB protein. An unsectored, white (Lac^+) colony resulted.

This screening assay was not completely specific for dominant lethal mutations. In particular, unsectored white colonies arose in a trivial way when a primary transductant was not infected by an M13 helper phage in a timely way. Since attachment of M13 to target cells is inefficient (35), this circumstance was impossible to prevent completely (see above). Such spurious unsectored colonies should contain the parental *dnaB34*(Am) plasmid. To identify and eliminate such colonies at an early stage in the analysis, plasmids obtained from mutant candidates were tested for their ability to complement the temperature sensitivity of a *dnaB*(Ts) *supE* strain. Only those plasmids that were negative in this test were examined in detail. Even among this doubly screened group, only a portion of the isolates showed a dominant lethal phenotype on retesting (see below). Twelve mutants with a dominant lethal phenotype obtained through this double screening are described below.

Phenotypic characterization of mutants. Two properties of the mutants were examined: their ability to exert a lethal effect at various temperatures and their ability to exert a lethal effect in host strains whose chromosomal genotype was either $dnaB^+$ or *dnaB22*(Ts) (Table 2). Two kinds of lethal effects were observed. In some cases, such as that for *dnaB129*, the transducing titer in the nonpermissive host was reduced by 3 or more orders of magnitude compared with the value in the permissive host. The rare colonies that did form in the nonpermissive host were normal in size and presumably reflected revertants in the plasmid population. Thus, we concluded that the *dnaB129* plasmid transductants utterly failed to form colonies. In other cases, such as that for *dnaB152* in the $dnaB^+$ host, the titer was within 1 or 2 orders of magnitude of the permissive titer, but the colony size was reduced (30°C) or normal (37 and 42°C). We conclude that *dnaB152* plasmid transductants are able to form, but grow slowly at 30°C. By the transductional method used in this study, there is considerable variation in titer of a control (nonlethal) plasmid, even within a single experiment (Table 2), and for this reason we do not consider single order-of-magnitude differences significant.

The patterns of lethal effects shown by the various mutants can be summarized by the following two rules. (i) At 30°C, which is ordinarily a permissive temperature for the *dnaB*(Ts) strain, the severity of the lethal effect was at least as great in the *dnaB*(Ts) strain as in the $dnaB^+$ strain. (ii) In the $dnaB^+$ strain, the severity of the lethal effect was at least as great at 30°C as it was at 37 or 42°C. By combining these two rules, it is possible to rank the *dnaB* mutations in groups, as shown in Table 2, in order of phenotype severity.

Mapping and sequencing of the mutations. As a consequence of the double screening by which they were isolated, all 12 mutant *dnaB* genes exhibited a failure to complement a *dnaB*(Ts) mutant at 42°C; they also exhibited dominant lethality. As a point of departure for mapping the determinants of dominant lethality, we assumed that lethality and noncomplementation both resulted from a single mutation in each mutant (see below). Such an assumption was necessary, because only the noncomplementation phenotype afforded a selection for rare wild-type recombinants in crosses with deletions derived from $dnaB^+$. In contrast, the behavior of deletions in the dominant lethality assay was the same as that of the wild-type.

The 17 deletions used in these mapping experiments entered *dnaB* from the 5′ side (8 deletions) or the 3′ side (9

TABLE 2. Transduction by dominant lethal plasmids as a function of host genotype and temperature

dnaB dominant allele[a]	Amino acid change	Transduction to ampicillin resistance of hosts with the following genotype, temp (°C)[b]:			
		dnaB$^+$, 30	*dnaB*$^+$, 37	*dnaB*$^+$, 42	*dnaB*(Ts), 30
129	Arg-231 → Cys	<0.00002	0.00003	0.00004	<0.00002
130	Ile-135 → Asn	0.0002	<0.001*	<0.001*	<0.00003
131	Leu-156 → Pro	<0.0002	0.0002	0.006*	<0.0002
145	Ile-141 → Thr	0.001*(mi)	0.003*	0.08	0.00002
153	Ile-360 → Thr	0.006*(mi)	0.2	0.2	0.00001
152	Tyr-343 → Cys	0.02* (mi)	0.2	0.2	<0.00001
135	Ser-364 → Phe	0.02* (mi)	0.3	0.1	<0.00001
146	Asp-82 → Asn	0.06* (mi)	0.3	0.2	<0.00005
132	Asn-117 → Ser	0.2* (mi)	0.2*	0.04*	0.0002*(mi)
155	Ala-106 → Val	0.3* (sm)	0.4*	0.3*	0.01* (mi)
151	Ile-417 → Met	0.3* (sm)	0.6*	0.1*	0.07* (mi)
133	Ala-106 → Val	0.4*	3*	0.04*	0.2* (sm)
None[c]	None	0.4	0.3	0.07	0.01

[a] Braces indicate mutants whose lethalities are judged to be approximately the same.

[b] Values are the ampicillin transducing titer measured in a *supE* host strain [RM990 for *dnaB*$^+$ or RM1031 for *dnaB*(Ts)] relative to total ampicillin transducing titer as measured in a *sup*$^+$ strain (RM1094) at 37°C. All plasmids carried the *dnaB34*(Am) mutation. All values except those indicated by an asterisk were obtained from a single experiment and are approximately similar to values obtained in repeated measurements. Asterisks indicate values calculated from the results of a separate, comparable experiment with its own control titers (lethal plasmid on the permissive host and the nonlethal parental plasmid on all hosts). Colonies were judged to be normal in size, unless indicated as mi (minute, barely visible to the unaided eye at 24 h) or sm (small but readily visible at 24 h). When no transductants were obtained at the lowest practical dilution of transducing lysate, the upper limit of the transducing titer is indicated by a less than symbol (<).

[c] The nonlethal parental plasmid pFF157.

deletions) (Fig. 2). By identifying the longest deletion on each side with which each mutation would recombine to give the wild type, both the 5′ and 3′ limits of an interval in which the mutation must lie were determined. The presence of the *dnaB34*(Am) mutation in all of the mutant genes did not interfere with the scoring of recombinants, which took place in a *dnaB*(Ts) *supE* host strain. Each mutation (or, more exactly, the determinant of its noncomplementation phenotype) mapped to a single interval, which was then sequenced. For each sequenced interval, only a single-nucleotide difference from the wild type was found; in each case, the nucleotide change altered the encoded amino acid. The inferred amino acid changes in DnaB protein are given in Table 2. Among the 12 mutants, all of which were obtained from a single mutagenized preparation of pFF157, 11 different mutations were found. The single repetition (Ala-106 → Val) was something of a puzzle, since its phenotype was different in its two isolates (as *dnaB133* and *dnaB155*). We assume that one or the other of these mutant genes contains a second mutation that modifies the phenotype. Such a modifier mutation, unless it conferred a noncomplementing phenotype in its own right, would not have been detected by the mapping procedures used in this study.

DISCUSSION

The potential of some bacterial genes to mutate to a dominant negative form has long been known. Often, the existence of such mutants has been taken to indicate that the encoded protein functions as a multimer, even though this is not the only possible explanation for such mutations (18). For some proteins whose biochemical modes of action are well understood, however, the properties of dominant negative mutants can be explained satisfactorily on this basis (e.g., several procaryotic repressors [18]). We sought dominant negative mutations in *dnaB* as a way of defining the residues that are required for functions other than oligomerization. Since DnaB is an essential cellular protein, dominant negative mutations are also dominant lethal mutations, and expression of these mutant genes must be made conditional to allow for their propagation. We conditioned their

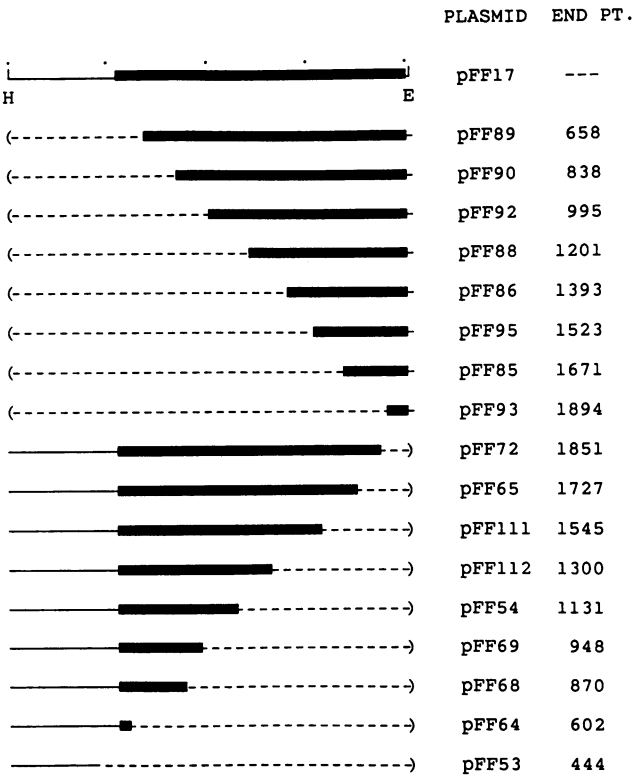

FIG. 2. Deletions used in mapping experiments. The 2,00′/-base-pair restriction fragment containing *dnaB*$^+$ is shown on the top line, with the *dnaB* structural gene indicated by the heavy bar. The direction of *dnaB* transcription was from left to right. Intervals of 500 base pairs are marked by dots above the line, and the *Hin*dIII and *Eco*RI ends of the fragment are indicated by H and E, respectively. Deletions are shown to scale on the remaining lines, with the deleted material indicated by dashes. The deletion endpoints (END PT.) are defined as the terminal deleted nucleotide, with numbering beginning at the *Hin*dIII cleavage site in the strand with the same polarity as that of the *dnaB* message.

expression on the presence of an amber suppressor mutation in the host cells by the inclusion of an amber mutation in *dnaB*. In addition, practical considerations required that we consider only those *dnaB* mutations that conferred a noncomplementation phenotype as candidate dominant mutations.

The primary result reported here is the isolation and sequence identification of dominant lethal mutations in *dnaB*. To put this result in perspective, we note that many classical temperature-sensitive mutations affecting *dnaB* as well as other DNA replication genes have been reported in the literature. Such mutations have been invaluable in facilitating protein purification by complementation of deficient extracts and in defining function by reconstitution assays with purified proteins. Yet, such mutations have been of little value for detailed structure-function studies of these important proteins. The alternative of designing critical mutations on the basis of X-ray crystal structure, such as has been done with DNA polymerase I (19), is prevented by the lack of crystals or crystallographic data for virtually all DNA replication proteins. Thus, the mutations described here represent a valuable resource for structure-function studies of DnaB, and the approach used to isolate these mutations represents a design that could be applied to other DNA replication proteins or, more generally, to any essential protein of the cell.

Physiological basis of lethality. Since synthesis of the lethal protein is not conditional within a single cell type, it has not been possible to carry out physiological experiments to determine how the lethal protein blocks cell growth. We assume that the proximate cause is interference with DNA replication, but the transductional test that we applied did not distinguish interference with host DNA replication from interference with replication of the *dnaB* plasmid itself. If host DNA cannot be replicated, no colony can form. If plasmid replication is specifically blocked, then cell division produces plasmid-free segregants which are sensitive to ampicillin. Substantial differences in the way the host chromosome and plasmid pUC8 (derived from ColE1) establish replication forks (for reviews, see references 23, 27, and 34) could provide a basis for mutations that inhibit replication of one molecule without affecting the other. Mutations in *dnaB* are known, for example, that block replication of phage λ DNA without affecting host DNA replication (16) and vice versa (20, 21). Determination of the physiological target of the mutations described here will require transfer of the mutant *dnaB* genes to a chromosomal location or a DnaB-independent replicon.

Molecular target of lethality. The deficiencies in the dominant lethal *dnaB* proteins and their biochemical mechanisms of cell growth inhibition remain to be determined. A few clues can be garnered from the phenotypic properties of the mutants and the location of the mutations within *dnaB*. For most of the mutations, the degree of inhibition at 30°C was greater in a *dnaB22*(Ts) host than in a *dnaB*$^+$ host. We offer two hypotheses to explain this effect. In the first hypothesis, the dominant lethal DnaB protein competes with functional DnaB for some limiting cellular component. According to this hypothesis, the *dnaB22* protein is present in lesser amounts than the *dnaB*$^+$ protein or is a less effective competitor than the *dnaB*$^+$ protein, and therefore, the *dnaB22* strain is more strongly inhibited. The alternative hypothesis supposes that the *dnaB*$^+$ protein moderates, or the *dnaB22* protein exacerbates, the lethality of the dominant DnaB protein, perhaps by forming mixed oligomers, as has been suggested for the moderating effect of the phage P1 *ban* protein on *dnaB252*(Ts) (20). Either of these explanations implies that the dominant DnaB protein exerts its inhibiting effect by interfering with the functional DnaB protein, as we suspect is also true of dominant proteins that are not sensitive to the host *dnaB* genotype.

The locations of some of the dominant mutations suggest the basis of their deficiencies. Arg-231 → Cys is located within the likely ATP-binding motif and affects a residue that is conserved in P22 gene 12, a *dnaB* analog (8, 38). This change could affect ATP binding or hydrolysis.

Ile-360 → Thr affects a residue that is conserved in P22 gene 12 and in T7 helicase primase. This conserved residue is part of an extended common sequence in these three proteins that may be responsible for a function that is shared by the three proteins, such as helicase or DNA binding. Ser-364 → Phe and Ile-417 → Met are also located in this region, but they are not conserved in all three proteins.

Asp-82 → Asn, Ala-106 → Val, and Asn-117 → Ser are all located in the amino domain of DnaB, as defined by trypsin digestion. Since this domain is dispensable for DnaB hexamer formation and ATPase activity, but is necessary for replication activity, these changes could affect a critical replication function such as interaction with DnaC or primase. These mutant proteins are candidates for mixed oligomer formation with functional DnaB.

Ile-135 → Asn, Ile-141 → Thr, and Leu-156 → Pro are located in the linker between the two domains. This region undergoes a change in conformation in connection with ATP hydrolysis, as reflected in its different sensitivity to trypsin in the ATPγS-bound form compared with the ADP-bound forms (29). Thus, in DnaB · ADP, this region has significant exposure to solvent (i.e., to trypsin). On this basis, it is surprising to find that all three mutations define critical hydrophobic residues. This finding may indicate that under physiological conditions this region of the protein is involved in the binding of another protein. Like the proteins discussed above, these three mutant DnaB proteins are likely to form mixed oligomers with functional DnaB.

Temperature sensitivity in dominant lethal DnaB proteins. Several of the dominant lethal proteins prevented or slowed the growth of DnaB$^+$ cells at 30°C but had little effect on cells of the same genotype at a higher temperature (Table 2). Thus, these dominant proteins (Asp-82 → Asn, Ala-106 → Val as *dnaB155*, Asn-117 → Ser, Tyr-343 → Cys, Ile-360 → Thr, Ser-364 → Phe, Ile-417 → Met, and perhaps, Ile-141 → Thr) are temperature sensitive. Apparently, temperature sensitivity in DnaB can be produced by mutations affecting either trypsin-resistant domain.

Sensitivity of the primary screening assay. As mentioned above, many white colonies obtained in the original screen did not carry a dominant lethal *dnaB* gene. Most such colonies proved to contain the parental plasmid pFF157. Occasionally, however, such a colony contained a plasmid bearing a recessive mutant *dnaB* gene that could not complement a chromosomal *dnaB22*(Ts) mutation. Although there is no obvious reason why such a mutant should have produced a white colony in the first place, we offer two possible explanations. The first is that such mutants were picked by chance among the several hundred white colonies analyzed to obtain the 12 mutants that we described. The second possibility is that the sectored colony assay provides a more sensitive measure of dominance than the transductional test (Table 2) applied to purified isolates. The latter explanation might also account for the recovery at 37°C of mutations whose dominance, on retesting, was seen clearly only at 30°C.

Additional mutations in dominant lethal DnaB. To map the dominant mutations, we had to assume that each mutation was also responsible for the noncomplementation phenotype of the mutants. Although our results do not prove the validity of this assumption, they impose an important limitation on the possibility that any of the *dnaB* alleles could be more complex than the single identified mutations. Specifically, none of the mutant genes could possess two mutations, each of which independently conferred a noncomplementation phenotype. Had this been the case, we would have found two mutations in the identified interval or we would have obtained anomalous deletion mapping behavior, because the identified map interval would have to be large enough to encompass the sites of both mutations. Therefore, if separate mutations are responsible for dominant lethality and noncomplementation, the mutation responsible for dominant lethality must not exhibit noncomplementation when present as a single mutation. This circumstance seems improbable to us, because it implies the existence of a DnaB protein that interferes with the function of the *dnaB*$^+$ protein but that restores function to (or is restored to function by) the *dnaB22*(Ts) protein at 42°C. Consequently, the changes listed in Table 2 likely are responsible for the dominant lethal phenotype.

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