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Suppression of *dnaC* Alleles by the *dnaB* Analog (*ban* Protein) of Bacteriophage P1

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The *dnaB* analog protein produced by the *ban* gene of bacteriophage P1 was shown to suppress several *Escherichia coli dnaC* alleles. Suppression of *dnaC7* temperature sensitivity in P1 lysogens of a *dnaC7* mutant was complete at all temperatures. For the *dnaC2* and *dnaC28* alleles, suppression was observed only at intermediate temperatures. Though these intermediate temperatures were sufficient to completely restrict the mutants, at higher temperatures the suppression was not observed. No suppression of the *dnaC1* allele was detected. These results have implications concerning the requirement for the *dnaB-dnaC* complex at the various stages of deoxyribonucleic acid replication.

Evidence that the single initiation-defective *dnaB* allele, *dnaB252*, can be suppressed by increasing the gene dosage of *dnaC*$^+$ is presented in the accompanying paper (7). Since this result implies a direct functional interaction between the *dnaB* and *dnaC* gene products in vivo, the possibility that *dnaC* mutants may be suppressed by the *dnaB* analog protein (*ban* protein) encoded by bacteriophage P1 was investigated.

We were aware of data showing that P1 *bac-1 ban*$^+$ (derepressed for *ban* expression) failed to suppress the temperature-sensitive, colony-forming phenotypes of *dnaC1* and of *dnaC28* at the standard restrictive temperatures (40 and 42°C, respectively) (2). P1 *bac-1* lysogens of the *dnaC* mutants were, therefore, analyzed at temperatures below 40°C but still sufficient to block growth and DNA synthesis. Under these conditions suppression of temperature sensitivity was easily detected. The allele specificity of the suppression suggests a direct interaction between the *dnaB* analog protein and the *dnaC* product.

(These experiments were done by R.A.S. in partial fulfillment of the Ph.D. requirements of Columbia University.)

MATERIALS AND METHODS

Bacteria and bacteriophage. Bacterial strains were all *Escherichia coli* K-12 and are listed in Table 1. P1Cm-1 *bac-1* and P1Cm-1 *bac-1 ban-1* were obtained from M. Yarmolinsky. P1Cm-1 *bac-1 crr-14* was isolated by selecting for cold resistance (growth at 25°C) of a *dnaB*::Tn*10* (P1Cm-1 *bac-1*) lysogen (R. A. Sclafani, J. A. Wechsler, and H. Schuster, Mol. Gen. Genet., in press) and subsequently purified and checked by the method of Touati-Schwartz (8). The Cm-1 and allele numbers are deleted from the P1 notation hereafter for convenience.

Media. Penassay broth (antibiotic medium no 3; Difco Laboratories) was supplemented with thymine (20 μg/ml). Solid nutrient medium was the same, but contained 1.5% agar. Bacterial strains were made lysogenic for the appropriate P1 phage by the method of D'Ari et al. (2).

Incorporation kinetics. Cultures were grown overnight at the permissive temperature in Penassay broth supplemented with thymine (4 μg/ml), subcultured into the same medium, and grown to the exponential phase. To begin the experiments, cultures were diluted to 5×10^6 cells per ml in the same medium containing [^{3}H]thymine at 8.25 μCi/μg and grown at the permissive temperature for 60 min (90 min for strain CT28-3b) before the shift to the appropriate restrictive temperature. Samples (50 μl) were taken at intervals starting with the dilution into medium containing label and processed for measurement of radioisotope incorporation as described previously (10).

RESULTS

The *ban* gene of bacteriophage P1 codes for a *dnaB* analog protein. Derepression of this gene resulting from a mutation in the *bac* gene (*bac* = *dnaB* analog control) of P1 allows *dnaB* mutants lysogenic for P1 *bac* to grow at the restrictive temperature (2). This suppression is cold sensitive. Selection for P1 *bac* suppression at low temperature commonly results in the isolation of P1 with a second mutation, *crr* (8). In cases which have been examined P1 *bac crr* lysogens produce 4 to 8 times more *ban* protein than does the P1 *bac* parent (3, 5).

The DNA synthesis defect of *dnaC2* at 38°C was suppressed equally well by P1 *bac* and P1

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bac crr prophage, but was not suppressed by P1 *bac ban* (Fig. 1a). The temporary inhibition of DNA synthesis after the shift to restrictive temperature was characteristic of P1 *bac* suppression and probably reflects a requirement for a reassortment of *dnaB* or *ban* proteins (or both) in the replication complex. At 40°C, P1 *bac crr* suppression was markedly better than that of P1 *bac* (Fig. 1b). Plating efficiencies at the restrictive temperatures displayed the same hierarchical pattern (Table 2). Though DNA synthesis, over the time examined, in the P1 *bac crr* and P1 *bac* lysogens approached the wild-type rate and 50% of the wild-type rate, respectively, the plating efficiencies were only 0.06 to 0.09%. This represented an increase in plating efficiency of more than 3 orders of magnitude, but implied that growth was still substantially unbalanced.

Suppression of the *dnaC7* allele by either P1 *bac* or P1 *bac crr* was complete with respect to both DNA synthesis and viability (Fig. 2 and Table 2). This suppression of *dnaC7* has also been observed by Yarmolinsky and co-workers (M. Yarmolinsky, personal communication).

The *dnaC28* allele was more stringent, and 25°C must be used as the permissive temperature. At 37°C the presence of any of the P1 *bac* prophages failed to cause suppression. At 34°C, however, both P1 *bac* and P1 *bac crr* lysogens of *dnaC28* showed substantial suppression of the DNA synthesis defect, though the rate of DNA synthesis was decreased by nearly 50% (Fig. 3). The lowered plating efficiency of the P1 *bac crr* lysogen again implied that growth was somewhat unbalanced.

No suppression by any P1 *bac* prophage was detected with the *dnaC1* allele.

TABLE 1. *Bacterial strains*

Strain	Genotype	Reference
PC1	*leu thy deoC str dnaC1*	(1)
PC2	*leu thy deoC str dnaC2*	(1)
PC7	*leu thy deoC str dnaC7*	(1)
CT28-3b	*thr leu thi his pro arg thy deoB* or *C str dnaC28*	(6)

TABLE 2. *Efficiencies of plating*[a]

Strain	Colonies per plate at:			
	34°C	37°C	38°C	40°C
PC2 (nonlysogen)			4.3×10^{-4}	$<10^{-7}$
PC2 (P1 *bac ban*)			5.5×10^{-4}	$<10^{-7}$
PC2 (P1 *bac*)			0.47	6.5×10^{-4}
PC2 (P1 *bac crr*)			0.87	8.6×10^{-4}
PC7 (nonlysogen)			4×10^{-4}	1.2×10^{-5}
PC7 (P1 *bac ban*)			4.1×10^{-4}	7×10^{-5}
PC7 (P1 *bac*)			1.0	0.84
PC7 (P1 *bac crr*)			1.0	0.83
CT28-3b (nonlysogen)	1.2×10^{-4}	$<10^{-7}$		
CT28-3b (P1 *bac ban*)	1.2×10^{-3}	$<10^{-7}$		
CT28-3b (P1 *bac*)	1.1	$<10^{-7}$		
CT28-3b (P1 *bac crr*)	0.21	$<10^{-7}$		

[a] Strains were grown in log phase, diluted, and plated on nutrient medium. The number of colonies on plates at the permissive temperature (30°C for PC2 and PC7, 25°C for CT28-3b) is taken as unity.

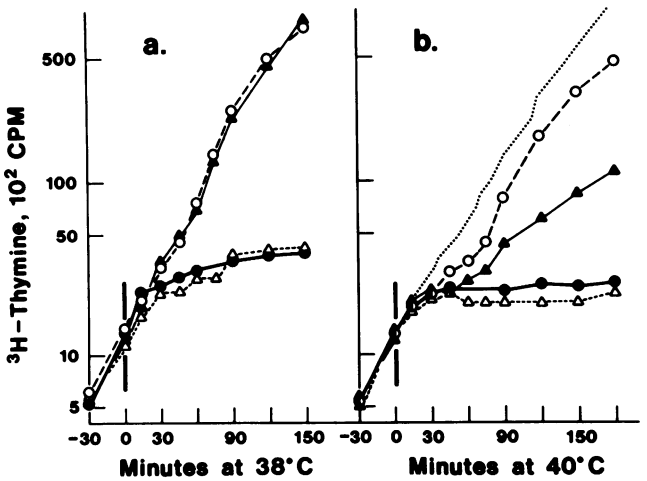

FIG. 1. *DNA synthesis of dnaC2 derivatives at (a) 38°C and (b) 40°C. Cultures were grown as described in the text and shifted as indicated. ●, PC2; △, PC2 (P1 bac ban); ▲, PC2 (P1 bac); ○, PC2 (P1 bac crr); (·····), DNA synthesis of any of the strains at 30°C.*

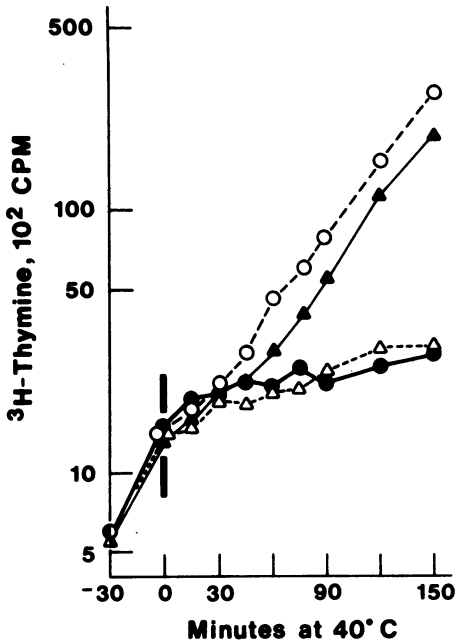

FIG. 2. *DNA synthesis of dnaC7 derivatives at 40°C. Cultures were grown as described in the text and shifted as indicated.* ●, *PC7;* △, *PC7 (P1 bac ban);* ▲, *PC7 (P1 bac);* ○, *PC7 (P1 bac crr).*

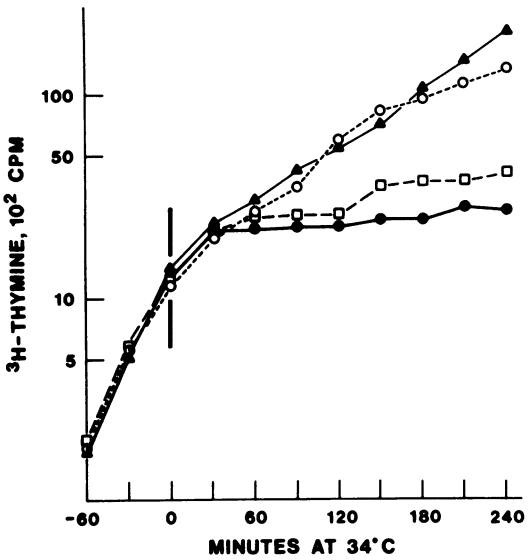

FIG. 3. *DNA synthesis of dnaC28 derivatives at 34°C. Cultures were grown as described in the text and shifted as indicated.* ●, *CT28-3b;* □, *CT28-3b (P1 bac ban);* ▲, *CT28-3b (P1 bac);* ○, *CT28-3b (P1 bac crr).*

DISCUSSION

The *dnaB* analog protein encoded by the *ban* gene of bacteriophage P1 can substitute completely for the *dnaB* protein in vivo (2, 4, 8; Sclafani et al., in press). The data presented in this paper show that *dnaC* mutations can be suppressed by the *ban* protein; suppression varied from none with *dnaC1* to complete with *dnaC7*.

The *ban* product cross-reacts with antibody to *dnaB* and is similar in size, but the proteins are not identical (5). The *ban* and *dnaB* genes are also not similar in sequence, as no homology between P1 and a λ *dnaB* transducing phage is detectable by Southern blot hybridization (S. Projan and R. Sclafani, unpublished data). The *ban* product exhibits two functional differences from the *dnaB* protein.

(i) Substitution of *dnaB* by *ban* in P1 *bac* lysogens of an unsuppressed *dnaB266* amber mutant carrying strain results in cryosensitive DNA synthesis (2). Experiments with another amber mutant as well as *dnaB*::Tn*10* insertion mutations indicate that the *ban* protein is itself cold sensitive (Sclafani et al., in press).

(ii) In P1 *bac* lysogens of the *dnaB* mutants above, the *ban* protein cannot substitute for the *dnaB* protein to allow λ bacteriophage DNA synthesis at any temperature (2, 8; Sclafani and Wechsler, submitted for publication). The P1 *bac crr* phage can support production of λ and restore cryoresistant growth to these strains, and presumably, acts by increasing the amount of *ban*$^+$ product over P1 *bac* lysogens (3, 5). This difference between *ban* and *dnaB* products can be attributed to their having different affinities for other proteins in the replication complex (8). Thus, the *ban* protein can be viewed as if it were an altered *dnaB* protein which retains the functional activity required for chromosomal DNA synthesis.

The allele-specific suppression of *dnaC* mutations by *ban* is, therefore, an example of extragenic suppression and implies that the *dnaB* product can be altered to compensate for *dnaC* defects.

A simple explanation of the partial suppression of *dnaC* mutants by *ban* is that *ban*$^+$-*dnaC* protein complexes are more thermostable than are *dnaB*$^+$-*dnaC* protein complexes. Depending on the particular *dnaC* allele, the formation of *ban*$^+$-*dnaC* protein complexes may range from inefficient to very efficient. In cases where complex formation is relatively inefficient, the overproduction of *ban* product by P1 *bac crr* should result in improved suppression compared with that of P1 *bac*. This is the pattern observed with *dnaC2*.

The 10-fold improvement in plating efficiency of CT28-3b (P1 *bac ban*) compared with that of

the nonlysogenic parent at 34°C (Table 2) presumably reflects mutual compensatory suppression between the mutant *ban* and mutant *dnaC* proteins.

An additional conclusion is generated by the fact that *ban* suppression was observed with the initiation-defective *dnaC* alleles, *dnaC2* and *dnaC28*, and also with the elongation-defective *dnaC7*. Since *ban* protein is an analog of *dnaB* protein, the *dnaB-dnaC* protein complex must, by extension, function both in initiation and in elongation.

It would not be entirely unreasonable to suggest, from these results, that the *ban* protein has a *dnaC*-like activity in addition to its well-documented *dnaB* activity. Since *ban* protein is very similar in size to *dnaB* protein, forms functional heteromultimers with *dnaB* protein, and is precipitated by antibody to *dnaB* (4, 5), it seems unlikely that the protein could also accommodate a *dnaC*-like activity. Also, the patterns of suppression by P1 *bac* and P1 *bac crr* bear no apparent relationship to the phenotypes of the *dnaC* mutants (9).

Finally, though the strains used in this work contained both the *dnaB*$^+$ gene and the P1 *ban*$^+$ gene, complete inactivation of the *dnaB* gene in a P1 *bac crr* lysogen of PC2 (*dnaC2*) had no effect on DNA synthesis or viability (data not shown). The *dnaB* gene was inactivated by insertion of Tn*10* (details of *dnaB*::Tn*10* insertions will be reported elsewhere). Thus, the *ban* product can completely replace *dnaB* and simultaneously partially suppress a *dnaC* allele.

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