OVERPRODUCTION OF dna GENE PRODUCTS BY *ESCHERICHIA COLI* STRAINS CARRYING HYBRID ColE1 PLASMIDS

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SUMMARY A bank of 2,000 *E. coli* strains carrying ColE1 plasmids into which small random segments of the *E. coli* chromosome are inserted [Clarke, L., and Carbon, J. (1975) *Proc. Nat. Acad. Sci. USA*, 72, 4361-4365] was screened for those hybrids transferring thermoresistance to dna temperature-sensitive (ts) mutants. One or more was found to transfer dnaB, dnaC(D), dnaE, and dnaZ; none was found to transfer dnaG. These donor strains each overproduce by 3- to 10-fold the product of the gene whose defect is corrected, except in the case of dnaE. The strain donating dnaE does not overproduce DNA polymerase III.

The conversion of ϕX174 single-stranded DNA to a double-stranded form by crude extracts of uninfected *E. coli* (the "ϕX system") (1, 2) provides an assay of the dnaB, dnaC(D), dnaE, dnaG, and dnaZ gene products by an *in vitro* analog of the *in vivo* complementation test (3-7). Using this assay, the dnaB, dnaC(D), dnaG, and dnaZ gene products have been purified (3-8). The dnaB gene product has ribonucleoside triphosphatase activity which is stimulated by single-stranded DNA (5). The dnaC(D) gene product interacts physically and functionally with the dnaB gene product (9). These two proteins function in the formation of an initiation complex in the ϕX system (9, 10). The dnaE gene product is DNA polymerase III (11). The dnaZ gene product acts in the elongation of primed DNA in conjunction with other elongation factors and DNA polymerase II or III (7). In the ϕX system, dnaZ and dnaG function in the reaction after formation of the initiation complex (7, 10).

Each of these proteins is present only in very small amounts in extracts of wild-type strains. The dnaB gene product required 40,000-fold purification (5) and DNA polymerase III required 4,500-fold purification (12) to reach homogeneity; dnaC(D), dnaG, and dnaZ gene products have been purified 4,000-fold,

10,000-fold, and over 10,000-fold, respectively (6, 3, 7). Thus, it would be desirable to obtain strains which overproduce one or more of these proteins.

Molecular cloning facilitates the isolation of large amounts of the DNA, the RNA transcripts, and the protein gene products of the amplified chromosome segments (13-16). By linking random fragments of sonicated whole *E. coli* DNA to ColE1 DNA *in vitro* and transforming an F^+ strain to colicin E1-resistance, Clarke and Carbon (16) have prepared a bank of *E. coli* strains, most of which carry hybrid ColE1 plasmids. The hybrid plasmids in the 2,000 strains in this bank probably cover most of the *E. coli* chromosome.

We have screened this bank for strains transferring the dna genes B, C(D), E, G, or Z, and report here the specific overproduction of dnaB, dnaC, or dnaZ proteins in different strains of this type.

MATERIALS AND METHODS

Bacterial strains are listed in Table 1. Strains from the Clarke and Carbon bank are indicated by MV12 (the recA F^+ derivative of C600 transformed by Clarke and Carbon with the ColE1 hybrids) followed by an identification number.

Screening of *E. coli* strains containing hybrid ColE1 plasmids for those carrying dna genes was accomplished as follows: Patches of each of the 2,000 F^+ MV12/ColE1 streptomycin-sensitive strains were replica-plated to lawns (21) of each of the F^- dna ts streptomycin-resistant strains on LB agar (1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5% agar). After 30 minutes at 37° to allow transfer of potential hybrid plasmids in the F-mediated matings, plates were sprayed with 5% streptomycin sulfate to kill the donors and incubated for 24 hours at 42° to allow selective growth of recipients which had acquired a hybrid ColE1 plasmid correcting the temperature-sensitive defect. Strains found to transfer dna^+ were purified by single colony isolation and retested for transfer and other markers. In most cases, patches were first treated with colicin E1 to select cells which retained the ColE1 hybrid plasmid from those which had lost it. Crude colicin E1 was the filter-sterilized supernatant from an overnight culture of the colicinogenic strain JF390 (obtained from John Foulds) grown in LB medum. In each case cells which had received the dna^+ phenotype were repurified by single colony isolation and retested for streptomycin resistance, temperature resistance, and other markers.

For preparation of extracts, cells were grown in H-broth (8) with shaking at 37° to $A_{650} = 1.2$. They were collected by centrifugation and resuspended in 10% sucrose, 0.05 M Tris-Cl (pH 7.5) in a volume (ml) equal to the weight of the cells (gm), and frozen in dry ice-ethanol. Cells were lysed by incubating thawed cells for 30 minutes at 0° in centrifuge tubes with 0.2 mg/ml lysozyme, 0.01 M dithiothreitol, 0.005 M EDTA, and 0.15 M NaCl. Lysates were centrifuged for 45 minutes at 40,000 rpm in a Spinco 40 rotor. The clear supernatants were adjusted to 4% streptomycin sulfate with a 20% solution; the precipitate was collected by centrifugation. The supernatants were adjusted to 45% or 50% of saturation in ammonium sulfate by the addition of saturated neutralized ammonium sulfate, and the precipitates were collected. The precipitates for experiments 3, 5, 6, and 7 were resuspended in 0.02 M potassium phosphate (pH 6.5), containing 10% glycerol, 1 mM EDTA, and 1 mM dithiothreitol; those for experi-

TABLE 1. *Strains of E. coli K12*

Strain Designation	Relevant Genotype	Reference
475	F⁻ thr leu strA dnaB391 ts	(17); J. Tomizawa
PC221	F⁻ his thy arg malA xyl strA dnaC2 ts	(18)
E486	F⁻ thr leu thy lac tonA met strA dnaE486 ts	(17)
E293	F⁻ thr leu thy lac tonA strA dnaE293 ts	(17)
BT308	F⁻ thr leu thy lac tonA strA dnaG308 ts	(19)
NY731	F⁻ thy leu metE rif strA dnaG3 ts	(18)
AX727	F⁻ lac strA dnaZ ts	(20)
MV12/28	F⁺/ΔtrpE thr leu recA/ColE1-dnaB⁺	This work and (16)
MV12/29	F⁺/ΔtrpE thr leu recA/ColE1-dnaC⁺	This work and (16)
MV12/20	F⁺/ΔtrpE thr leu recA/ColE1-dnaC⁺	This work and (16)
MV12/26	F⁺/ΔtrpE thr leu recA/ColE1-dnaE⁺	This work and (16)
MV12/27	F⁺/ΔtrpE thr leu recA/ColE1-dnaZ⁺	This work and (16)
MV12/16	F⁺/ΔtrpE thr leu recA/ColE1-dnaZ⁺	This work and (16)
MV12/15	F⁺/ΔtrpE thr leu recA/ColE1 [a]	This work and (16)

[a] This strain carries none of the dna genes on its ColE1 plasmid.

ment 4 were resuspended in 0.02 M Tris-HCl (pH 7.5) containing 10% glycerol, 1 mM EDTA, and 1 mM dithiothreitol. Precipitates for use in experiments 1 and 2 were resuspended in 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 0.05 M Tris-Cl (pH 7.5), and 40% saturated ammonium sulfate; precipitates were collected by centrifugation and resuspended in 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol, and 0.02 M Tris-Cl (pH 7.5). All fractions within a given experiment were prepared at the same time in identical fashion.

Assays of dna gene products and DNA polymerase were performed as previously described (7, 8, 22).

RESULTS

The bank of 2,000 *E. coli* strains carrying random segments [average length 8×10^6 (16)] of the *E. coli* chromosome on hybrid ColE1 plasmids was screened for transfer of temperature resistance to dnaB ts, dnaC ts, dnaE ts, dnaG ts, or dnaZ ts recipients. One strain transferred dnaB⁺, two transferred dnaC⁺, one transferred dnaE⁺, none transferred dnaG⁺, and two transferred dnaZ⁺ (see Table 2). Single colonies which transferred temperature resistance were puri-

TABLE 2. *Transfer of dna+ markers from strains carrying ColE1 hybrids to dna ts mutants* [a]

		Recipient				
	Strain:	475	PC221	E486	BT308 [c]	AX727
Donor Strain	dna ts:	B	C	E	G	Z
MV12/28		+	-	-	-	-
MV12/29		-	+	-	-	-
MV12/20		-	+	-	-	-
MV12/26		-	-	+ [b]	-	-
MV12/27		-	-	-	-	+
MV12/16		-	-	-	-	+

[a] Ability to donate dna+ markers was judged by appearance of thermoresistant recipients after matings with donors (see "Materials and Methods").

[b] MV12/26 also transferred thermoresistance to E293, another dnaE ts mutant.

[c] The same results were obtained with NY731, a different dnaG ts mutant.

fied from each donor patch, in some cases by selecting for colicin E1-resistance. Some patches contained a mixture of colicin-sensitive nontransferring and colicin-resistant transferring cells. Each patch transferred temperature resistance to only one of the five dna ts mutants tested (Table 2). This was expected since the dna genes are widely scattered on the *E. coli* map (17) and each ColE1 hybrid carries only a small part of the host genome. However, specific donation of thermoresistance to a dna ts mutant is not proof that the dna+ allele has been donated. Further evidence for this can be obtained by examining donating strains for overproduction of the specific dna gene product.

Partially purified fractions (prepared as described in "Materials and Methods") of strains donating thermoresistance to specific dna ts mutants were assayed for dnaB, dnaC(D), and dnaZ gene products by *in vitro* complementation assays dependent on $\phi X174$ DNA and for DNA polymerase III with DNase-treated salmon sperm DNA as primer-template (Table 3). Fractions of strains correcting dnaB ts, dnaC ts, or dnaZ ts showed elevated levels (3- to 12-fold) only of the

TABLE 3. *Elevated levels of dna gene products in extracts of strains carrying ColE1 hybrid plasmids*

Strain; ColE1 Plasmid [a]	Activity Assayed (U/mg) [b]			
	dnaB	dnaC	DNA Polymerase III	dnaZ
Experiment 1: 0-40% AS [c]				
MV12/15; ColE1	8.3	-	-	44
MV12/16; ColE1-dnaZ$^+$	4.4	-	-	84
MV12/27; ColE1-dnaZ$^+$	4.9	-	-	130
Experiment 2: 0-40% AS				
MV12/26; ColE1-dnaE$^+$	-	-	200	19
MV12/27; ColE1-dnaZ$^+$	-	-	370	220
Experiment 3: 0-45% AS				
MV12/28; ColE1-dnaB$^+$	4.4	0.51	-	3.3
MV12/27; ColE1-dnaZ$^+$	0.9	0.36	-	11
Experiment 4: 0-45% AS				
MV12/29; ColE1-dnaC$^+$	-	1.4	-	1.1
MV12/27; ColE1-dnaZ$^+$	-	0.16	-	6.7
Experiment 5: 0-45% AS				
MV12/28; ColE1-dnaB$^+$	9.0	-	70	-
MV12/26; ColE1-dnaE$^+$	1.8	-	88	-
Experiment 6: 0-45% AS				
MV12/26; ColE1-dnaE$^+$	-	0.20	110	1.9
MV12/29; ColE1-dnaC$^+$	-	2.20	100	2.1
Experiment 7: 0-50% AS				
MV12/28; ColE1-dnaB$^+$	6.9	0.22	-	1.5
MV12/29; ColE1-dnaC$^+$	1.8	2.6	-	2.3
MV12/27; ColE1-dnaZ$^+$	0.9	0.32	-	4.9

[a] The dna gene indicates that whose ts phenotype is corrected by the particular ColE1 hybrid plasmid (see Table 2).

[b] One unit is the amount which stimulates the incorporation of 1 nmole of dTMP in 20 minutes at 30° under the conditions of the assays.

[c] Ammonium sulfate (AS) fractions were prepared as described in "Materials and Methods."

product of the dna gene whose defect was corrected. Comparisons of specific activities between different experiments in Table 3 are not possible because of the different methods of fractionation used in different experiments.

Calculations of total activity recovered per gram of cells indicated similarly elevated levels (3- to 10-fold) of dnaB, dnaC(D), and dnaZ gene products in extracts of the strains carrying the appropriate ColE1 hybrid plasmids. Complementation assays of mixtures of fractions prepared from the various strains gave additive results, indicating that neither activators nor inhibitors could account for the observed differences.

Protein fractions of the strain donating dnaE$^+$ did not have significantly elevated DNA polymerase III levels† when compared with fractions of strains donating dnaZ$^+$, dnaB$^+$, or dnaC$^+$. Thermoresistant DNA polymerase III activity was detected in extracts of *E. coli* E486 dnaE ts carrying the ColE1 plasmid which rendered this strain temperature-resistant for growth; no DNA polymerase III activity was detected in extracts of E486 dnaE ts.

DISCUSSION

The strains described here will be useful in the purification of dnaB, dnaC(D), and dnaZ gene products because each is overproduced 3- to 10-fold. Since overproduction occurs in exponentially growing cells and does not require any special maneuvers, it will be possible to use this approach in large-scale fermentations.

There are several possible explanations why elevated levels of DNA polymerase III were not detected in the strain transferring temperature-resistance to dnaE ts mutants. (a) The dnaE gene product may be only one of the subunits of DNA polymerase III [see (12)], and its overproduction would not necessarily yield more polymerase unless the other subunit were also overproduced. (b) The particular hybrid plasmid used may, for some reason, be present at a low copy number per genome. (c) Assay of DNA polymerase III in the relatively crude fractions examined is an unreliable indicator of actual polymerase

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† The preliminary fractionation removed most of the DNA polymerase I, since over 90% of the DNA polymerase activity in these fractions was sensitive to N-ethylmaleimide. The polymerase activity was inhibited about 90% by the addition of 0.1 M KCl, indicating that DNA polymerase II was not a substantial portion of the total polymerase activity in these fractions (23).

content. (d) Overproduction of DNA polymerase III may be prevented by some regulatory mechanism. In this regard a specialized transducing λ phage carrying the dnaE gene also failed to overproduce DNA polymerase III on induction (24).

Our inability to find a hybrid plasmid carrying the dnaG$^+$ gene might be due to chance failure of this part of the *E. coli* genome to be represented in the collection or inherent instability of plasmids carrying the dnaG region.

These strains, each carrying one of the dna genes, should also be useful in attempts to identify new dna genes; they could be used like small F' factors to rapidly map new dna ts mutants.

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