JOURNAL OF BACTERIOLOGY, Apr. 1984, p. 216–221
0021-9193/84/040216-06$02.00/0

Vol. 158, No. 1

Map Location of the *pcbA* Mutation and Physiology of the Mutant

SHARON K. BRYAN AND ROBB E. MOSES*

Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Received 6 September 1983/Accepted 5 December 1983

Many temperature-resistant revertants of a *polA1 polB polCts* (HS432) strain are PolI$^+$ (by either suppression of the *polA1* amber allele or intragenic reversion) but remain *polCts* (contain a temperature-sensitive DNA polymerase III). It appears that DNA replication in such temperature-resistant revertants depends on an extragenic mutation, *pcbA*, already present in the parent strain and not linked to any of the DNA polymerase loci. This allele allows DNA replication dependent on DNA polymerase I and bypasses a temperature-sensitive DNA polymerase III (*polC* bypass), so that reversion to PolI$^+$ makes the strain temperature resistant. This pathway of DNA replication also supports phage and plasmid DNA replication. At restrictive temperature, these mutants display a normal response to UV irradiation but show increased sensitivity to the alkylating agent methyl methanesulfonate. We have located *pcbA* linked to *dnaA*.

DNA replication in *Escherichia coli* requires a number of enzymatic functions (10, 17). Among these are the synthesis activity of DNA polymerase III (7) and the 5′→3′ exonucleolytic activity of DNA polymerase I (9), although the synthetic activity of this enzyme is not required at normal levels (5). It is not clear why DNA polymerase III is specifically required for DNA replication. The enzyme in holoenzyme configuration has a high processivity (6), and it can interact with several proteins. However, DNA polymerase I has similar enzymatic functions and can utilize ribo- or deoxyribonucleotide primers (10). We might expect that DNA polymerase III has some unusual facility in a step, perhaps repeated frequently, making it an essential element of DNA replication.

Numerous *polA*$^+$ *polCts* strains are temperature sensitive. We have isolated temperature-resistant revertants of a particular *polA1 polCts* strain (HS432) in which the temperature resistance phenotype is dependent on the PolI$^+$ phenotype (10, 16). Our evidence is that the strain already contained an extragenic suppressor of the *polCts* temperature-sensitive phenotype, *pcbA*, dependent on DNA polymerase I activity. Thus in strain HS432, introduction of a PolI$^+$ phenotype produces a temperature-resistant phenotype. The PolI$^+$ phenotype may result from suppression of the *polA1* nonsense mutation or introduction of a wild-type *polA* allele; it makes no difference, either one produces a temperature-resistant phenotype. Subsequent introduction of a temperature-sensitive DNA polymerase I into the temperature-resistant revertants made DNA synthesis temperature sensitive, indicating a strict requirement for DNA polymerase I in replication at restrictive temperature in the temperature-resistant revertants. This suggests that DNA polymerase I can interact with the product of the *pcbA* gene to permit replication.

The phenotypic suppression of temperature-sensitive DNA synthesis by DNA polymerase I extends to phage and plasmid DNA replication in these strains. DNA repair at 43°C after exposure to UV radiation is normal in *pcbA* strains. However, there is increased sensitivity to the alkylating agent, methyl methanesulfonate (MMS), suggesting a role for DNA polymerase III in repair of damage by that agent.

We show here that *pcbA* exists as a transducible genetic element, suppressing the temperature sensitivity phenotype of *polCts* strains when DNA polymerase I activity is present. The *pcbA* mutation maps in the region of *dnaA*.

MATERIALS AND METHODS

Bacterial strains. The strains used are listed in Table 1.

P1 transduction. P1 transduction was done as we previously described (16).

Culture methods. Cells were grown on L broth. For phage lambda, lambda Y medium was used. Selection for auxotrophic markers was done on M9 medium. For MMS testing, a 6-mm circle of Whatman 3MM filter paper was saturated with 10 μl of a 1:10 dilution of stock MMS and placed on a soft-agar overlay of the strain. After overnight incubation, the zone of clearing was measured (14). For UV-sensitivity testing, mid-log cells were diluted to 1×10^8 cells per ml in M9 salts, irradiated for various times, and spread on L broth plates. After growth, colonies were counted. Alternatively, a streak of 8×10^8 cells per ml was exposed to various doses of UV.

Transformation. Transformation of bacteria by plasmid DNA was performed by the method of Morrison (13). Selection was on either tetracycline (12 μg/ml) or ampicillin plates (50 μg/ml).

Curing of F factor. Cells were cured on acridine orange by the method of Beutin and Achtman (1). Curing was verified by fd or MS2 phage testing.

Thymidine incorporation. The 50-ml cultures in L broth contained 0.25 mCi of thymidine. At indicated intervals, 0.1-ml portions were withdrawn, precipitated with 10% trichloroacetic acid, and washed over Whatman GF/C filters, and retained radioactivity was determined after drying.

Materials. Growth media were purchased from Difco Laboratories. [^{3}H]thymidine was purchased from Schwarz/Mann.

Phage testing. Titers for each phage were determined on permissive hosts. Infection was done in the appropriate medium, and plaques were determined by soft-agar overlay. Between 10^2 and 10^4 phage infectious units were added to 10^9 cells to be tested. This culture was then assayed for plaques by soft-agar overlay at 32 and 43°C. Ratios were determined for Table 2. Resulting titers were frequently less than on the optimal permissive host.

For selection of λ lysogens, the phage stock was spotted on a soft-agar overlay of the strain to be tested. Lysogens were picked from the center of the plaque and tested for

* Corresponding author.

TABLE 1. Bacterial strains used

Strain	Relevant genotype (phenotype)	Source
HS432	*polA1 polB100 polC1026 pcbA* (temperature sensitive)	H. Shizuya
W3110	Wild type	J. Cairns
E486	*polA*$^+$ *polC486* (temperature sensitive)	C. McHenry
E511	*polA*$^+$ *polC511* (temperature sensitive)	Y. Hirota
P3478	*polA1*	J. Cairns
N4177	*gyrBts* (temperature sensitive)	M. Gellert
E508	*polA*$^+$ *dnaA508* (temperature sensitive)	J. Wechsler
CRT46	*polA*$^+$ *dnaA46* (temperature sensitive)	Y. Hirota
HC194	*dnaNts* (temperature sensitive)	C. McHenry
JW355	Tn*10* linked to *dnaA*$^+$	B. Bachmann
JC9239	*recF143*	J. Clark
CSM61	*polA1 polB100 polC1026 pcbA sup* (PolI$^+$) (temperature resistant)	This laboratory
SCM6	*polA1 polB100 polC1026 pcbA sup* (PolI$^+$)	This laboratory
CSM14	*polA*$^+$ *polB100 polC1026 pcbA* (temperature resistant)	This laboratory
HM1	HS432 *polC*$^+$ (temperature resistant)	This laboratory
HM16	HS432 *polA*$^+$ (temperature resistant)	This laboratory
HM6	CSM61 F$^-$	This laboratory
RM734	CSM61 F$^-$	This laboratory
HM29	HS432 F$^-$	This laboratory
ES16	E486 *pcbA*$^+$ linked to Tn*10* from 61P-14 (temperature sensitive)	This laboratory
ER11	E486 *pcbA* linked to Tn*10* from 61P-14 (temperature resistant)	This laboratory
CRT4638	*polA1 dnaAts* (temperature sensitive)	Y. Hirota
CRT266	*dnaBts* (temperature sensitive)	Y. Hirota
AX727	*dnaZts* (temperature sensitive)	J. Walker
61P-13	E511 with Tn*10* linked to *pcbA* (temperature resistant)	This laboratory
61P-14	E511 with Tn*10* linked to *pcbA* (temperature resistant)	This laboratory
CM1062	*metE polA*$^+$	W. Kelley
Gllal	*ilv metB ampA1*	B. Bachmann
AB2569	*pro his argHI metA28*	B. Bachmann
PC2	*dnaCts* (temperature sensitive)	Y. Hirota

immunity. The lysogens were then tested for the temperature resistance phenotype.

RESULTS

Growth of *polCts* cells at restrictive temperature. We have reported that derivatives of strain HS432 (*polA1 polB100 polCts pcbA*) containing spontaneously arising suppressors of the amber *polA1* mutation can grow at 43°C, even though they still contain a temperature-sensitive DNA polymerase III (14, 16). Whether the strain becomes PolI$^+$ by a naturally arising suppressor, an introduced amber suppressor, or a mutation to *polA*$^+$ does not matter. Replication is dependent on the presence of an active DNA polymerase I and the *pcbA* allele. Figure 1 contains typical data from such strains. Comparison is made with a *polC*$^+$ derivative of strain HS432 (HM1) and the parent strain. As expected, a *polC*$^+$ gene converts strain HS432 to a temperature-resistant phenotype. Strain CSM61 contains a naturally arising suppressor of the *polA1* nonsense allele.

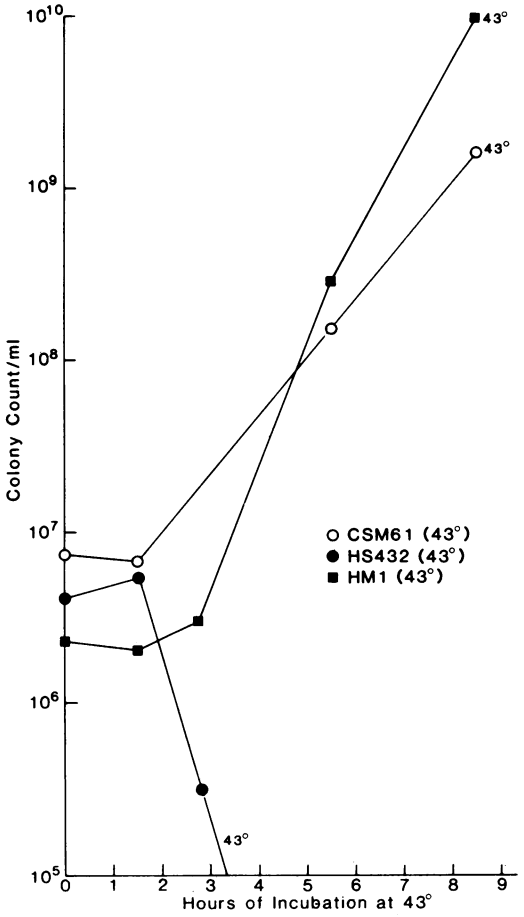

FIG. 1. Growth of *pcbA* strains. Colony counts of liquid cultures growing in L broth. The cultures were shifted from 32 to 43°C at time zero.

TABLE 2. Phage growth on *pcbA* strains

Strain	Growth of phage[a]:					
	λγ5	G4	φX	λ	T7[b]	M13
W3110	1.3	N.G.	N.G.	1.7	1.07	N.G.
HS432	N.G.	<0.001	<0.001	<0.001		<0.001
HM29 (HS432 F$^-$)					<0.001	
CSM61	0.96	<0.001	1.4	1.4		0.95
HM6 (CSM61F$^-$)					1.3	

[a] N.G., No growth of phage at either temperature; numbers are titer at 43°C divided by titer at 32°C.

[b] T7 only grows on F$^-$.

[^{3}H]thymidine incorporation is shown in Fig. 2. Incorporation in strains CSM61 and HM1 appears normal compared with a wild-type strain (not shown). The *pcbA* mutation suppresses the temperature sensitivity phenotype of three *polCts* alleles [*polC1026*(Ts), *polC486*(Ts), and *polC511*(Ts)] with equal efficiency, and the cells containing it plate with equal efficiency at 32 and 43°C for all three alleles.

Growth of phage and plasmids at restrictive temperature. Since our data support the argument that DNA replication is supported in some key step(s) by DNA polymerase I in *pcbA* mutants, we were interested in whether phage and plasmid replication could also be supported at restrictive temperatures. Phage can replicate at 43°C in strain CSM61, a spontaneous PolI$^+$ *polCts* temperature-resistant revertant of strain HS432 (due to a suppressor mutation). Phage G4 is only poorly supported, but in view of the normal phage M13 and φX174 replication, that is possibly a strain-specific

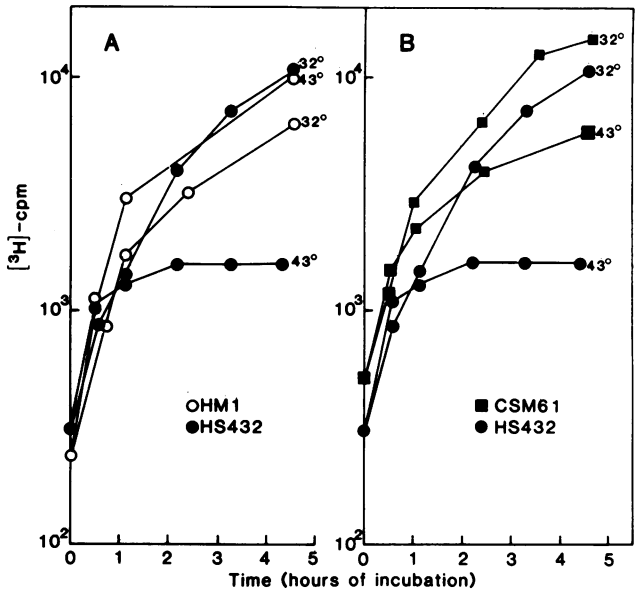

FIG. 2. Incorporation of [^{3}H]thymidine. Incorporation was done as described in the text.

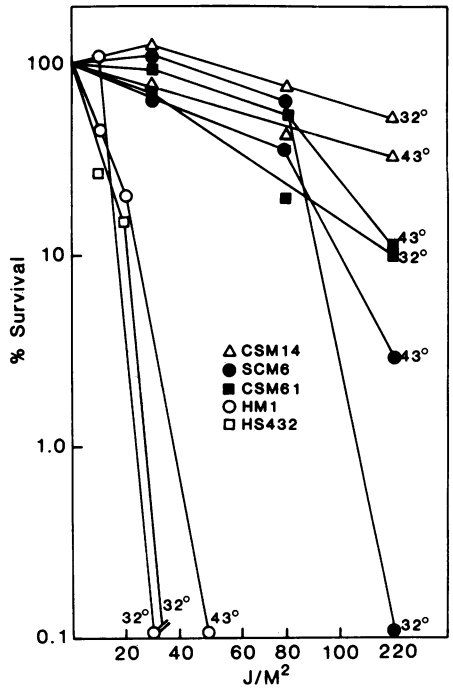

FIG. 3. UV survival of *pcbA* strains. Cells were grown and irradiated as described in the text.

function. The data are reported as the ratio of titer at the two temperatures to normalize for efficiency of infection. A value of <0.001 indicates phage growth at 32°C, but 10^{-3} or less growth at 43°C. In the case of strains HS432 and HM29, this reflects a failure of the cells to grow at 43°C. Our results demonstrate that the DNA polymerase I-dependent pathway can support phage replication. The failure of a λγ mutant to grow on strain HS432 even at permissive temperature is a reflection of a requirement for DNA polymerase I. Growth of these λ on the temperature-resistant revertant strain CSM61 is verification of a PolI$^+$ phenotype due to suppression of the *polA1* allele.

Table 3 contains information derived from growth of plasmid pBR322 on strain CSM61. This plasmid requires both DNA polymerase I and III for replication (3). Transformed colonies were picked at 32°C on either ampicillin or tetracycline plates. They were then restreaked and grown at 43°C with or without drug. As evidenced by drug resistance, pBR322 replicates in *pcbA* cells grown at 43°C. If the original selection was at 43°C, transformation efficiency was the same as that shown for 32°C. Growth of transformants at 43°C without drug selection maintained the plasmid; that is, the transformants remained drug resistant on subsequent plating, suggesting stable plasmid retention. We conclude that the DNA polymerase I can serve requirements for plasmid and DNA replication under conditions in which DNA polymerase III is inactive. Microcolony extracts and analysis of plasmid DNA by agarose gel electrophoresis showed similar amounts of plasmid DNA in strain CSM61 grown at 32 and 43°C. The amount was similar to that seen in strain W3110. This indicates that drug resistance was not due to a single-copy gene integrated into the chromosome and that the plasmid replicated extrachromosomally.

TABLE 3. Replication of plasmid pBR322 strain CSM61

Condition	No. of colonies (%)
32°C plus ampicillin	75[a]
43°C	75 (100)
43°C plus ampicillin	75 (100)
32°C plus tetracycline	56
43°C plus tetracycline	56 (100)

[a] Independently selected transformed colonies were picked and streaked for growth at 43°C.

DNA repair. There is suggestive evidence that DNA polymerase III serves an ancillary role in DNA repair in *E. coli*. The *pcbA* temperature-resistant revertants described offer an opportunity to observe DNA repair under conditions where DNA polymerase III is inactive, i.e., at 43°C. Sensitivity to UV is normal in *pcbA polC*(Ts) mutants at 43°C, in which DNA polymerase I should be the only functional DNA polymerase for synthesis (Fig. 3). Wild-type cells (not shown) yield data similar to those for strain CSM14. This suggests little if any role for DNA polymerase III in cell survival, although data on the rate of DNA repair were not obtained. A strain with a naturally occurring amber suppressor (CSM61) showed less survival than did *polA*$^+$ strains when streaked on solid medium and irradiated, suggesting that lower levels of DNA polymerase I activity, as noted in extracts (14), result in less DNA repair. Resistance to MMS at 32°C correlates with the presence of a PolI$^+$ phenotype, but sensitivity to MMS is increased at 43°C (Fig. 4). This is surprising, since sensitivity to MMS is a hallmark of the presence of DNA polymerase I, as noted with strain P3478 (8). Since there is no indication that the *polA1* allele is temperature sensitive, this seems to indicate a role for DNA polymerase III in survival after MMS exposure.

***pcbA* is not on an F′.** The strain HS432 is F$^+$. Since it is possible that phenotypic suppression of a *polCts* mutant might arise from an episomal gene, we cured the temperature-resistant revertants of the F′ plasmid and rechecked the phenotype. Strains (e.g., HM6 and RM734), independently cured and selected, were still temperature resistant (9/9). We conclude that the temperature sensitivity suppression is not due to a gene on the F′ plasmid.

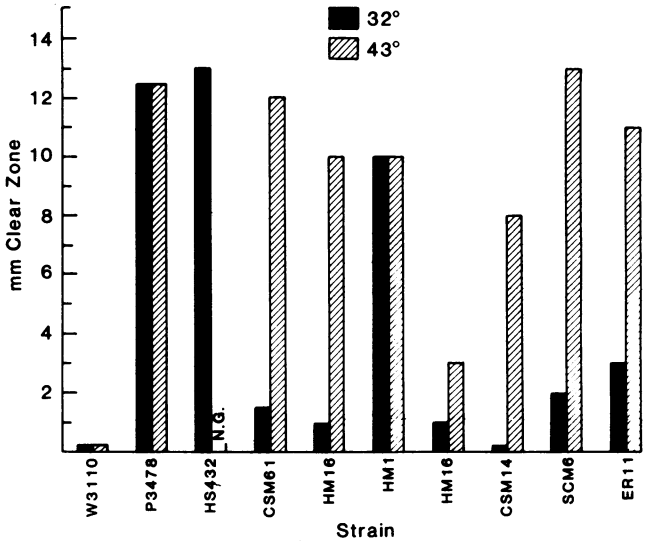

FIG. 4. MMS sensitivity of *pcbA* strains. Spot tests for MMS sensitivity were performed as described in the text. The sensitivity distance was measured from the edge of the paper disk to the edge of the zone of cell clearing.

***pcbA* is near *dnaA*.** We have previously reported that the *pcbA* mutation appeared to map in the region from 75 to 90 min by conjugation (15). Introduction of F' plasmids covering this region yielded largely temperature-resistant exconjugants, indicating that *pcbA* is dominant. We inserted Tn*10* randomly into strain CSM61 (*pcbA*) to generate Tn*10-pcbA* linkages, and after P1 phage transduction to an unrelated *polA*$^+$ *polCts* strain (E511), we selected for the temperature-resistant phenotype in Tet^r transductants (15). This gave us independent inserts of Tn*10* linked to *pcbA* (temperature-resistant phenotype in *polA*$^+$ *polCts* strains when Tet^r was introduced). Strains 61P-13 and 61P-14 are representative derivatives of E511. They were still *polCts*. The Tn*10-pcbA* linkage was verified by subsequent transduction of Tet^r from the 61P series to a *polA*$^+$ *polCts* strain with a resulting temperature resistance phenotype. The *pcbA* allele phenotypically suppressed several *polCts* alleles equally well.

By P1 transduction of *tet* from some of the 61P series to strains with convenient markers, we selected for Tet^r then checked for cotransduction of the second marker. After a number of trials, we found linkage to the *dnaA* region (Table 4). We could also establish linkage to other markers in the region. Of our independent Tn*10-pcbA* linkages, we have determined that eight (the number mapped) all map in the *dnaA* region with varying linkage to *dnaA*. Since we were using a drug resistance marker linked to *pcbA*, we could not establish definitive gene order. These results indicate that *pcbA* is a transducible allele in the region of *dnaA*.

In mapping the location of *pcbA*, we obtained data showing nonlinkage to numerous other markers (Table 4). Among these were a number of *dnats* mutants. An important conclusion can be drawn from these results: DNA replication occurring at restrictive temperature in *pcbA polCts* revertants (dependent on DNA polymerase I) requires several *dna* genes. That is, the *pcb* pathway of DNA replication requires many, if not all, of the genes normally required in DNA replication.

Tn*10-pcbA*$^+$ transduction. We confirmed that *pcbA* is in the *dnaA* region by using temperature-sensitive derivatives resulting from the above Tn*10* transduction to *polA*$^+$ *polC*(Ts) cells. Some cells received the Tet^r marker but remained temperature-sensitive (due to nontransduction of *pcbA* from the 61P series) and therefore were still *pcbA*$^+$. ES16 is such a strain. If the *tet* gene recombines legitimately, then it should be linked to *pcbA*$^+$. On subsequent transduction of Tet^r from strain ES16, we obtained some temperature-sensitive cells in PolI$^+$ *pcbA polCts* temperature-resistant strains (Table 5). This is substantiation that Tn*10* is linked to the *pcbA* allele in our insertions. Transduction of this insertion to *dnaAts* strains also resulted in complementation of the *dnaAts* marker showing that the *dnaA*$^+$ marker from strain ES16 could be recovered linked to Tet^r. There

TABLE 4. Transduction linkage of *pcbA*

Recipient strain	*tet* donor	% Linkage of *tet* to recipient marker[a]
E511 *polCts*	61P-13	30[b]
	61P-14	97
E486 *polCts*	61P-13	75
	61P-14	90
CRT4638 *polA1 dnaAts*	61P-14	20
CRT46 *dnaAts*	61P-13	40
	61P-14	20
HC194 *dnaNts*	61P-13	11
	61P-14	3
N4177 *gryBts*	61P-13	50
	61P-14	25
JC9239 *recF*	61P-13	14
CRT266 *dnaBts*	61P-13	None
AX727 *dnaZts*	61P-13	None
CM1062 *metE*	61P-14	None
Glla1 *amp*	61P-14	None
metB	61P-14	None
AB2569 *metA*	61P-14	None
argH	61P-14	None
PC2 *dnaCts*	61P-14	None

[a] More than 70 transductants were screened in all tests.
[b] For strains E511 and E486 the percentage is not linkage but conversion to temperature resistance.

TABLE 5. Transduction of *pcbA*

Recipient strain	No. of transductants screened	Donor	% Cotransduction[a]
E486	129	JW355 (Tn*10* linked to *dnaA*$^+$)	0
	34	ES16 (Tn*10* linked to *pcbA*$^+$)	0
CRT46	160	JW355	93
	97	ES16	89
CSM61	55	JW355	43
	55	ES16	20

[a] Cotransduction is defined as reversing the phenotype from temperature sensitivity to temperature resistance or vice versa.

was no conversion to a temperative-resistant phenotype in strain E486 (*polCts*), demonstrating that strain ES16 contained *pcbA*$^+$.

This trial also demonstrates the important point that temperature-resistant revertants such as strain CSM61 still contain *polC*(Ts), since temperature-sensitive transductants were recovered, although the Tn*10*::*pcbA* or Tn*10*::*dnaA* linkage was transferred from a strain with no temperature-sensitive gene in the Tn*10* region. Also this trial indicates that transduction of the *dnaA* region in general does not cause phenotypic reversal of *polCts* strains (JW355 or E486). It appears the Tn*10*::*pcbA* linkage is stable and can be moved serially.

The linkage to *dnaA* was verified with two separate *dnaAts* mutants, strains E508 and CRT46. Although the above data do not argue whether *pcbA* is an allele of *dnaA* or is another gene, it is worth noting that the insertion of the Tn*10-pcbA* linked to *dnaA*$^+$ suppresses the *dnaAts* phenotype. A potential problem not found was that *pcbA* could represent an allele of a temperature-sensitive gene which might not reverse the temperature-sensitive phenotype of that gene, leading to failure of linkage and failure to map.

It is also important to remember that the *pcbA* allele phenotypically suppressed multiple *polC*(Ts) alleles. This seems to rule out strain-specific gene interactions.

Verification of *pcbA-dnaA* linkage. In the preceding section, we presented evidence that the Tn*10-pcbA* linkages we established could cotransduce with *dnaA*. These results show that the Tn*10* linked to *pcbA* can be passed with stable linkage to *dnaA*.

We used another approach to verify our conclusion that *pcbA* lies in the region of *dnaA*. Strain JW355 contains Tn*10* linked to *dnaA* at over 90%. By P1 transduction, we moved the Tn*10* from strain JW355 into CSM61 (PolI$^+$ *polC*(Ts), *pcbA*, temperature resistant). If this Tn*10-dnaA* linkage moved a wild-type allele of *pbA* into CSM61, the phenotype of the strain should change to temperature sensitive. We found good linkage (Table 5). The linkage of Tn*10* to *dnaA* was greater than to *pcbA* (reversal of the temperature resistance phenotype in strain CSM61). This suggests that *pcbA* is not an allele of *dnaA* but does not make a conclusive argument.

Failure to complement *pcbA* with plasmid and lambda. Our trials also suggested that *pcbA* is dominant. We have used a specialized λ carrying the *dnaA* region (12) and plasmid pPB2 carrying the *dnaA* region (4) to transduce or transform *pcbA* temperature-resistant revertants (Tables 6 and 7). In no case did we recover temperature-sensitive cells. The result with the plasmid could be due to dominance of *pcbA*, *cis* action of *pcbA*, or lack of *pcbA*$^+$ on the plasmid. Our results show that λ*i*^{21}-*dnaA-2* also carries *gyrB*. Since this λ carries the region to which we have mapped *pcbA*, our result is compatible with *pcbA* being dominant, since λ lysogens do not revert to the temperature sensitivity phenotype. Of course this conclusion assumes that *pcbA*$^+$ is carried by the phage.

TABLE 6. λ *dnaA* Complementation

Strain	No. of λ *i*^{21}[a] lysogen	% Complementation[b]
CRT4638 *dnaAts*	11	100
HC194 *dnaNts*	10	100
N4177 *gyrBts*	6	100
CSM61 *pcbA polCts* PolI$^+$	5	0
HM6 *pcbA polCts* PolI$^+$	16	0

[a] λ *i*^{21} is λ i^{21}-*dnaA*-2 (12).
[b] Complementation is defined as reversing the phenotype from temperature sensitivity to temperature resistance or vice versa.

TABLE 7. Plasmid complementation

Strain	No. of colonies at 32°C	Growth at 43°C	% Complementation[a]
HC194 *dnaNts*	210	184	88
E508 *dnaAts*	126	112	89
E486 *polCts*	210	0	0
CSM61 *pcbA* (temperature resistant)	209	209	0

[a] Complementation is defined as reversing the phenotype from temperature sensitivity to temperature resistance or vice versa, by use of plasmid pPB2.

DISCUSSION

We observed, that most temperature-resistant revertants of a *polA1 polB100 polCts* strain (HS432) are phenotypically PolI$^+$. This occurs either by suppression of the amber *polA1* allele or by intragenic reversion to *polA*$^+$. These revertants remain *polCts*, as measured by biochemical and genetic means. The most economical hypothesis would be that the starting strain contained a mutation, *pcbA* (*polC* bypass), allowing DNA replication at restrictive temperature, dependent on DNA polymerase I synthetic activity, even though DNA polymerase III is temperature sensitive (Fig. 5). Representative percentages of each class are shown in our model. Although some temperature-resistant revertants of strain HS432 are *polC*$^+$ (and remain *polA1*) as expected, most are not, which is an unexpected result.

We have tested this hypothesis and found that we can define and map a transducible locus which confers a temperature resistance phenotype on *polC*(Ts) mutants if DNA polymerase I is present. The mutation is located close to *dnaA*. We conclude that there is evidence for a bypass pathway of DNA replication in which the synthetic activity of DNA polymerase I can fulfill a requirement for DNA polymerase III. DNA polymerase I is normally required for DNA replication, as shown by Konrad and Lehman (9). We have established that restoration of the polymerization activity of DNA polymerase I is required in *pcbA* mutants for a temperature-resistant phenotype. This suggests that DNA

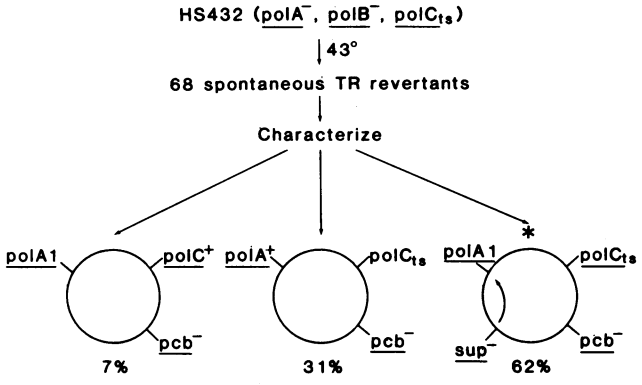

FIG. 5. Classes of TR revertants.

polymerase III is required for some specific step, such as primer utilization, and that modification of an accessory protein might allow DNA polymerase I to fulfill this role. The remaining proteins of host DNA replication are still required, showing conservation of function.

There is precedent for our conclusion. Blinkowa and Walker (2) reported extragenic phenotypic suppression of *dnaZts* alleles by mutations in the *dnaA* locus. Kuwabara and Uchida (11) described a suppressor of *dnaEts sueA*, mapping in the same region as *pcbA*. However, these authors have not defined dependence of the suppression on DNA polymerase I. Also, *sueA* seems to phenotypically suppress different *polCts* alleles with markedly varying efficiency. This is in contrast to *pcbA*, which suppresses several *polC*(Ts) alleles equally well. Burgers et al. (4) have shown that *dnaN*, mapping close to *dnaA*, produces the β subunit of holoenzyme DNA polymerase III.

Several important points bear emphasis: first, our results in no way rule out a requirement for nonsynthetic functions for DNA polymerase III in DNA replication, for example, a structural role to appose components in the replicon; and, second, *pcbA* is probably only a prototype. We were fortunate to use a strain with *pcbA* in the background. However, beginning with a *polCts pcbA*$^+$ strain, it is quite possible that additional *pcb* alleles will be found.

ACKNOWLEDGMENTS

This work was supported by grants from the Public Health Service (GM 19122) and the Robert A. Welch Foundation (Q-543).

We thank Thomas Stark for expert assistance.

LITERATURE CITED

1. **Beutin, L., and M. Achtman.** 1979. Two *Escherichia coli* chromosomal cistrons, *sfrA* and *sfrB*, which are needed for expression of F factor *tra* functions. J. Bacteriol. **139**:730–737.
2. **Blinkowa, A., and J. Walker.** 1983. Interactions of DNA replication factors in vivo as detected by introduction of suppressor alleles of *dnaA* into other temperature-sensitive *dna* mutants. J. Bacteriol. **153**:535–538.
3. **Bolivar, F., R. Rodriguez, P. Greene, M. Betlach, H. Heyneker, and H. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
4. **Burgers, P., A. Kornberg, and Y. Sakakibara.** 1981. The *dnaN* gene codes for the β subunit of DNA polymerase III holoenzyme of *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:5391–5395.
5. **DeLucia, P., and J. Cairns.** 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1164–1166.
6. **Fay, P. J., K. Johanson, C. McHenry, and R. Bambara.** 1981. Size classes of products synthesized processively by DNA polymerase III and DNA polymerase III holoenzyme of *Escherichia coli* J. Biol. Chem. **256**:976–983.
7. **Gefter, M. L., Y. Hirota, T. Kornberg, J. A. Wechsler, and C. Barnoux.** 1971. Analysis of DNA polymerases II and III in mutants of *Escherichia coli* thermosensitive for DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **68**:3150–3153.
8. **Gross, J., and M. Gross.** 1969. Genetic analysis of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1166–1169.
9. **Konrad, B., and I. R. Lehman.** 1974. A conditional lethal mutant of *Escherichia coli* K12 defective in the 5′ → 3′ exonuclease associated with DNA polymerase I. Proc. Natl. Acad. Sci. U.S.A. **71**:2048–2051.
10. **Kornberg, A.** 1980. DNA replication, p. 347–414. Freeman Publishing Co., Ltd., San Francisco.
11. **Kuwabara, N., and H. Uchida.** 1981. Functional cooperation of the *dnaE* and *dnaN* gene products in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:5764–5767.
12. **Miki, T., M. Kimura, S. Hiraga, T. Nagata, and T. Yura.** 1979. Cloning and physical mapping of the *dnaA* region of the *Escherichia coli* chromosome. J. Bacteriol. **140**:817–824.
13. **Morrison, D. A.** 1977. Transformation in *Escherichia coli*: cryogenic preservation of competent cells. J. Bacteriol. **132**:349–351.
14. **Niwa, O., S. Bryan, and R. E. Moses.** 1979. Replication at restrictive temperatures in *Escherichia coli* containing a *polC*ts mutation. Proc. Natl. Acad. Sci. U.S.A. **76**:5572–5576.
15. **Niwa, O., S. Bryan, and R. E. Moses.** 1980. DNA polymerase I-dependent replication, p. 597–604. *In* B. Alberts (ed.), Mechanistic studies of DNA replication and genetic recombination. Academic Press, Inc., New York.
16. **Niwa, O., S. Bryan, and R. E. Moses.** 1981. Alternate pathways of DNA replication: DNA polymerase I-dependent replication. Proc. Natl. Acad. Sci. U.S.A. **78**:7024–7027.
17. **Nossal, N.** 1983. Prokaryotic DNA replication systems. Annu. Rev. Biochem. **52**:581–615.