NOTES

Overproduction of DnaE Protein (α Subunit of DNA Polymerase III) Restores Viability in a Conditionally Inviable Escherichia coli Strain Deficient in DNA Polymerase I

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A polA12 recA718 double mutant of Escherichia coli, in which DNA polymerase I is temperature sensitive, was unable to maintain normal DNA synthesis or to form colonies on rich media at 42°C. Overproduction of DnaE protein, the polymerizing α subunit of DNA polymerase III, restored bacterial DNA replication and cell viability, as well as the PolI-dependent replication of the plasmid carrying dnaE.

Although it has long been known that recA polA1 double mutants of Escherichia coli are inviable (3), the essential role of RecA protein when DNA polymerase I (PolI) is defective has never been identified. The RecA protein encoded by recA718 causes moderate UV sensitivity but is competent in recombination, in SOS induction, and in all known RecA-mediated proteolytic activities (10, 17). We found that recA718 polA1 mutants were conditionally inviable. They grew slowly on minimal medium but could not form colonies on rich media, exhibiting a phenotype similar to that of recA+ polA-deleted strains (4).

Because the recA718 polA1 mutant is easily overgrown in liquid culture by suppressors or revertants of the polA1 or recA718 allele, we introduced the temperature-sensitive polA12 allele (13) into recA718 strain SC18 (17). This double mutant (strain SC18-12) was viable and stable at 30°C but did not form colonies on rich media at 37°C or 42°C. In this report, we show that overproduction of DnaE protein, the polymerizing α subunit of DNA polymerase III (PolIII), restored viability to the double mutant at high temperatures.

Table 1 compares the growth of the double-mutant strain SC18-12 with that of each of the single-mutant strains and with that of a polA12 strain from which recA has been deleted. The polA12 recA718 strain differed from each of the single mutants at 42°C in its inability to form colonies on nutrient agar and in its extremely slow growth on minimal medium. Similar results were obtained at 37°C. The polA12 recA-deleted strain was unconditionally inviable at 42°C and grew very poorly at 30°C. Figure 1 shows that the rate of DNA synthesis on nutrient agar, which even at 30°C was slightly slower in the recA718 polA12 strain SC18-12 than in either single mutant, decreased steadily in the double mutant after 1 h at 42°C. The rates of DNA synthesis in both single mutants were indistinguishable from those in the recA+ polA1 strain SC18-RP under the same conditions (data not shown).

All of the activities of PolA12 are extremely thermolabile in vitro, and even at permissive temperatures the mutant enzyme performs nick translation inefficiently, apparently because of poor coordination between its polymerizing and 5'-3' exonuclease activities (7). The conditional viability of strains from which the polA gene has been deleted (4) indicates that the normal role of PolII in DNA replication (degradation of RNA primers and gap-filling to connect Okazaki fragments) can be subsumed in its total absence, presumably by PolIII with the help of some other proteins, including RecA. Assuming that PolIII also takes over at least part of the normal PolII role in polA12 mutants at high temperatures, the small number of PolIII holoenzyme molecules normally present per cell, 10 to 20 (6), may be inadequate for this additional role, at least when RecA718 replaces RecA+*. This could be especially true on rich media, where PolIII must function at multiple replication forks. To test this possibility, we asked whether overproduction of DnaE, the polymerizing α subunit of PolIII, could compensate for the PolA12 defect and restore viability to strain SC18-12 under otherwise nonpermissive conditions. This

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Table 1. Growth of various E. coli strains

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<th>Straina</th>
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<td>polA</td>
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<td>SC18-RP</td>
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<td>SCDR-12</td>
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* All other strains were constructed by P1 transduction from strain SC18 (17).
† Log cultures grown in nutrient broth at 30°C were diluted and streaked at high cell densities (ca. 10⁶ cells per streak) and were also plated at low cell densities (ca. 10⁵ cells per plate). The growth of streaks and individual colonies was scored as follows: ++++, full confluent growth of streak and colonies of normal size in 1 day; +++, thin but confluent streak, colonies smaller than normal in 1 day; +, streak not confluent, colonies extremely small in 1 day; −, no growth of streak or colonies (except revertants) in 4 days.
subunit is a nonprocessive polymerase, likely to function well in filling small gaps. In fact, amplification of DnaE enhances gap filling by PolIII in vitro (9).

Plasmid pdnaE-OP1-pJF118HE, supplied by C. S. McHenry, carries the dnaE gene under the control of the tac promoter. In the presence of 10^{-4} M isopropyl-β-D-thiogalactopyranoside (IPTG), an inducer for genes controlled by this promoter, DnaE is amplified from this plasmid to become 0.5 to 1% of the total soluble cell protein (12). Figure 2 shows that strain SC18-12, transformed with this plasmid, remained inviable on rich medium that did not contain IPTG at 42°C but was restored to full viability at this temperature when plated on the same medium containing 10^{-4} M IPTG. The same result was obtained at 37°C (data not shown). Half as much IPTG was still effective, but viability was not restored at lower concentrations of the inducer. Furthermore, the plasmid plus IPTG (but not the plasmid without IPTG) markedly sped the otherwise slow growth of this strain on minimal medium at elevated temperatures. Figure 3 shows that the rate of DNA synthesis at 42°C in the double mutant containing the plasmid decreased steadily after 60 min on nutrient agar in the absence of IPTG, but increased normally in the presence of IPTG. We conclude that overproducing the α subunit of PolIII restores DNA replication and cell viability in the double mutant SC18-12, probably by providing an efficient DNA polymerizing activity that replaces or supplements the defective activity of PolA12.

The plasmid vector used to amplify DnaE was derived from pBR322 and has a ColEl-type origin of replication (12). It therefore requires PolII activity for the initiation of DNA replication (15). Such plasmids do not replicate at high temperatures in polA12 strains (5). Our results imply that the plasmid must have replicated at 42°C in strain SC18-12 on rich medium containing IPTG, an implication confirmed by the ampicillin resistance of the colonies obtained under these conditions. We conclude that DnaE, when overproduced, replaces or supplements PolA12 in performing the function in plasmid replication that normally requires PolII. This function is thought to be the extension of RNA primers at ColEl-type origins (15).

Joyce and Grindley showed that F' plasmids carrying either the polymerizing fragment or the 5'-3' exo nuclease fragment of PolII can cure the conditional inviability of polA-deleted recA^{+} strains (4), indicating that improved efficiency either in erasing RNA primers or in connecting Okazaki fragments is sufficient to permit survival on rich medium in the complete absence of PolII when RecA^{+} is present. We found that either of these F' plasmids, provided by C. Joyce, also cured the conditional inviability of strain SC18-12, promoting full growth on nutrient agar at 42°C (data not shown).

In contrast to the result with the polA12 recA718 strain, the temperature sensitivity of the polA12 recA^{+}-deleted strain was not cured by the plasmid carrying dnaE. Because both recB (13) and uvrB (14) mutations are also lethal when PolII is deficient, we constructed recB polA12 and uvrB polA12 double mutants and found that their unconditional inviabilities at 42°C were not alleviated by this plasmid. These negative results could mean either that overproduction of

FIG. 1. Rates of DNA synthesis on nutrient agar. Log-phase bacteria grown at 30°C in nutrient broth were plated on nutrient agar and incubated at 30°C for 1 h. At time zero, half of the plates were transferred to 42°C (right half of figure) and the other half remained at 30°C (left half of figure). At the times indicated, bacteria at both temperatures were pulse-labeled for 2 min with [3H]thymidine while on the plates, as described elsewhere (8). ○, strain SC18 (recA718 polA^{+}); ●, strain SC18 (recA^{+} polA12); □, strain SC18-12 (recA718 polA12).

FIG. 2. Growth of strain SC18-12/pdnaE-OP1-pJF118HE on nutrient agar. Rotary streaks were incubated at 42°C without (left) or with (right) 10^{-6} M IPTG in the medium. A loopful taken from a cell suspension containing ca. 10^{9} cells per ml was drawn slowly across the radius as the plate spun on a turntable.
DnaE does not restore plasmid replication in these strains or that overproduction of DnaE supports plasmid replication but fails to restore cell viability in a polA12 strain totally lacking in RecA, RecB, or UvrB function. Another possibility is that neither plasmid replication nor cell viability can be restored in any of these double mutants. These uncertainties could be resolved by assessing plasmid replication in vitro instead of in vivo with cell extracts or by overproducing DnaE from a plasmid not dependent upon PolI for its replication.

Slow joining of nascent DNA fragments at the replication fork occurs in various polA mutants (6) and has been demonstrated with our strain, SC18-12 (16). Presumably, failure to rejoin these fragments within some critical time is the cause of inviability in the absence of RecA. A possible role for RecA is to perform recombinational repair of the persistent gaps on the lagging strand, perhaps by a strand switch and copy-choose mechanism, using the newly synthesized daughter of the leading strand as template. Such a mechanism has been proposed to account for the required RecA role in recovery of DNA replication after UV irradiation (1), a process in which RecA718 is conditionally deficient (18). RecA718 may perform this type of recombination less efficiently than RecA+. Recombinational postreplication repair, another gap-filling mechanism requiring RecA, occurs much more slowly in recA718 mutants than in recA+ strains (11).

Fijalkowska et al. (2) showed that two other recombination-proficient recA alleles, recA441 and recA730, cause conditional lethality in combination with polA12 but only under conditions of recA441- or recA730-mediated constitutive expression of the SOS response. They suggested that cells undergoing continuous SOS expression may have an increased need for PolI activity, a need not satisfied by PolA12 at high temperatures. Although RecA718 does not normally cause constitutive SOS expression unless it is amplified by a recA(Def) allele (10), it may do so in PolI-deficient cells. If so, this explanation could account, at least in part, for the phenotype of SC18-12.

Expression of mammalian DNA polymerase β in strain SC18-12, like that of E. coli DnaE protein, restores full viability to this double mutant (16). Strain SC18-12 could provide an assay for other DNA polymerase activities capable of replacing or supplementing E. coli PolI.

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REFERENCES