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New Gene in *Escherichia coli* K-12 (*drpA*): Does Its Product Play a Role in RNA Synthesis?

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The mutation *drpA1* defines a new gene in *Escherichia coli* K-12 that maps at about 5.2 min. This mutation was obtained after enriching a population of cells for temperature sensitive *dna* mutations with the [^{3}H]thymidine "suicide" technique followed by screening for mutants defective in transposon Tn*5* precise excision. When growing cells carrying the *drpA1* allele were shifted to the nonpermissive temperature, we showed that DNA, RNA, and protein syntheses shut off quickly, with the cessation of RNA synthesis occurring first. A recombinant plasmid between pBR322 and an *Hin*dIII fragment from wild-type *E. coli* restores the growth defect in *drpA1* mutants. Using transposon Tn*5* mutagenesis of this plasmid, we have been able to correlate the presence of a 68-kilodalton protein, as observed with the maxicell technique, with the ability of this plasmid to restore growth to *drpA1* mutants.

The use of *Escherichia coli* K-12 in the genetic analyses of biological processes allows the isolation of mutations in genes of already known function. Thus, it may be possible to infer mechanistic details from the simple knowledge of the map position of a mutation (10). We have been using this approach to study precise excision, one of the genetic rearrangements associated with bacterial transposons (17). Transposons frequently cause gene-inactivating insertion mutations; precise excision is the reversion of these mutations with the concomitant loss of the transposon. For example, the transposon Tn*5*, which encodes a kanamycin resistance gene, can insert into the *lacZ* gene to give a Lac$^-$ Kan^r strain. A precise excision event yields Lac$^+$ Kan^s revertants often enough to allow the isolation of strains with mutations affecting this process (8, 11, 12).

In this paper we present the characterization of a mutant obtained in the course of isolating mutants that were defective in both chromosome replication and precise excision. We searched for such a mutant in an attempt to obtain direct evidence for the postulate that precise excision may occur during DNA replication.

The first mutation characterized in this series, *drpA1*, maps to a previously undescribed gene and is highly pleiotropic, affecting the biosynthesis of a variety of macromolecules, as well as precise excision. In this paper we present the genetic analysis and characterization of *drpA1*.

MATERIALS AND METHODS

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

Genetic techniques. All genetic manipulations were as described by Miller (22). Rich medium is tryptone-yeast extract broth, and minimal medium is M9 supplemented with 0.5% glucose. The Casamino supplement is 0.5% decolorized, vitamin-free Casamino Acids (Difco Laboratories) (24).

Transduction for *metD*$^+$ was performed by transducing a *metD metB* strain to growth on minimal medium supplemented with D-methonine and scoring only those that then failed to grow on minimal medium.

Mutant isolation. The mutant isolation procedure was adapted from that of Fangman and Novick (7). After strain SY270 was mutagenized with ethyl methanesulfonate, the cells were grown to the log phase at 30°C in minimal medium. The culture temperature was shifted to 42°C, 10 μCi of [^{3}H]thymidine was added, and the cells were grown for 2 h. Cells were harvested and frozen at −20°C. After 6 weeks a 3-log decrease in titer had occurred, and the cells were thawed and allowed to grow to saturation at 30°C. Dilutions were plated on MacConkey lactose agar medium, and low-papillating mutants were isolated as described previously (11, 12).

Plasmid constructions. Plasmid DNA purifications, restriction enzyme digestions, and ligations were as described by Maniatis et al. (21). pKFL1 was isolated by inserting *Hin*dIII-digested chromosomal DNA (from SY203) into pBR322 and selecting for complementation of *drpA1* in SY356.1. The pKFL1-containing clone was recognized as a colony that grew faster at 30°C than did SY356.1 and grew normally at 42°C. pKFL2 was isolated after cutting pKFL1 with *Bam*HI, religating, and selecting for an Amp^r transformant of SY356.1 that grew faster than did SY356.1 at 30°C and again grew normally at 42°C.

Preparation of maxicells and polyacrylamide gel electrophoresis. Maxicells were produced from SY918 (*recA*) by the methods of Sancar et al. (27) and Isberg et al. (14). SY918 was transformed with each of the pKFL series of plasmids. Cells were grown to an absorbancy at 600 nm of 0.5 in minimal medium, irradiated with UV light, incubated in growth medium for 24 h in the presence of cycloserine, and labeled with 10 μCi of [^{35}S]methionine for 30 min. Cells were then harvested and prepared for 0.1% sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis.

Biosynthetic assays. (i) DNA synthesis (pulse-labeling). *thyA deo* strains, SY732 and SY733, which had been grown up overnight in minimal M9 glucose (0.2%) with thymine (10 μg/ml) and Casamino Acids (0.15%) were diluted to a Klett reading of 10 in the same medium. The cells were grown at 30°C until the cell density reached a Klett reading of 40. The temperature was then shifted to 38°C; at each designated time point, 200 μl of cells were drawn out and added to 100 μl of prewarmed minimal medium containing 10 μCi of [^{3}H]thymidine (New England Nuclear Corp.). After 1 min,

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TABLE 1. Bacterial strains

Strain	Relevant properties	Source
AB1157	F⁻ *thr-1 leu-6 proA-2 his-4 argE3 str-31 ara-14*	J. Clark
MC1000	F⁻ Δ(*ara-leu*) Δ*lac galU rpsL*	M. Casadaban
MC4100	F⁻ *ara* Δ*lac rpsL*	M. Casadaban
KS519	F⁻ *metB metD proA* Δ*lac rpsL*	B. Konrad
SY203	F⁻ Δ(*lac-pro*)XIII *argE*(Am) *nalA* Rif^r	Laboratory collection
SY250	F⁻ *lacPUV5 proC rpsL*	Laboratory collection
SY270	SY203 *thyA* Str^r/F′ *lacZ*::Tn*5 pro*	Laboratory collection
SY356.1	SY270 *drpA1*	Laboratory collection
SY657	AB1157 *proA2 zai*::Tn*10*	Laboratory collection
SY658	AB1157 Δ(*lac-pro*)XIII *zai*::Tn*10 drpA1*	Laboratory collection
SY732	SY658 *thyA deo drpA1 strA*	This study
SY733	SY658 *thyA deo* Ts$^+$ *strA*	This study
SY735	SY203 *thyA zae-13*::Tn*10 drpA1*/F′ *pro lac*::Tn*10*	This study
SY736	AB1157 *zae-13*::Tn*10 drpA1*	This study
SY736.1	AB1157 *zae-13*::Tn*10* Ts$^+$	This study
SY747	AB1157 Pro$^+$ *zae-13*::Tn*10 drpA1*	This study
SY748	AB1157 Pro$^+$ *zae-13*::Tn*10* Ts$^+$	This study
SY844.2	SY203 *leu*::Tn*5*/F′ *rep*(Ts) Tn*10 lac*	Laboratory collection
SY889	MC4100 *zae*::Tn*10*	This study
SY891	SY889/F′ Tn*10 rep-114*(Ts) *lac*	This study
SY893.2	Hfr: Lac$^+$ Tn*10*/*zae-13*::Tn*10*	This study
SY909	SY658 *drpA1 nalA*	This study
SY918	F⁻ Δ*lacZ*M445 Δ(*recA-srl*) *xyl mtl his argE*/F′ *lacI*^{Q} *lacZ*::Tn*5*	This study
SY929	AB1157 *metD zae-13*::Tn*10 drpA1*	This study

600 μl of cold 10% trichloroacetic acid (TCA) was added, and the precipitates were collected on Whatman GF/C filters (2.4 cm), washed, and counted.

(ii) DNA and protein synthesis (continuous labeling). SY732 and SY733 were grown as described above. At a Klett reading of 40, 3 ml of cells was added to 7.5 μCi ^{14}C-amino acids (mixture of labeled amino acids) and 4 μCi of [^{3}H]thymidine and grown at 38°C. At each time point, 200 μl of sample was added to 500 μl of cold 10% TCA. The precipitates were collected on filters as described above.

(iii) RNA synthesis (pulse-labeling). SY747 and SY748 were grown as above to a Klett reading of 40 at 30°C. At that time, the temperature was shifted to 38°C; at each time point, 200 μl of sample was added to 100 μl of medium containing 10 μCi of [^{3}H]uridine (New England Nuclear). After 1 min of labeling, 700 μl of cold 10% TCA was added, and the precipitate was collected.

DNA synthesis in toluenized cells in vitro. The DNA synthesis procedure of Moses and Richardson (23) was used. SY747 and SY748 were grown in 200 ml of rich broth at 30°C to an absorbancy at 600 nm of 0.5, and the cultures were divided in half; one half was grown at 40°C for 40 min, and the other half was grown at 30°C. The cells were harvested and suspended in 5 ml of 50 mM potassium phosphate (pH 7.4) buffer. Toluene (50 μl) was added, and the cells were gently shaken for 10 min at 30°C. The toluene-treated cells were washed once and suspended in 2 ml of the same phosphate buffer. Toluene-treated cells (0.3 ml) were added to 3 ml of a reaction mixture that contained 10 μCi of [α-^{32}P]dATP (Amersham Radiochemicals), 70 mM potassium phosphate buffer (pH 7.4), 13 mM $MgSO_4$, 1.3 mM ATP, and 33 μM dATP, dGTP, dTTP, and dCTP. A second reaction mixture without ATP was used as a control. The assay was incubated at 30°C; at each time point 0.3 ml was added to 0.7 ml of cold 10% TCA, and the TCA precipitates were collected and counted.

RESULTS

Isolation of the *drpA1* mutant. The *drpA* mutations were isolated in the course of a search for temperature-sensitive mutants that were defective in both precise excision at the permissive temperature of 30°C and nucleic acid synthesis at the nonpermissive temperature of 42°C. A population of bacteria that had been enriched by the [^{3}H]thymidine suicide technique for temperature-sensitive *dna* mutants (7) was screened for precise excision mutants. A *thyA lacZ*::Tn*5*-containing strain (SY270) was mutagenized with ethyl methanesulfonate and labeled at 42°C with [^{3}H]thymidine as described above. The mutagenized and labeled cells were harvested, frozen, and stored at −20°C for 3 weeks. Bacteria in which DNA is labeled with [^{3}H]thymidine are killed as the ^{3}H decays, whereas mutants that fail to incorporate the label at 42°C survive. Surviving cells were grown at 30°C and then plated on MacConkey lactose agar media. The Lac⁻ parental strain makes white colonies that give rise to numerous red Lac$^+$ papillae, which represent precise excision events of the Tn*5* in *lacZ*. We searched for clones that gave very few Lac$^+$ papillae. These were then screened for variants that were also temperature sensitive for growth. Those that failed to grow at 42°C were then tested for their precise excision frequency at 30°C by measuring the number of Lac$^+$ clones that arose from the total population. We found 16 temperature-sensitive mutants whose precise excision frequency was 5- to 15-fold lower than that of the ancestral strain, SY270. Most of the subsequent characterization was concentrated on one of these mutants, called *drpA1*. This particular strain was reduced fivefold for precise excision.

Mutational analysis shows that the temperature-sensitive and precise excision phenotypes of these mutants arise by a single mutational event. When strain SY356.1, which contains *drpA1*, is plated at 42°C, about one spontaneous revertant appears for every 2×10^7 to 5×10^7 cells plated. Of eight temperature-resistant revertants tested, we found that all gave the Lac$^+$ excision phenotype of the ancestral SY270, as measured by calculating the frequency from the number of Lac$^+$ clones that grew on minimal lactose medium (data not shown). The simplest explanation for this result is that a single mutational event produces both the temperature-sensitive and precise excision phenotypes. In addition, in the course of mapping *drpA1* (see below), we found that the precise excision phenotype, as determined by colony papillation, cotransduced 28 of 28 times with the temperature-sensitive phenotype.

Mapping *drpA1*. The *drpA1* mutation was mapped by first inserting a Tn*10* transposon into a site that was linked by P1 transduction to the temperature-sensitive allele in SY356.1 by the procedure of Kleckner et al. (18). An approximate map position for *drpA1* was obtained when we found that this Tn*10* was closely linked to Δ(*lac-pro*)$_{XIII}$. In a series of two-factor P1 crosses testing linkage of the *ts* allele in *drpA1* and the Tn*10* insertion, which was consequently designated *zai*::Tn*10*, to *metD*, *proC*, and *proA*, we obtained the linkage data shown in Fig. 1. It should be noted that *drpA1* is cotransducible with *metD* and *proA*.

In addition, we obtained from E. Cox a mutant with *zae-13*::Tn*10*, which is known to be 99% linked to a *mutD*

allele of *dnaQ*. We showed that *zae-13*::Tn*10* is located between *metD* and *drpA1*. This was done by integrating an F' carrying Tn*10* into *zae-13*::Tn*10* and showing that the integrated F' broke the linkage between *metD* and *drpA*. This Hfr was constructed by the procedure of Chumley et al. (5). An F' *rep-114*(Ts) *lac*(Tn*10*) plasmid was transferred into SY889 (containing *zae-13*::Tn*10* and Δ*lac*) at 30°C by selecting for a Lac$^+$ exconjugant to give SY891. An Hfr was then isolated by growing this strain at 42°C and picking a Lac$^+$ colony to give SY893.2. We found that when P1 was grown on this Hfr (SY893.2) and used as a donor to transduce SY929 that *metD* and *drpA* were no longer cotransducible, but that *proA* and *drpA* were still cotransducible. In further support of this map order this Hfr (SY893.2) conjugally transferred *metD*$^+$ and *leu*$^+$ with high efficiency into the *metD leu* recipient (SY929), but transferred *drpA*$^+$ and *pro*$^+$ inefficiently into the same recipient. *drpA* must be to the left of *proA* because *zai*::Tn*10* is linked to *proC* and this Tn*10* and *drpA* must be on opposite sides of the Δ(*lac-pro*)XIII deletion. The best map position places *drpA* at 5.1 to 5.3 min.

We have not ordered *drpA* directly with respect to *dnaQ* and *rnh*, which are essential genes affecting nucleic acid metabolism known to be in this region (4, 16, 20). However, on the basis of linkage to *zae-13*::Tn*10*, these two appear to be at a different locus. In addition, other lines of evidence indicate that *drpA* is in neither of these genes. *dnaQ* encodes a subunit of DNA polymerase III holoenzyme. Mutations in this gene have a mutator phenotype (28). *drpA1* has no mutator phenotype at 30°C, even though the mutation significantly slows cell growth at this temperature. *rnh* encodes RNase H, an enzyme that degrades RNA chains in RNA-DNA duplex structures (4). Enzyme extracts from *drpA1* cells grown at a variety of temperatures have normal levels of RNase H activities (this laboratory, unpublished data; Robert Crouch, personal communication). Finally (see below), the properties of a recombinant plasmid that complements *drpA1* also indicates that *drpA* is neither *dnaQ* nor *rnh*.

Properties of recombinant plasmids that complement *drpA1*. We constructed a clone with fragments of DNA from wild-type *E. coli*, which complements *drpA1*. *Hin*dIII-digested chromosomal DNA cloned into the *Hin*dIII site of pBR322 was transformed into *drpA1*, and clones were isolated that could complement the temperature-sensitive defect at 42°C. Each complementing clone contained a plasmid, called pKFL1, that carried an insert 4.5 kilobases long. It should be noted that we have not unambiguously proven that this plasmid contains the *drpA* gene, although each plasmid isolated contained a 4.5-kilobase insertion. There is a formal possibility that this plasmid contains some other gene from the ancestral strain which, when carried on a high copy number plasmid, suppresses the *drpA1* phenotype.

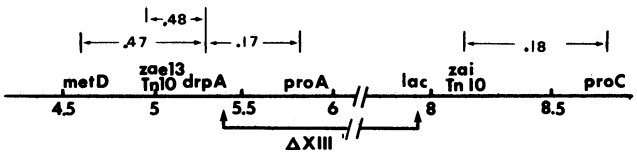

FIG. 1. Linkage map of the *drpA* mutant. Percentages above the line give P1 cotransduction frequencies. *drpA* is 60% linked to *zai*::Tn*10* when the *lac-pro* deletion XIII is present in both the donor and the recipient, but is completely unlinked (0 of 100) in the absence of the deletion. *metD* and *drpA* are unlinked (0 of 208) when F'*rep*(Ts)*lac* (Tn*10*) is integrated at *zae-13*::Tn*10* (893.2) in the donor. Linkages were determined by the following crosses. Between *metD* and *drpA* the strain KS519 was the recipient, SY356.1 was the donor, and *metD*$^+$ transductants were selected at 30°C and then screened for their temperature sensitivity. Between *zae-13*::Tn*10* and *drpA*, SY736 was the recipient, MC1000 was the donor, growth at 42°C was selected, and the transductants were screened for tetracycline resistance. Between *drpA* and *proA* SY929 was the recipient, SY893.2 was the donor, and Pro$^+$ transductants were selected and then screened for temperature sensitivity. Between *zai*::Tn*10* and *proC* SY250 was the recipient, SY658 was the donor, and Pro$^+$ transductants were selected and then screened for tetracycline resistance. Distances should not be inferred from the crosses involving a Tn*10* insertion; they frequently disrupt linkages.

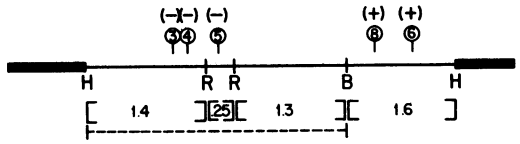

FIG. 2. Physical map of pKFL1 and derivatives. The numbered circles above the line designate the positions of the respective Tn*5* insertions. The Tn*5* insertion in pKFL7 apparently rendered that plasmid too unstable to accurately map the site of insertion. The dotted line below the line designates the bacterial sequences present in pKFL2. Restriction enzyme cutting sites: R, *Eco*RI; H, *Hin*dIII; B, *Bam*HI. The bracketed numbers below the line are distances in kilobases. (+) and (−) indicate whether or not the respective Tn*5* insertion complements *drpA1*. Heavy line, pBR322 sequences.

The *drpA*$^+$ *Hin*dIII fragment contained two *Eco*RI sites and one *Bam*HI site (Fig. 2). We subcloned a DNA fragment between one of the *Hin*dIII sites and the *Bam*HI site into pBR322 that successfully complemented *drpA1* (pKFL2, Fig. 2). We were unable to subclone a fragment from the *Hin*dIII site to the *Eco*RI site that would complement *drpA1*. This suggests that an *Eco*RI site lies in or near the *drpA* gene.

To further locate the *drpA*$^+$ gene we inserted transposon Tn*5* into pKFL1 and sought pKFL1::Tn*5* plasmids that either did or did not complement *drpA1*. This was done by infecting a lambda phage carrying Tn*5* into a *drpA* strain carrying pKFL1 and selecting for kanamycin-resistant clones. A few thousand independent kanamycin-resistant colonies were pooled, and plasmid DNA was isolated and transformed into *drpA1*, selecting for simultaneous kanamycin- and ampicillin-resistant clones. Two classes of Kan^r Amp^r clones were found: those that grew at 42°C (+) and those that did not (−). Three from each class were picked and the location of the Tn*5* insertion in each plasmid was physically mapped. Mapping was done by digesting plasmid DNA with *Hin*dIII, which cuts pKFL1 twice and Tn*5* twice (in its inverted stems); with *Eco*RI, which cuts in pKFL1 three places, but not in Tn*5*; and with *Hin*dIII and *Eco*RI simultaneously. From the sizes of the various fragments, the map in Fig. 2 was constructed. The three Tn*5* insertions either near or between the two *Eco*RI cutting sites in the inserted *Hin*dIII fragment failed to complement *drpA1*, whereas those insertions distant from this region did complement *drpA1*. We tentatively place the *drpA* gene in the region spanning the two *Eco*RI sites.

If pKFL1 does, in fact, carry the *drpA* gene, then it seems even more unlikely that *drpA1* is an allele of *dnaQ* or *rnh*. The restriction map of an *Eco*RI fragment that carries these two genes and their known location within that fragment (20) are entirely different from the map presented in Fig. 2.

***drpA*$^+$ plasmids encode a 68-kd protein.** We identified a 68-kilodalton (kd) protein that is encoded by the *drpA*$^+$

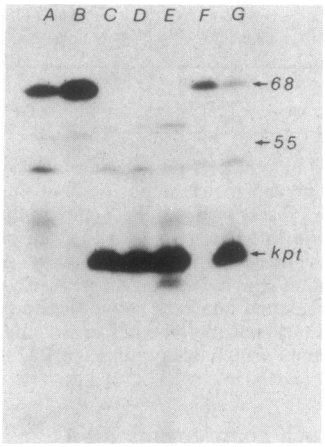

FIG. 3. Proteins synthesized from pKFL1 and derivatives. An autoradiogram of a sodium dodecyl sulfate-polyacrylamide gel separation of labeled maxicell proteins is shown. Lanes represent cells carrying (A) pKFL1, (B) pKFL2, (C) pKFL3, (D) pKFL4, (E) pKFL5, (F) pKFL6, and (G) pKFL8. kpt designates kanamycin phosphotransferase, which is encoded in Tn*5*. The absence of kpt in lane F probably reflects the extreme instability of that Tn*5*; presumably the Tn*5* in this maxicell strain transposed to the chromosome, so its proteins are not visible.

plasmids (pKFL1, pKFL2, pKFL6, pKFL7, and pKFL8), but which is absent from those plasmids that have pKFL1::Tn*5* insertions that fail to complement *drpA1* (pKFL3, pKFL4, and pKFL5). This identification was made with the maxicell technique, a procedure whereby [^{35}S]methionine labels proteins encoded by small plasmids rather than those encoded by the bacterial chromosome (27). Figure 3 shows the autoradiograph of the sodium dodecyl sulfate-polyacrylamide gel that was used to fractionate the preferentially labeled plasmid proteins. Strains that carry those plasmids that are *drpA*$^+$ (Fig. 3, lanes a, b, f, and g) synthesize a prominent 68-kd protein. This protein is absent in the pKFL1::Tn*5* derivatives that fail to complement *drpA1* (Fig. 3, lanes c, d, and e). The most straightforward explanation for this result is that the 68-kd protein is the product of *drpA*. We cannot eliminate the possibility that the 68-kd protein is encoded by a gene in an operon that also contains *drpA* (whose product remains unseen) and that loss of the 68-kd protein is caused by polarity or some other secondary effect. This 68-kd protein is encoded on the 2.9-kilobase *Hin*dIII-to-*Bam*HI fragment (Fig. 2), which is large enough to encode an additional 40 kd of polypeptide.

RNase H and the *dnaQ* protein are 43 and 25 kd, respectively. No proteins of that size are evident in the maxicells carrying any of the pKFL plasmids.

drpA1 mutant is defective in macromolecular synthesis. The *drpA1* mutant was isolated from a population of cells that had been enriched for mutations defective in DNA synthesis. We were next interested in showing that the *drpA1* mutant was, in fact, defective in DNA synthesis and in ascertaining whether the *drpA* product is directly involved in the DNA synthetic apparatus.

The in vivo rates of DNA synthesis in the isogenic *drpA1* (SY732) and *drpA*$^+$ (SY733) strains were performed by growing the two strains at 30°C, shifting the temperature to 42°C, and then measuring incorporation of [^{3}H]thymidine pulses into 10% TCA-insoluble material (29). We found that incorporation of ^{3}H in *drpA1* ceased immediately after the temperature shift (data not shown). To measure the rate at which DNA synthesis was reduced, the assay temperature was lowered to 38°C. At this temperature incorporation of ^{3}H into the *drpA1* strain was significantly reduced 20 min after the temperature was shifted (Fig. 4).

Since the above result may be due to a general collapse of macromolecular synthesis at the nonpermissive temperature, we next tested the rates of protein and DNA synthesis

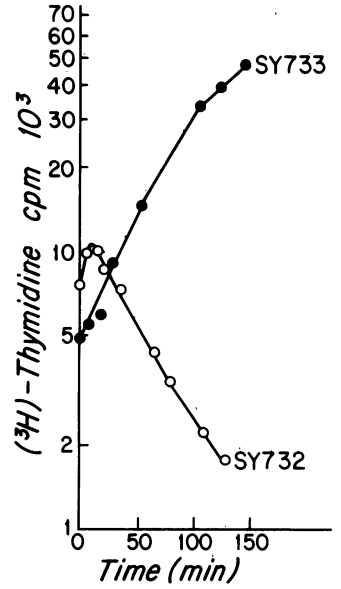

FIG. 4. Rates of DNA synthesis in the *drpA1* mutant (SY732) and the wild type (SY733). The two strains were grown at 30°C, and at zero time the temperature was shifted to 38°C. At each time point, a sample of the culture was added to culture medium containing [^{3}H]thymine and incubated (pulsed) for 1 min. Incorporation of radioactivity into 10% TCA-insoluble material is displayed.

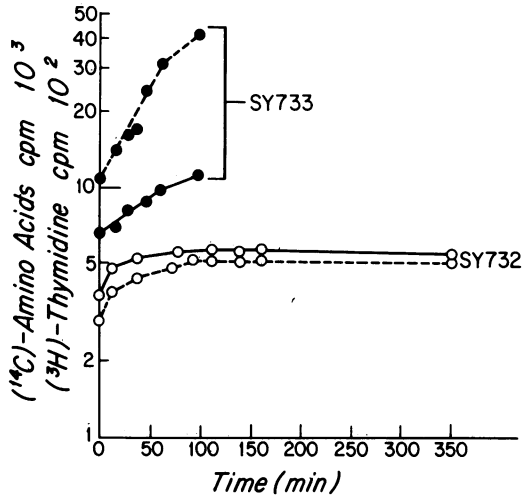

FIG. 5. Steady-state accumulation of protein or DNA in the *drpA1* mutant (SY732) and the wild type (SY733). The two strains were grown at 30°C, and at zero time their temperature was shifted to 38°C and their growth medium was changed to include ^{14}C-amino acids and [^{3}H]thymine. At each time point a sample of this culture was removed, and incorporation of ^{14}C (broken line) and ^{3}H (solid line) into 10% TCA-insoluble material was determined.

in the *drpA1* strain. The *drpA*$^+$ strain (SY733) and the *drpA1* strain (SY732) were continuously labeled with both [^{3}H]thymidine and ^{14}C-amino acids after the temperature was shifted to 38°C. At different time points (Fig. 5), incorporation of ^{3}H or ^{14}C into the TCA precipitates was counted. Incorporation of both ^{3}H and ^{14}C was reduced to the same extent in the *drpA1* mutant after the temperature shift, indicating that both DNA and protein syntheses are equally defective in the mutant (Fig. 5). Next we measured the rate of RNA synthesis in the *drpA1* mutant. The experiment in Fig. 6 shows the incorporation of pulses of [^{3}H]uridine into growing cells after the shift to 38°C; RNA synthesis abruptly stops in the mutant. In fact, the rate of RNA synthesis decreases even more rapidly in the mutant than does the rate of DNA synthesis (compare Fig. 6 with Fig. 4). We then performed a double-label experiment in which we monitored the simultaneous uptake of both [^{14}C]uridine and [^{3}H]thymidine into SY732 and SY733 and confirmed this difference. Synthesis of RNA shuts off much more abruptly than does synthesis of DNA when the *drpA1* mutant is shifted to 38°C (data not shown). The most straightforward explanation for this result is that *drpA1* primarily affects the ability of the cell to conduct RNA synthesis.

***drpA1* does not affect biosynthesis of metabolite precursors.** The fact that RNA synthesis shuts down sooner than either protein synthesis or DNA synthesis when the *drpA1* strain is shifted from 30°C to 38°C would suggest that the *drpA* product is directly involved in RNA synthesis and that DNA and protein syntheses stop as a consequence of this. An alternative explanation is that the *drpA* product is required in the biosynthesis or maintenance of precursors for the synthesis of RNA and DNA. We have examined this possibility in a number of different ways, but have found no evidence to support it.

The *drpA* mutant has normal amounts of ATP in vivo either when growing at 30°C or after the temperature is shifted to 40°C. This measurement was made on cells that had been labeled with $^{32}PO_4$ and extracted with 10% TCA; the resulting extract was chromatographed with polyethyleneimine thin-layer chromatography by the procedures of Randerath and Randerath (25, 26) and Bochner and Ames (2, 3) (data not shown). In addition, we showed that the *drpA1* mutant does not leak ATP or other phosphorylated nucleotides into its growth medium at 42°C (6). The possibility of an ATP deficit was pursued further because it has been reported that a temperature-sensitive mutation in the gene for adenylate kinase isolated in *E. coli* B blocks DNA, RNA, and protein syntheses (9). The activity of this enzyme apparently recycles ADP back into ATP. We therefore assayed adenylate kinase activities in cell-free extracts from both the ancestral *drpA*$^+$ and *drpA1* strains. Extracts were prepared from cells grown at 30°C or from cells shifted to 40°C and were assayed at both 30 and 42°C. Adenylate kinase activities were the same in extracts prepared either from *drpA1* or from *drpA*$^+$ strains (data not shown).

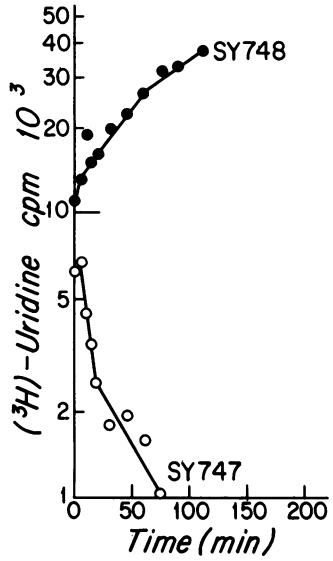

FIG. 6. Rate of RNA synthesis in the *drpA1* mutant (SY747) and the wild type (SY748). The two strains were grown at 30°C, and at zero time their temperature was shifted to 38°C. At each time point a sample of cells was added to a medium containing [^{3}H]uridine for 1 min. Incorporation of radioactivity into 10% TCA-insoluble material is displayed.

An essential enzyme in RNA and DNA biosynthesis is UMP kinase, which converts UMP to UTP. The structural gene for this enzyme has not been mapped precisely in *E. coli*, but appears to be located between 4 and 5 min (13). To test whether the *drpA1* mutant could correspond to this gene, we measured the levels of UMP kinase both in vivo and in vitro. The in vivo measurement was done by pulse-labeling *drpA1* and *drpA*$^+$ strains with [^{3}H]uridine after shifting the temperature to 42°C and then extracting nucleotides and chromatographing them on polyethyleneimine-cellulose thin-layer plates (1). Both strains converted the ^{3}H into approximately the same amounts of UMP, UDP, and UTP as viewed by autoradiography. Similar results were obtained with an in vitro assay devised by Justensen and Neuhard (15) (data not shown). The genetic map position of *drpA* eliminates other known genes whose products affect synthesis of metabolic precursors to polynucleotides.

The strongest piece of evidence indicating that the levels of ATP and the other nucleotide triphosphate precursors are not the cause of the defect in the *drpA1* mutant is inferred from the results of the assay of DNA synthesis in toluenized cells. In this assay bacterial cells are made permeable by treatment with toluene, which causes the leakage of endogenous ATP and precursors. To see incorporation of precursor ([α-^{32}P]dATP) into TCA-insoluble material in this system, ATP and the four deoxynucleotide triphosphates must be added. Therefore, if the *drpA1* mutant is defective in precursor biosynthesis, this defect should be overcome in toluenized cells when these precursors are added.

In establishing the conditions for this assay using the wild-type control, we could only see incorporation of label into TCA-insoluble material when the assay temperature was 30°; there was no detectable activity at 35 or 40°C. This result was reported earlier by Moses and Richardson (23). We therefore assayed both the ancestral SY747 and SY748 at 30°C. Cells to be assayed either were grown at 30°C or were grown at 30°C and then shifted to 42°C for 40 min before harvesting (Fig. 7). There are two types of activity in this system: an ATP-dependent activity that corresponds to DNA replication and an ATP-independent activity that corresponds to DNA repair synthesis (23). The ATP-independent activity was the same in both *drpA*$^+$ and *drpA1* strains; however, the ATP-dependent activity was different. Wild-type cells whose temperature had been shifted to 42°C before harvesting had less activity than those that had been grown just at 30°C; the ATP-stimulated activity was reduced by 60%. In *drpA1* cells, on the other hand, the 42°C

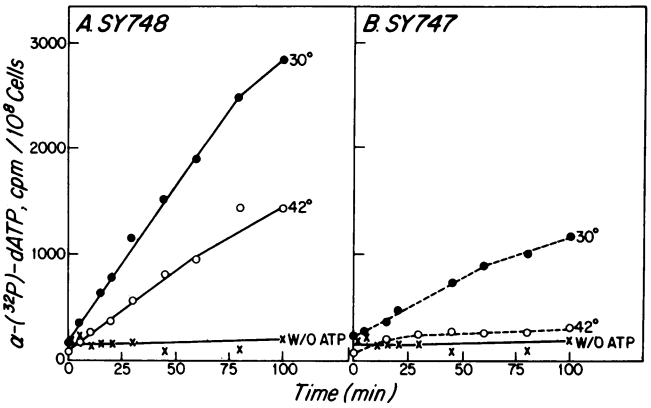

FIG. 7. ATP-dependent DNA synthesis in toluenized cells from the *drpA1* mutant (A) and the wild type (B). All assays were performed at 30°C. Cells for the assay were grown either at 30°C (●) or for 40 min at 42°C (○) before harvesting. The time gives length of the assay period and cpm gives radioactivity in 10% TCA precipitates.

temperature shift caused at least an 86% reduction in activity. More strikingly, the specific activity in *drpA1* cells grown at 42°C was only 13% of that found in the wild-type cells grown at 42°C. Therefore, since DNA replication was still defective even after the addition of deoxynucleotide triphosphates and ATP, it is probable that *drpA1* does not affect the nucleotide pool.

The fact that toluenized cells from *drpA1* have a significant defect in DNA synthesis suggests that *drpA* may play a direct role in DNA synthesis as well as affecting DNA synthesis indirectly through RNA synthesis.

DISCUSSION

The *drpA1* mutation defines a new gene that maps between 5.1 and 5.3 min on the *E. coli* chromosome. We have constructed a hybrid plasmid between pBR322 and *drpA*$^+$ *E. coli* DNA (called pKFL1) that restores the ability of the *drpA1* strain to grow at 42°C. A series of Tn*5* insertions in pKFL1 was isolated that destroy pKFL1's ability to restore growth to the *drpA1* strain at 42°C. These same Tn*5* insertions eliminate a 68-kd protein that is synthesized from pKFL1. We are tentatively assigning the structural gene for *drpA* to pKFL1.

Growing cultures of the *drpA1* mutant, after being shifted to high temperature, experience an abrupt stop in RNA synthesis, whereas protein and DNA syntheses take longer to stop. On the basis of this result we suggest that the primary defect in the *drpA1* mutant is in RNA synthesis, with the *drpA* protein defining a previously unsuspected component involved in this process. A mutant of similar phenotype has been isolated by Liebke and Speyer (19). The map positions of the two mutations, however, are entirely different. Because a defect in DNA synthesis is also observed in the partial in vitro assay, which uses toluenized cells, the *drpA1* mutation may directly affect DNA replication as well. We are considering two possible explanations for these results. One is that the wild-type *drpA* product is part of the enzymatic apparatus for synthesis of RNA, and that this product is also used in DNA replication. A second possibility is that *drpA* protein is involved in maintaining proper chromosome structure and that the defect in the *drpA1* mutant affects RNA synthesis most acutely. A third possibility is that the *drpA1* protein is an inhibitor of RNA and DNA synthesis. This, however, is unlikely because the mutation is recessive to the wild type.

The *drpA1* mutation was obtained in the course of isolating mutants in which transposon precise excision was affected. An object of these studies is to obtain mutations in *E. coli* genes of known function to infer details of the precise excision mechanism. This aspect of the analysis must wait until we have a better understanding of *drpA* gene function.

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