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# Novel Mutants of *Escherichia coli* That Accumulate Very Small DNA Replicative Intermediates

**("hyper-rec mutants"/DNA replication/recombination)**

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**ABSTRACT** **A new group of mutants has been isolated which, during short pulses, incorporate [$^3$H]thymidine into DNA fragments that are substantially smaller than Okazaki fragments. These small fragments can be chased into DNA of high-molecular-weight, and thus may be precursors in DNA replication. During longer pulses, label also appears in DNA of higher molecular weight, although at an abnormally slow rate. The mutations map at a previously undescribed locus (*dnaS*) at 72 min on the *E. coli* chromosome.**

DNA replication in *Escherichia coli* appears to be a discontinuous process whereby daughter strands are synthesized as short fragments that are joined by DNA ligase to form covalently continuous strands (1–5). Intervening steps might include removal of an RNA primer on the 5′ end of each fragment (6), perhaps by the 5′ → 3′ exonuclease of DNA polymerase I (7) and filling of gaps between fragments also by DNA polymerase I (8, 9). Among the evidence in support of this model are kinetic studies which show that during short pulses, [$^3$H]thymidine is incorporated predominantly into fragments about 1000 nucleotides long (Okazaki fragments) and later appears in DNA of high-molecular-weight (1, 2). The life-time of these normally short-lived fragments is greatly lengthened by deficiencies in DNA polymerase I, its associated 5′ → 3′ exonuclease, or in DNA ligase (3–5, 7–9).

This paper describes a novel group of mutants in which short pulses of [$^3$H]thymidine are incorporated into fragments substantially smaller than the Okazaki fragments found in wild-type strains. These mutations are tentatively assigned to a new locus, *dnaS*, which is linked to *pyrE* at 72 minutes on the *E. coli* map.

## MATERIALS AND METHODS

*Phage and Bacterial Strains.* All bacterial strains are derived from *E. coli* K12. Genetic nomenclature is from Taylor and Trotter (10).

Strains KS391 (Hfr Hayes *thi*$^-$*lac*MS286$\phi$80*d*IIlacBK1) and KS418 (F$^-$*met*B$^-$*ara*$^-$*thi*$^-$*lac*MS286$\phi$*d*IIlacBK1) were used in isolating *dnaS* mutations; both of these are diploid for *lac*. $\phi$80*d*IIlac is a defective prophage inserted at *att80*, that carries the *lac* operon but not $\phi$80*imm* (11), and *lac*MS286 is a deletion including *lacY* and *lacZ*, furnished by M. Malamy. *lac*BK1 is a deletion of part of *lacZ* that does not overlap *lac*MS286. Strains RS5087, RS5083, RS5091, and KS474 were constructed by transducing strain KS468 (F$^-$ *metB*$^-$*thi*$^-$*pyrE*$^-$*lac*MS286$\phi$80*d*IIlacBK1*str*$^r$) to *pyrE*$^+$ and *dnaS1*, *dnaS2*, *dnaS3*, and *dnaS*$^+$, respectively (see below). Phage P1*vir* was provided by J. Shapiro, λ and λ *red3* by Dr. Freifelder, and the *recA*1 mutation by A. J. Clark.

*Scoring the "Hyper-rec" Phenotype.* Strains carrying *lac*MS286$\phi$80*d*IIlacBK1 were tested for the "hyper-rec" character by streaking to single colonies on lactose tetrazolium indicator plates. These strains are lactose negative, and initially form red colonies. On prolonged incubation, white lactose positive papillae, each representing a clone of recombinant (*lac*$^-$$\phi$80-*d*IIlac$^+$) cells, appear on the surface of colonies. Colonies showing conspicuously more papillae than wild type were scored as hyper-rec.

*Other Procedures.* Bacterial matings, transductions with phage P1*vir*, mutagenesis with ethylmethane sulfonate and nitrosoguanidine, plating of λ phage, and preparation of lactose tetrazolium plates were as described by Miller (12). Assays of polymerase and 5′ → 3′ exonuclease activities of DNA polymerase I have been described previously (7); the assay of DNA ligase is given in Modrich and Lehman (13). Cultures were pulse-labeled with [$^3$H]thymidine or [$^3$H]-thymine, and the DNA was extracted and centrifuged in alkaline sucrose gradients as previously described (7). This extraction procedure recovers more than 90% of the [$^3$H]-thymidine incorporated into acid-insoluble material. Sucrose gradients were layered with 300 μl of extract, and [$^{32}$P]DNA from phage $\phi$X174, kindly provided by Lee Rowen, was used as a 16S sedimentation velocity marker.

## RESULTS

*Isolation of dnaS Mutants.* Strains carrying *dnaS1*, *dnaS2*, and *dnaS3* were isolated by screening hyper-rec mutants, obtained at 30° as described by Konrad and Lehman (7), successively for temperature-sensitive conditional lethality at 44°, capacity to form filaments at 44°, and abnormal accumulation of isotope in small DNA fragments following short pulses of [$^3$H]thymidine. Mutations *dnaS1* and *dnaS3* were induced by ethylmethane sulfonate, and *dnaS2* by nitrosoguanidine (12). Details of this procedure, which has also been used to isolate strains defective in DNA ligase and DNA polymerase I (refs. 4 and 7, and unpublished results), will be given in a subsequent publication. Although these *dnaS* mutants were isolated as conditionally lethal at 44°, in each

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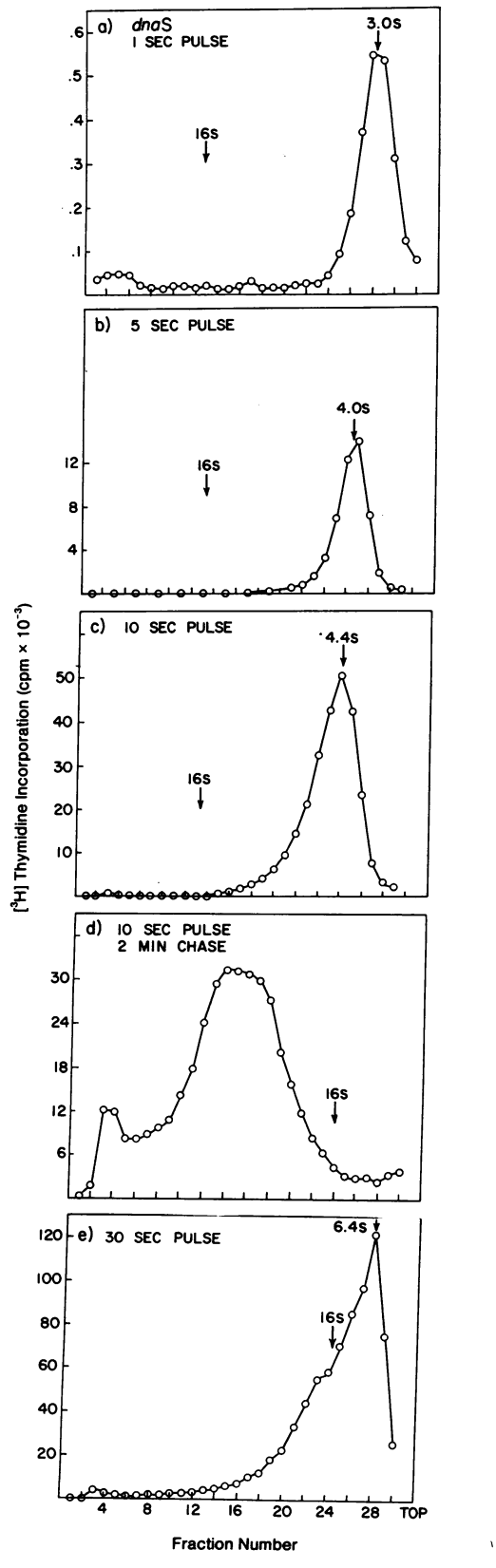


FIG. 1. Sedimentation profiles in alkaline sucrose gradients of [$^3$H]thymidine-labeled DNA extracted from a *dnaS1* mutant (RS5087). (a)–(c), gradients were fractionated after 15 hr of centrifugation; (d)–(e), gradients were fractionated after 4 hr of centrifugation. In (d), the 10-second pulse was followed by a 2-min chase with 100 μg/ml of unlabeled thymidine. All pulses were at 44°. The sedimentation profiles shown in (c) and (d) include 272,900 and 393,250 cpm, respectively. The sedimentation profile of DNA labeled during a 30-sec pulse (e) yielded a peak sedimentation coefficient of 6.8 S when the gradient was fractionated after 15 hr of centrifugation.

case secondary mutations were responsible for the lethality, since this property was lost on transfer of the *dnaS* mutation to a wild-type background (KS468).

The following results were obtained with a *dnaS1* mutant (RS5087) but qualitatively similar results were found with *dnaS2* and *dnaS3* mutants.

*Accumulation of Short DNA Fragments in dnaS Mutants.* At 44° the *dnaS* mutant incorporated [$^3$H]thymidine into DNA fragments with sedimentation coefficients in alkaline sucrose gradients progressing from 3.0 S for a 1-sec pulse, to 4.4 S for a 10-sec pulse (Fig. 1a, b, and c), indicating that they were considerably smaller in size than the 5.1S to 8.2S Okazaki fragments labeled during equivalent pulses with a wild-type strain (Fig. 2a, b, and c) or accumulated by strains with deficiencies in DNA ligase or polymerase I. The [$^3$H]-thymidine in these smaller fragments, like that in Okazaki fragments, could be chased into higher molecular weight DNA by unlabeled thymidine (Figs. 1d and 2d). During longer pulses, the rate of appearance of [$^3$H]thymidine in high-molecular-weight DNA was considerably retarded in the *dnaS* mutant (Figs. 1e and 2e) [see Konrad *et al.*, Fig. 7 (4), and Konrad and Lehman, Figs. 2 and 3 (7) for comparable data with strains deficient in DNA ligase and the 5′ → 3′ exonuclease of DNA polymerase I, respectively]. The *dnaS1* mutant also incorporated label into small DNA fragments during short pulses of [$^3$H]thymine under steady state conditions (data not shown).

Figs. 3 and 4 show the results of subjecting the DNA labeled during 1-sec and 5-sec pulses in the *dnaS1* and wild-type strains to prolonged sedimentation (24–30 hr) in alkaline sucrose gradients. Both sedimentation profiles of DNA from the mutant strain had a single peak with little material at the position corresponding to the major peaks for the wild-type strain.

A 2S peak that is partially resolved in Fig. 3b is more clearly seen in sedimentation profiles of DNA isolated from *dnaS1* or wild-type strains pulsed with [$^3$H]thymidine at 24° (Fig. 3c), since at this lower temperature, the sedimentation coefficient of pulse label in Okazaki fragments was greater for pulses yielding comparable incorporation of [$^3$H]thymidine into DNA (Fig. 3c and other data not shown). We find that the label in this 2S peak cannot be readily chased into large DNA; thus, its relationship to DNA replication is unclear. Wang and Sternglanz (14) have described a 2S DNA fragment which is rapidly labeled by [$^3$H]thymine pulses of *Bacillus subtilis* and which also cannot be chased readily into larger DNA.

*Other Characteristics of dnaS Mutants.* The levels of DNA ligase and DNA polymerase I in extracts of the mutant *dnaS* and wild-type strains were indistinguishable (1.05 versus 1.06 units/mg for DNA ligase and 1.47 versus 1.46 units/mg for DNA polymerase I). Assay of the 5′ → 3′ exonuclease activity of DNA polymerase I in partially purified extracts of the *dnaS* mutant also gave an essentially normal value

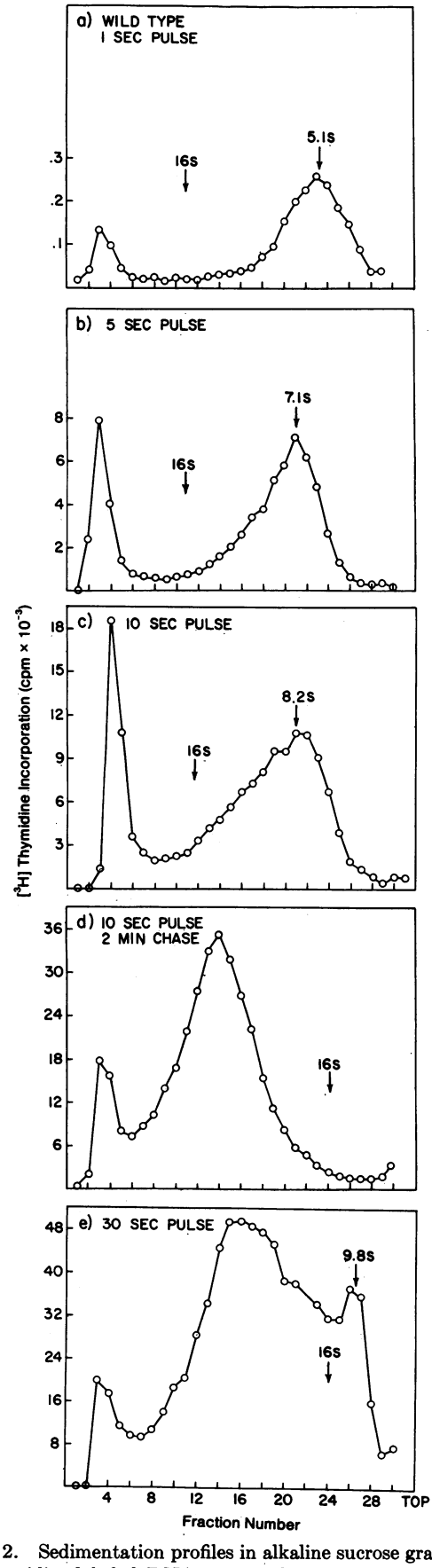


FIG. 2. Sedimentation profiles in alkaline sucrose gradients of [$^3$H]thymidine-labeled DNA extracted from a wild-type strain (KS474). (a–c), gradients were fractionated after 15 hr of centrifugation; (d)–(e), gradients were fractionated after 4 hr of centrifugation. In (d), the 10-second pulse was followed by a 2-min chase with 100 μg/ml of unlabeled thymidine. All pulses were at 44°. The sedimentation profiles shown in (c) and (d) include 228,900 and 365,200 cpm, respectively.

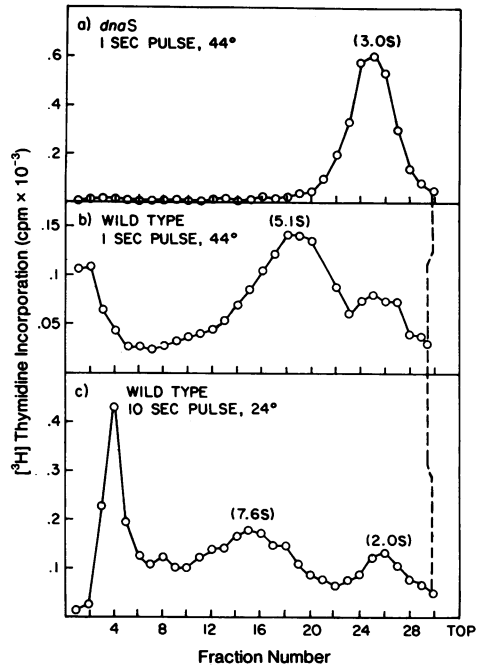


FIG. 3. Sedimentation profiles in alkaline sucrose gradients of [$^3$H]thymidine-labeled DNA extracted from: (a) *dnaS1* mutant (RS5087), (b) wild-type strain (KS474), and (c) wild-type strain (KS474). The durations of the pulses and temperatures are indicated in the figure. Gradients were run simultaneously and fractionated after 24 hr of centrifugation. Sedimentation coefficients of the peaks are taken from the sedimentation profiles, obtained with samples of the same DNA extracts, shown in Figs. 1a and 2a, and similar profiles for the pulse at 24°. The dashed line connects the menisci.

(a total of 27 units for the *dnaS* mutant as compared to 44 units for the wild-type strain). Besides accumulating very small fragments, these mutants differed phenotypically from DNA polymerase I and DNA ligase mutants in their resistance to methyl methanesulfonate, their slight sensitivity to ultraviolet irradiation, and their capacity to support plaque formation by λ phage defective in general recombination (λ*red*$^-$) (4, 5, 7, 15–17). Strains with both *dnaS1* and *recA*$^-$ mutations are viable.

*The dnaS Locus Is Closely Linked to pyrE at 72 Min.* The hyper-rec phenotype associated with the *dnaS1* mutation was transferred as an early marker by Hfr strains PK3 and KL228 but not by Hfr strain R1 (18). This localized *dnaS1* between the origins of PK3 and R1 (Fig. 5). Further mapping within this interval by generalized transduction with phage P1 established very close linkage between *dnaS*1 and the *pyrE* locus at 72 min, and less close linkage to the *cysE* locus (18) (Fig. 5b). We have not determined the order of *pyrE* and *dnaS*. The linkage of *dnaS1* and of *dnaS2* and *dnaS3* to *pyrE* was confirmed by demonstrating that the capacity to accumulate very small fragments as well as the hyper-rec character was cotransduced with *pyrE*. We tentatively assign all of these mutations to the same locus. The *dnaS1* mutation

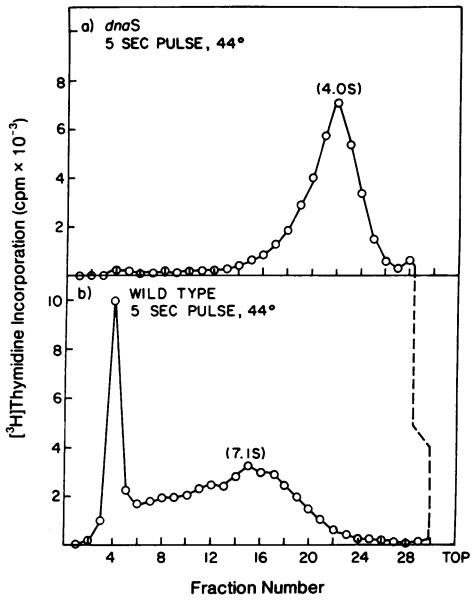


FIG. 4. Sedimentation profiles in alkaline sucrose gradients of [³H]thymidine-labeled DNA extracted from: (a) *dnaS1* mutant (RS5087) (b) wild-type strain (KS474). The durations of pulses and temperatures are indicated in the figures. Gradients were run simultaneously and fractionated after 30 hr of centrifugation. Sedimentation coefficients of the peaks are taken from the sedimentation profiles, obtained with samples of the same DNA extracts, shown in Figs. 1b and 2b. The dashed line connects the menisci.

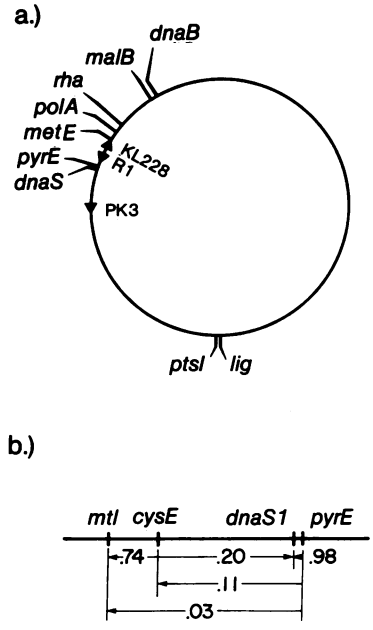


FIG. 5. Position of the *dnaS* locus on the *E. coli* map. (a) The position of *dnaS* relative to *dnaB* and to *polA* and *lig*, the structural genes for DNA polymerase I and DNA ligase, respectively (10); (b) cotransduction frequencies via phage P1 between loci near *dnaS1*. These were determined in the following crosses: *mtl-cysE* and *cysE-dnaS1*, 90 transductants scored; *cysE-pyrE*, 55 transductants scored; and *pyrE-mtl* and *pyrE-dnaS1*, 193 transductants scored. In each case the arrow points away from the marker selected. Loci have been ordered assuming *cysE* and *pyrE* lie on the right of *mtl* (10). These data do not establish the order of *pyrE* and *dnaS1*. The origins of Hfr strains PK3, R1, and KL228 are as given by Low (18).

is recessive, since introduction of an episome carrying the wild-type locus (F'111) restored the wild-type phenotype to a *dnaS1* mutant (18).

## DISCUSSION

We have described a group of *E. coli* mutants (*dnaS*) with a novel defect in DNA replication. During short pulses, these mutants incorporate [³H]thymidine into DNA fragments much smaller than the Okazaki fragments which are found in wild-type strains and that are accumulated by mutants defective in DNA ligase or DNA polymerase I. The [³H]-thymidine can be chased into high-molecular-weight DNA; thus, these fragments may also be intermediates in DNA replication. DNA replicative intermediates of comparable size have been found in *dnaB* mutants [Lark, K. G. & Wechsler, J. (1975) *J. Mol. Biol.* **92**, 142–163] and in ether-treated cells (19), as well as in various eukaryotic cells (20–22). Slower incorporation of [³H]thymidine into high-molecular-weight DNA by *dnaS* mutants indicates that *dnaS* fragments persist longer than Okazaki fragments and suggests the mutants are also defective in joining these fragments. The *dnaS* mutations are closely linked to *pyrE* at 72 minutes on the *E. coli* map. At least one of them is recessive.

If *dnaS* fragments occur as intermediates in wild-type *E. coli*, we can envision them converted to Okazaki fragments either via joining by an unknown ligase or via extension by chain propagation. The latter mechanism would require that a substantial portion of the pulse-labeled fragments be nearly as large as Okazaki fragments. However, we find that the smallest sizes of *dnaS* and Okazaki fragments that can be pulse labeled and distinguished as replicative material have sedimentation coefficients of about 3 S and 5 S, respectively. The 5S fragments are probably incomplete, since strains defective in DNA ligase incorporate [³H]thymidine into 7.3S Okazaki fragments under these conditions but over a range of longer pulse times (Konrad, unpublished observations). Nevertheless, the 3S fragments include only a small amount of 5S material. Similarly, the 4S fragments labeled during a longer pulse include little 7S material.

The *dnaS* mutations might also alter a component of the replication machinery in such a way as to shorten the length of Okazaki fragments. If this were the case, *dnaS* fragments would represent abnormally short Okazaki fragments that only occur in these mutants. Alternatively, *dnaS* mutations might generate repairable discontinuities, perhaps nicks or intercalated abnormal nucleotides, which lead to fragmentation under the conditions we use for extracting DNA.

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