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RNase H Is Not Involved in the Induction of Stable DNA Replication in *Escherichia coli*

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***rnh* mutations of *Escherichia coli* inactivating RNase H activity allow the initiation of rounds of DNA replication in the absence of protein synthesis (stable DNA replication). However, levels of RNase H did not change during or after the induction of stable DNA replication in *rnh*$^+$ strains by incubation with nalidixic acid or UV irradiation.**

The ability of *Escherichia coli* to initiate rounds of chromosome replication in the absence of continued protein synthesis (8, 9) can be induced by treatments that elicit the *recA lexA*-regulated SOS response (see reference 21 for a review) and has been termed induced stable DNA replication (iSDR) (12). iSDR is considerably more resistant to DNA damage caused by UV radiation than is normal replication (12) and appears to be mutagenic (14). It has been postulated that the independence of initiation from continued protein synthesis, increased tolerance to UV damage, and decreased fidelity of iSDR may be a consequence of a modification in the replisome (12, 14). Recently, in *lexA*(Def) mutants constitutively expressing all *lexA*-controlled genes, expression of iSDR has been shown to require the activated form of the RecA protein (RecA*) (22). Initiation of iSDR expression by activation of the RecA protein in *lexA*(Def) strains can occur in the absence of concomitant protein synthesis. Because the characteristic requirement for RecA* without protein synthesis is shared by SOS mutagenesis and iSDR, it has been speculated that a replisome modified by RecA* is responsible for both iSDR and SOS mutagenesis (22). Stable DNA replication (cSDR) is constitutively expressed in *rnh* mutants lacking RNase H activity (4, 7, 16, 19). Both iSDR and cSDR require a functional RecA protein for sustained replication (14, 20). It was therefore of interest to determine whether SDR in *rnh*$^+$ strains was also mediated by RNase H. We report here that levels of RNase H activity did not significantly change during and after iSDR in *rnh*$^+$ cells.

AQ170, a *thyA705 deo-25* derivative of AB1157 (12), was grown at 37°C in M9 salts-glucose supplemented with required amino acids (50 μg/ml), thymine (4 μg/ml), and thiamine-HCl (2 μg/ml) to approximately 2×10^8 cells per ml. SDR was induced either by incubating cells with nalidixic acid (40 μg/ml) for 40 min or by UV irradiation (at 40 J/m^2) followed by incubation for 40 min in growing medium (Fig. 1). RNase H was extracted, as described by Carl et al. (1), from cells immediately after induction treatments or 60 min after the addition of chloramphenicol (CM; 150 μg/ml). RNase H was assayed by using φX174 DNA-[^{32}P]RNA hybrid (a kind gift from R. J. Crouch) as described by Kanaya and Crouch (5), or polyadenylate-polydeoxythymidylate [poly(rA-dT)] as the substrate. RNase H activity was expressed in units defined as moles of acid-soluble nucleotides per 15 min at 37°C. The poly(rA-dT) substrate was prepared by using [^{3}H]ATP (New England Nuclear Corp. Boston, Mass.; 50.3 Ci/mmol) and poly(dT) (P-L Biochemicals, Inc., Milwaukee, Wis.) as template by the method of Crouch (2). Protein was determined by the method of Lowry et al. (17).

SDR was readily induced in strain AQ170 (*rnh*$^+$) by incubation with nalidixic acid, a known SOS inducer (Fig. 1a). The specific activity of RNase H during the nalidixic acid treatment did not significantly change (40 and 46 U/mg of protein with and without treatment, respectively). A period of incubation after UV irradiation, which also induced SDR (Fig. 1b), did not affect the levels of RNase H activity in the treated cells (54 U/mg of protein). The specific activity of RNase H in cells that had been incubated with CM with or without inducing treatments increased slightly compared with activity in control cells (56, 80, or 75 U/mg of protein with 60 min in CM after, respectively, incubation with nalidixic acid, UV irradiation, or no UV irradiation). This may be attributed to a slower turnover of RNase H relative to some other more labile proteins in the absence of protein synthesis. We concluded from these results that the induction of SDR does not proceed via a direct inactivation of RNase H.

We next considered the possibility that the inductive treatment might elicit synthesis of an inhibitor of RNase H and that the inhibitor activity might have been removed during the partial purification of the enzyme. We therefore attempted to detect inhibitory activity in induced cells by examining the effect of the addition of crude extract prepared from induced cells on a partially purified RNase H preparation (data not shown). Crude extract was prepared by a gentle nondetergent procedure from cells that had been either incubated with nalidixic acid or irradiated with UV. A total of ca. 1.5×10^9 cells were harvested by centrifugation and suspended in 1 ml of Tris hydrochloride (50 mM [pH 8.0])–5 mM EDTA–10% (wt/vol) sucrose. The cell suspension was subjected to freeze-thaw treatment. Then, KCl (at a final concentration of 250 mM), dithiothreitol (2 mM), and lysozyme (200 mg/ml) were added and incubated for 30 min at 0°C and then for 2 min at 30°C. The lysate was centrifuged at 4°C for 15 min to remove cell debris. A slight inhibition of RNase H activity was detected with crude extract from induced cells (data not shown). The degree of inhibition varied between 4 and 31% from one preparation to another. However, similar extents of inhibition were also observed with crude extract prepared from uninduced cells (data not shown). Therefore, the observed inhibition by induced cell

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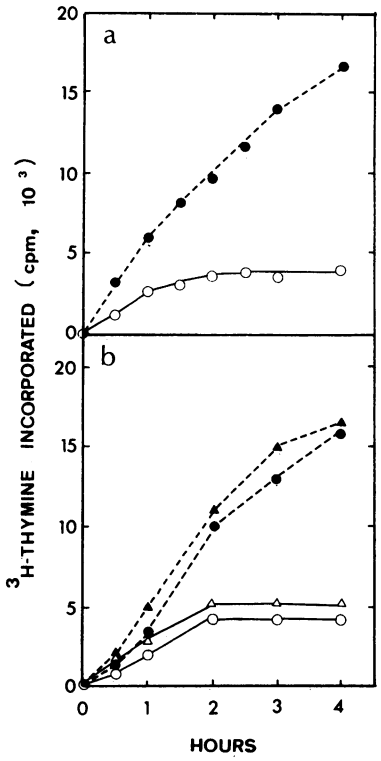

FIG. 1. DNA replication in the presence of CM after treatment with nalidixic acid or UV irradiation. (a) A culture of AQ170 was grown at 37°C to 2 × 10^8 cells per ml. The culture was split into two parts: one was incubated with nalidixic acid (40 μg/ml), and the other was incubated without the drug. After 60 min, the nalidixic acid was removed by filtration (9), and the cells were washed with M9 buffer and suspended in drug-free medium. [^{3}H]thymine (at a final concentration of 5 μCi/4 μg per ml; New England Nuclear Corp.) and CM (150 μg/ml) were added to both the drug-treated (●) and untreated (○) cells. Radioactivity in acid-insoluble material was determined for samples (0.1 ml) taken at intervals, as described previously (7). (b) AQ170 was transformed with pSK760 and pBR322 to Ap^r yielding AQ1792 and AQ1793, respectively. Portions of AQ1792 (○) and AQ1793 (△) cultures were irradiated with UV at 40 J/m^2. The cells were then diluted 1:1 with 2 × supplemented M9 medium and incubated for 40 min in the dark before the addition of [^{3}H]thymine and CM. DNA synthesis in the presence of CM in UV-irradiated (●, ▲) and nonirradiated (○, △) cells were determined as described for panel a.

extract was not due to the presence of a specific diffusible inhibitor presumed to be synthesized as an SOS response.

The results of the above in vitro experiments were confirmed by an in vivo experiment (Fig. 1b). We reasoned that if the induction of SDR resulted from the neutralization of cellular RNase H by an inhibitor, the presence of an excess amount of RNase H would raise the threshold level of induction. Cells harboring plasmid pSK760, a derivative of pBR322 carrying the *rnh*$^+$ gene, overproduced the enzyme by 8- to 15-fold (data not shown; 5). Inducibility of SDR with UV at 40 J/m^2 was indistinguishable between cells harboring pSK760 and pBR322 (Fig. 1b). UV irradiation at a smaller dose (15 J/m^2) did not change the result (data not shown).

These results did not completely rule out the possible presence of inhibitory activity in induced cells if an induced factor binds to a specific target of RNase H. For example, RNase H may normally remove RNA in a certain type of DNA-RNA hybrid, and a factor synthesized in induced cells may bind to such a structure, depriving RNase H of an access to the site. Such a protective factor might be encoded by one of the *din* (damage inducible) genes which are derepressed during the SOS induction (6). We examined the *dinA*, *dinB*, *dinD*, and *dinF* genes, the functions of which in the SOS response have not been determined (21). Mutations inactivating the gene would be expected to render the mutant noninducible for SDR. In fact, SDR was readily induced by nalidixic acid treatment in all four *din*::Mu *dl*(Ap *lac*) mutants in which one of the above *din* genes had been inactivated by an insertion of a derivative of Mu phage (data not shown).

It has been reported that mutants lacking RNase H activity can support a replication mutant of ColE1 but that a particular *rnh* mutant having a residual level of RNase H activity (0.08% that of wild type) at 30°C failed to express this phenotype (18). This suggests that RNase H activity must decrease to a very low level before this effect is manifested. The extents of decrease in RNase H activity detected in the above experiments were hardly near the threshold level. Thus, it appears that neither the direct nor the indirect inactivation of RNase H is the signal which leads to SDR following DNA damage or replication arrest.

Our finding that RNase H is the mediator of cSDR but not of iSDR is not very surprising. Both iSDR and cSDR can be characterized by independence from continued protein synthesis for initiation and the requirement of a functional RecA protein for sustained replication. However, the similarity ends there. Whereas iSDR is resistant to UV irradiation and is mutagenic (12, 14), cSDR is normally UV sensitive and not significantly error prone (T. Kogoma, unpublished data). cSDR has been shown to occur from several sites on the chromosome (*oriK*) distinct from *oriC* (3); iSDR is most likely initiated from *oriC* (8). Furthermore, cSDR can become the reproductive DNA replication in cells in which the *oriC dnaA*-dependent initiation pathway is completely blocked (10, 11). Thus, *rnh* mutants can dispense with the *oriC* site or the DnaA protein (13). On the other hand, our attempt to inactivate the *oriC* site or *dnaA* gene in *recA441 lexA*(Def) mutants at 42°C has not been successful (T. Torrey and T. Kogoma, unpublished data). In such mutants, iSDR is expected to be constitutively expressed owing to the derepression of all *lexA*-controlled genes and the activation of the RecA441 protein at 42°C (22). It is likely, therefore, that these two apparently similar replication activities arise from two distinct types of alterations in the *E. coli* DNA replication system. The alteration leading to cSDR appears to lie in the DNA duplex structure and does not necessarily involve changes in the properties of the DNA replication mechinery. That is, the absence of RNase H activity may result in stabilization of a locally melted duplex involving a DNA-RNA hybrid structure. Such a structure (R loop) could become a site for the initiation of replication by the normal replisome (3). On the other hand, the alteration giving rise to iSDR may involve a modification of the replisome structure. RecA* may be directly or indirectly responsible for the modification (22).

A mutation (*sdrT*) mapping elsewhere also gives rise to a form of cSDR that appears to be error prone (15). *sdrT* cells contain as much RNase H activity as *sdrT*$^+$ (*rnh*$^+$) cells (H. Bialy, C. A. Lark, and T. Kogoma, unpublished data). In contrast to *sdrA* (*rnh*) mutants in which a functional RecA protein becomes indispensable for cell survival only when the *dnaA*$^+$ *oriC*$^+$ initiation pathway is inoperational (11), *sdrT* mutants require active RecA protein for both cSDR and cell survival (15). These additional observations support the

above notion that the error-prone type of SDR (iSDR in *rnh*$^+$ cells and cSDR in *sdrT*$^-$ cells) results from a modification of the replisome.

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