Effect of Rifampicin and Uracil Depletion on Bacteriophage φX174 DNA Synthesis in an E. coli dnaH\textsuperscript{ts} Mutant

Atsushi Higashi, Junji Morita, Hiroshi Sakai and Tohru Komano

Laboratory of Biochemistry, Department of Agricultural Chemistry, Kyoto University, Kyoto

Received May 30, 1977

The replication of bacteriophage φX174 DNA occurs in three distinct stages: i) complementary strand synthesis, ii) replicative form (RF) replication, and iii) progeny single-stranded DNA synthesis. The first stage is catalyzed by pre-existing host cell enzyme(s); i.e., it requires no phage-induced protein synthesis.\textsuperscript{1,2} The initiation of the synthesis of the complementary DNA strand is shown to require RNA synthesis,\textsuperscript{2} and this synthesis is resistant to rifampicin, a specific inhibitor of the host cell RNA polymerase,\textsuperscript{3} both in vitro\textsuperscript{7} and in vivo.\textsuperscript{4,5} It is reported that φX174 single-stranded DNA can be converted to RF in vivo by a rifampicin-sensitive process if the normal pathway is blocked by a dnaC mutation.\textsuperscript{6} The E. coli dnaC protein is required for the initiation of cycles of chromosome replication\textsuperscript{8} as well as for the replication of φX174 RF.\textsuperscript{7} An E. coli dnaH function is also required for the replication of φX174 RF DNA\textsuperscript{8} as well as for the initiation of cycles of chromosome replication in vivo like dnaC protein.\textsuperscript{8} In cell-free extracts of E. coli, the dnaH gene product is essential for the conversion of single-stranded DNA to the RF.\textsuperscript{10} However, φX174 parental RF is formed normally in a dnaH-defective mutant of E. coli, so that the dnaH function is considered not to be required solely for the initiation of parental RF synthesis in vivo.\textsuperscript{9} Similar process as observed in a dnaC mutation is also expected to be involved in a dnaH mutation. Therefore, under a dnaH-defective condition, where only parental RF is made,\textsuperscript{9} the inhibitory effect of RNA synthesis on φX174 parental RF formation has been investigated.

E. coli HF4704S (her\textsuperscript{−}, thy\textsuperscript{−}, dnaH\textsuperscript{ts}), HF4704SU, a uracil-requiring derivative of HF4704S after N-methyl-N’-nitro-N-nitrosoguanidine mutagenesis, and a lysis defective mutant phage φX174 am3 were used. Materials and Methods used here are essentially the same as reported previously\textsuperscript{11}: synchronized cells were treated with mitomycin C, and infected with \textsuperscript{32}P-labeled φX174 am3 in the presence or absence of thymine-\textsuperscript{3}H at 43°C. After 20 min at 43°C, the cells were lysed with lysozyme and sodium dodecyl sulfate, and the lysate was extracted with phenol. DNA extracted was sedimented through linear neutral and alkaline 5 to 20% sucrose gradients. Rifampicin was added at a concentration of 0.2 mg/ml at 10 min before infection. No phage was produced when E. coli HF4704SU was infected with φX174 after starvation for 30 min or more at 27°C. When the strain was infected with phage after uracil starvation for 90 min or more at 27°C, no thymine-\textsuperscript{3}H incorporation into DNA was observed within at least 40 min after infection, and after then thymine-\textsuperscript{3}H was incorporated at a very reduced rate. Accordingly, nucleotides required for phage growth were depleted by starvation for 30 min at 27°C, and those required for DNA synthesis were also depleted for 90 min at 27°C. The turnover of RNA in the cells did not occur within 40 min after phage infection.

E. coli HF4704S was infected with φX174 under a dnaH-defective condition, and DNA was analyzed by neutral (Fig. 1a) or alkaline (Fig. 1b) 5 to 20% sucrose gradient centrifugation. Total amount of thymine-\textsuperscript{3}H incorporated into DNA in the presence of rifampicin was about 20% to those in the absence of rifampicin. \textsuperscript{32}P-label was sedimented at the positions of single-stranded DNA and RF in a neutral sucrose gradient, and of bottom and single-stranded DNA in an alkaline gradient. \textsuperscript{3}H-label was sedimented mainly at the RF position in a neutral gradient, and at single-stranded DNA position in an alkaline gradient. Total amount of \textsuperscript{3}H-label was more than that of \textsuperscript{32}P-label. Consequently, parental double stranded duplex (RF) was formed, which was mostly RFII (one of the RF strand was nicked.)

E. coli HF4704SU was infected with φX174 after uracil starvation for 70 min at 43°C. After uracil starvation, most of \textsuperscript{32}P-label was sedimented slower than single-stranded DNA position, while main peak of the label appeared at the position of single-stranded DNA in the presence of uracil (Fig. 2), suggesting that in the absence of uracil, parental RF might be formed though further replication was blocked.

The host cell dnaH function was not required for the synthesis of the φX174 complementary DNA strand of parental RF in vivo when rifampicin was present at a concentration that inhibited DNA-dependent RNA polymerase, and even when RNA synthesis was inhibited by uracil depletion. Consequently, it is most likely that E. coli dnaH function might not be involved in primer RNA synthesis of φX174 parental RF formation in the cell, despite the requirement for in vitro conversion of φX174 single-stranded DNA to the RF.\textsuperscript{10} In addition, a longish primer containing all four RNA bases might be dispensable in the cell as indicated by Hurwitz and his colleagues that in the in vitro system for φX174 DNA synthesis, only one of the ribonucleo-
Sucrose Gradient Centrifugation of Intracellular Phage DNA.

When *E. coli* HF4704S cells were grown to $1 \times 10^8$ cells/ml in TPG–CA–20T medium at 27°C, the culture was transferred to 43°C followed by incubation for 70 min, treated with mitomycin C, washed with SB, and resuspended in TPG–CA–2T medium prewarmed to 43°C. Rifampicin was added at a concentration of 0.2 mg/ml followed by incubation for 10 min at 43°C, and the cells were infected with $^{32}$P-labeled φX174 am3 at a multiplicity of infection (m.o.i.) of 5 in the presence of 2 µCi thymine-$^3$H/ml at 43°C. After incubation for 20 min at 43°C, DNA was extracted and sedimented through linear 5 to 20% sucrose gradients. a) The DNA was sedimented in a neutral condition in RPS-40 rotor of a Hitachi ultracentrifuge for 7 hr at 32,400 rpm at 4°C. b) Fractions 10 to 22 in a) were pooled, DNA was precipitated with ethanol with carrier tRNA, resuspended in Tris-EDTA, denaturated with alkali, and sedimented in an alkaline sucrose gradient underlavered with 0.5 ml of 60% sucrose cushion at 32,400 rpm in the same rotor at 4°C. The arrows indicate the marker single-stranded (SS) and RE DNA positions. $^3$H-counts were corrected for specific activity and the content of thymine base in the complementary strand of φX174 RF DNA. $^{32}$P-counts were corrected for the specific activity and the disintegration.

A. Higashi, J. Morita, H. Sakai and T. Komano

REFERENCES

4) S. Ailverstein and D. BiIlen, ibid., 247, 383 (1971).