Detection of Thermolability of Cell
Extracts from *dnaH*^{ts} Mutant of
Escherichia coli in DNA
Synthesis *in vitro*

Hiroshi SAKAI, Seiji HASHIMOTO,
Tsuyoshi FUJIWARA and Tohru KOMANO

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

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Conversion of single-stranded circular DNA of a bacteriophage ϕ X174, fd or M13 to the double-stranded DNA (replicative form II; RFII) by using extracts of *E. coli* has been investigated in a DNA-synthesizing system *in vitro*.^{1~3)} In the *in vitro* DNA-synthesizing system with ϕ X174 DNA as a template, the reaction is insensitive to rifampicin, and dependent on the gene products of *dnaB*, *dnaC(D)*, *dnaE* and *dnaG*, whereas the reaction with M13 or fd DNA is rifampicin-sensitive, and requires only RNA polymerase and the *dnaE* gene product (DNA polymerase III).^{1~3)} The ϕ X174 DNA-dependent activity is thus distinct from fd or M13 DNA-dependent one.

The ϕ X174 DNA-primed reaction has been available for investigating the role of the gene products involved in DNA replication and for purifying them.^{4~8)} Results of these studies indicate that still more protein factors are required for the ϕ X174 DNA-dependent reaction *in vitro*.

Recently, a new gene of *E. coli* has been identified, which is assumed to be involved in the initiation of chromosomal DNA replication and is designated as *dnaH*.⁹⁾ It has been reported also that the progeny RF synthesis of bacteriophage ϕ X174 requires the function specified by *dnaH* gene *in vivo*.¹⁰⁾ In the present report, *in vitro* detection of DNA-synthesizing activity of cell extracts from *dnaH*^{ts} and *dnaH*⁺ bacteria is described.

E. coli BT1000 (*F*⁺, *EndI*⁻, *Sup*⁻, *Str*^r, *thyA*⁻,

polA1⁻), *E. coli* S1814 (a *dnaH*^{ts} derivative of BT1000),⁹⁾ bacteriophage ϕ X174 and ϕ X174 DNA were used. The preparation of crude extracts and the reaction mixture were essentially the same as the reported method⁸⁾: crude extracts were prepared at 0°C by lysing the cells with lysozyme and Brij 58 followed by centrifuging the lysates at 50,000×*g* for 30 min. The reaction mixture contained 0.01 M MgCl₂, 0.05 M Tris-HCl (pH 7.5), 0.015 mM ϕ X174 single-stranded circular DNA (as nucleotides), 0.05 mM each of dATP, dGTP, dCTP and ³H-dTTP (0.4 Ci/mmol), 0.6 mM ATP and 0.1 mM each of UTP, GTP and CTP. Each assay mixture (0.05 ml) contained an appropriate amount of cell extracts. All samples were assayed for activity at 25°C for 20 min. The conversion of ϕ X174 single-stranded circular DNA to the RFII could be assayed by measuring the amount of ³H-dTMP incorporated into acid-precipitable material. Total radioactivity in nucleotide incorporated into acid-precipitable material was approximately identical to that found in the RF region of sucrose density gradients.

Crude extracts of BT1000 and S1814 incorporated ³H-dTMP into an alkaline-resistant, RNase-resistant and DNase-sensitive product in a reaction dependent on the exogenously added ϕ X174 single-stranded circular DNA. The ϕ X174 DNA-dependent activity was resistant to 10 µg/ml of rifampicin. In the absence of the exogenously added ϕ X174 DNA, the amount of ³H-dTMP incorporated was reduced to 8 percent of that in the presence of phage DNA, for which the values given in Fig. 1 and Fig. 2 had been corrected.

Extracts from S1814 exhibited the same stability as those from BT1000 when preincubated at 27°C (Fig. 1). In contrast, the activity in the extracts of S1814 was more unstable than that in the extracts of BT1000 when preincubated at 43°C (Fig. 2).

The rapid decrease in ϕ X174 DNA-dependent activity was observed when the extracts from S1814 was preincubated at 43°C. The extracts from BT1000 were active even when preincubated at 43°C (Fig. 2). These results

FIG. 1. ϕ X174 DNA-Dependent Activity in Extracts from BT1000 ($dnaH^+$) and S1814 ($dnaH^{ts}$).

Cells were grown to a concentration of 0.35 in absorbance at 610 nm at 27°C in TKB medium¹¹ containing 20 μ g thymine/ml and 0.5 mg Tris base/ml, harvested and treated as described in the text. The extracts were preincubated at 27°C and at the times indicated, 0.02 ml aliquots were withdrawn and placed in assay tubes with 0.03 ml of the reaction mixture. The activity was expressed as percent of the initial activity. 100% for the extracts of BT1000 corresponded to 3.6 pmol of dTMP incorporated per 20 min; 100% for S1814 corresponded to 4.3 pmol of dTMP. ○—○, activity in extracts of BT1000; ×—×, activity in extracts of S1814.

indicated that $dnaH^{ts}$ extracts of S1814 were quite thermolabile in the ϕ X174 DNA-dependent DNA-synthesizing activity and that $dnaH^+$ extracts of BT1000 were rather thermostable.

The thermolability observed above may be attributed most likely to the thermolabile product of $dnaH^{ts}$ gene. Although a novel factor, presumably the $dnaH$ gene product, is essential to the conversion of added ϕ X174 DNA to the RFII *in vitro*, the corresponding process *in vivo* (parental RF formation) does not require the function specified by $dnaH$ gene as reported previously.¹⁰ This *in vitro* reaction, therefore, may be different from the formation of parental RF *in vivo*. The *in vitro* and *in vivo* role of the $dnaH$ gene product should be examined in more detail.

FIG. 2. Heat Inactivation at 43°C of Extracts from BT1000 ($dnaH^+$) and S1814 ($dnaH^{ts}$) in the ϕ X174 DNA System.

Experimental details were the same as in the legend to Fig. 1. The extracts were preincubated at 43°C. The activity was expressed as described in the legend to Fig. 1. 100% for the extracts of BT1000 corresponded to 1.5 pmol of dTMP per 20 min at 25°C; 100% for the extracts of S1814 corresponded to 1.4 pmol of dTMP per 20 min at 25°C. ○—○, activity in extracts of BT1000; ×—×, activity in extracts of S1814.

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