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AN ADDITIONAL ROLE OF TRANSCRIPTIONAL ACTIVATION OF *oriλ* IN THE REGULATION OF λ PLASMID REPLICATION IN *ESCHERICHIA COLI*

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Summary. Initiation of replication of plasmids derived from coliphage λ *in vivo* is dependent on transcription at or near the replication origin, *oriλ*. However, this transcriptional activation is dispensable for λ plasmid DNA replication reconstituted *in vitro* from purified λ and *Escherichia coli* proteins. It was proposed previously that histone-like protein HU interferes with the assembly or function of the pre-primosomal complex, and transcription at or near *oriλ* abolishes HU-mediated inhibition of λ DNA replication. We found that during λ plasmid replication driven by the previously assembled replication complex (in amino acid-starved *relA* mutants), when the inhibition by HU protein should not be observed, the synthesis of λ DNA was still dependent on transcriptional activation. Moreover, in *hupA hupB* double mutants the transcription is necessary for the initiation of λ plasmid DNA replication perpetuated by the replication complex inherited by one of two daughter copies after a replication round. We conclude that transcriptional activation of *oriλ* has an additional role in the initiation of λ plasmid DNA replication beside the abolition of HU-mediated inhibition.

The dependence of λ plasmid DNA replication *in vivo* on transcription at or near *oriλ* (so called transcriptional activation of origin) in known since long (1). RNA polymerase function is also necessary for λ plasmid DNA replication *in vitro* in the system containing purified λ replication proteins (λO and λP) and a part of crude lysate of *Escherichia coli* cells, called Fraction II (2). However, initiation of λ DNA replication reconstituted with purified λ and *E. coli* replication proteins does not require transcriptional activation of *oriλ* (3). Mensa-Wilmot *et al.* (4) demonstrated that histone-like protein HU, present in the Fraction II, inhibits λ DNA replication *in*

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vitro. They found that when the *ori*λ template was transcribed by RNA polymerase, the HU-mediated inhibition of λ DNA replication was abolished. Therefore, they proposed that the transcriptional activation counteracts the inhibition of replication by HU protein. HU protein interferes with the assembly or function of the pre-primosomal complex formed at *ori*λ, as prior formation of an *ori*λ·λO·λP·DnaB nucleoprotein structure bypasses the inhibitory effect of HU on the initiation of λ plasmid DNA replication *in vitro* (4).

λ plasmid DNA replication is inhibited in amino acid-starved wild type strains (*i.e.* during the stringent response), whereas it proceeds for several hours in amino acid-starved *relA* mutants (*i.e.* during the relaxed response) (5, 6). This replication is perpetuated by the replication complex assembled before the onset of amino acid starvation and inherited by one of two daughter copies after each replication round (7). The complex contains the λO protein, which is protected from ClpP/ClpX protease by other elements of this complex (8), as well as λP and DnaB proteins (9). It seems that this type of replication of λ plasmid DNA is not restricted to amino acid-starved *relA* mutants; in λ plasmid-harboring bacteria growing in nutrient medium, every second plasmid circle bears a replication complex that originates from the preceding round of replication (7). Nevertheless, the *in vivo* system described above allows to investigate exclusively the replication driven by the "old" replication complex. The stable fraction of the λO protein, and thus the inherited replication complex, exists in both *relA*$^-$ and *relA*$^+$ strains (8, 9). It was proposed that the inhibition of λ plasmid replication in amino acid-starved *rel*$^+$ strains results from inhibition of RNA polymerase by guanosine 5'-diphosphate-3'-diphosphate (ppGpp), an effector of the stringent response (6). Since no protein synthesis is necessary for λ plasmid replication driven by the "old" replication complex (5, 6), the transcriptional activation of *ori*λ is the only transcription which could carry weight in this replication.

In the light of the above described λ plasmid DNA replication driven by the inherited replication complex, it was possible to verify the suggestion, based on *in vitro* experiments, that the role of transcriptional activation of *ori*λ consists of the abolishing of HU-mediated inhibition. If this hypothesis were true, λ plasmid replication perpetuated by the inherited replication complex should proceed during the relaxed as well as stringent response in mutants devoid of HU protein (*hupA hupB*), irrespective of eventual inhibition of RNA polymerase function. However, our results, presented below, indicate an existence of additional function of transcriptional activation of *ori*λ *in vivo*.

MATERIALS AND METHODS

Bacterial strains and λ plasmid. *Escherichia coli* K-12 strains are listed in Table 1. The λ plasmid, pAS1, has been already described (10).

Culture media and amino acid starvation. LB medium was used during construction of bacterial strains. Minimal medium 2, described earlier (6), was used in all experiments. Isoleucine starvation was induced by addition 1 mg/ml of L-valine to medium 2, according to the method described previously (6).

Table 1. *Escherichia coli* K-12 strains

Strain	Genotype	Reference
A5422	*galK hupA*16::*kan*	(14)
A5423	*galK hupB*11::*cat*	(14)
CP78	*leu arg thr his thi*	(15)
CP79	as CP78 but *relA relX*	(15)
FE1	as CP78 but *hupA*16::*kan*	This work, by P1 transduction from A5422
FE2	as CP79 but *hupA*16::*kan*	This work, by P1 transduction from A5422
FE3	as CP78 but *hupB*11::*cat*	This work, by P1 transduction from A5423
FE4	as CP79 but *hupB*11::*cat*	This work, by P1 transduction from A5423
FE5	as FE1 but *hupB*11::*cat*	This work, by P1 transduction from A5423
FE6	as FE2 but *hupB*11::*cat*	This work, by P1 transduction from A5423

P1 transduction. P1 transduction was performed as described by Silhavy *et al.* (11).
Measurement of λ plasmid DNA replication. The replication of λ plasmid was measured by estimation of the change in relative plasmid content per milliliter of bacterial culture with time. The method described by Herman *et al.* (12) was used but 8 ml samples of bacterial culture were withdrawn at indicated times.

RESULTS

Since the incorporation of radioactive thymidine may be inadequate as a measure of DNA replication when comparing the stringent and relaxed response (6, 13), we measured λ plasmid replication by estimation of the change in relative plasmid content per milliliter of bacterial culture with time. The samples of the same volume (8 ml) of bacterial culture were withdrawn at indicated times. Following DNA isolation and agarose gel electrophoresis the relative amount of plasmid DNA in the bands on an electrophoregram was estimated densitometrically. Therefore, if the replication of plasmid DNA were stopped, the relative content of plasmid DNA per milliliter of the culture should stay constant. On the other hand, an increase in the plasmid content indicates continued plasmid DNA replication.

We found that the replication of λ plasmid was inhibited in isoleucine-starved *relA*$^+$ strain containing functional HU protein as well as in the same strain but harboring *hupA*$^-$ and *hupB*$^-$ mutations (Fig.1 A and C). The replication proceeded during the relaxed response in both *relA*$^-$ *hup*$^+$ and *relA*$^-$ *hupA*$^-$ *hupB*$^-$ strains (Fig.1 B and D). However, in all investigated strains, the replication of λ plasmid was strongly inhibited by rifampicin, an inhibitor of RNA polymerase (Fig.1). These results indicate that transcriptional activation of *ori*λ is indispensable for λ plasmid replication *in vivo* even when HU function is absent.

DISCUSSION

HU-mediated inhibition of λ plasmid DNA replication was observed *in vitro* only when the pre-primosomal nucleoprotein complex *ori*λ·λO·λP·DnaB has not been

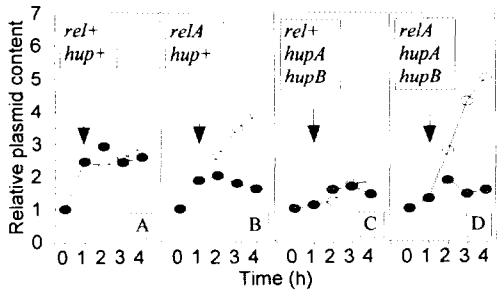

Figure 1. Relative content of λ plasmid (pAS1) per milliliter of bacterial culture in *Escherichia coli relA*$^+$ *hupA*$^+$ *hupB*$^+$ (A), *relA*$^-$ *hupA*$^+$ *hupB*$^+$ (B), *relA*$^+$ *hupA*$^-$ *hupB*$^-$ (C) and *relA*$^-$ *hupA*$^-$ *hupB*$^-$ (D) strains starved for isoleucine (empty circles), and in the same strains starved for isoleucine and treated with rifampicin (filled circles). The addition of L-valine (to 1 mg/ml), which induces isoleucine starvation, and rifampicin (to 25 μg/ml), an inhibitor of RNA polymerase, is indicated by the arrow.

formed previously (4). Transcriptional activation of *ori*λ was able to abolish this inhibition. The transcription was dispensable for λ DNA replication *in vitro* when HU protein was absent or the complex *ori*λ·λO·λP·DnaB was assembled before HU action. Therefore, it seemed that transcriptional activation of *ori*λ is necessary only for abolishing HU-mediated inhibition of replication. The finding that the replication of λ plasmid *in vivo* can be driven by the previously assembled replication complex which is inherited by one of two daughter plasmid copies after each replication round (7), allowed us to prove above mentioned hypothesis. Contrary to this prediction, we found that the function of RNA polymerase was indispensable for λ plasmid replication *in vivo* in conditions where no protein synthesis was necessary, the replication complex has been formed previously, and no functional HU protein was present. We conclude that transcriptional activation of *ori*λ has double role in the initiation of λ DNA replication. The first role would be that proposed earlier by Mensa-Wilmot *et al.* (4) *i.e.* the abolishing of the inhibition of the assembly or function of pre-primosomal complex mediated by HU protein. The second role would be necessary after the assembly of the *ori*λ·λO·λP·DnaB complex. It was proposed recently (10) that transcriptional activation of *ori*λ might be crucial for adequate positioning of two replication complexes, a prerequisite for bidirectional replication. It is worth to remind that λ plasmid DNA replication proceeds bidirectionally *in vivo* but unidirectionally in *in vitro* system reconstituted with purified replication proteins (3).

Mensa-Wilmot *et al.* (4) demonstrated that other histone-like protein of *E. coli*, IHF, was able to inhibit λ DNA replication *in vitro*, although at considerably higher concentrations than HU. The authors concluded that it is unlikely that IHF plays a major role in regulating λ DNA replication *in vivo* (4). Nevertheless, one may presume that in our experiments IHF might substitute HU in the inhibition of λ plasmid replication and the effect of *hupA* and *hupB* mutations might be masked. Thus, RNA

polymerase function would be necessary for abolishing IHF inhibition. However, if this were true, we should still observe λ plasmid replication in amino acid-starved bacteria treated with rifampicin. The replication complex has been assembled before the onset of amino acid starvation and eventual action of IHF after this step should not interfere with λ plasmid replication, thus RNA polymerase function should be dispensable. That was not the case, as the replication was strongly inhibited by rifampicin (Fig.1).

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