Replication of coliphage lambda DNA *

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Abstract

A general scheme of λ phage and plasmid DNA replication in Escherichia coli is presented, and results of in vivo experiments from the authors' laboratory are superimposed. The initiator AO functions in the assembly of the replication complex (RC) at oriA, making it a stable component of this structure. ClpP/ClpX protease-specific action on AO does not affect the regulation of replication; it only degrades the surplus of synthesized AO. The initiator AO becomes protected from proteolysis at a distinct step of the pathway of RC assembly. The once-assembled RC is inherited by one of two λ plasmid daughter copies at each round of circle-to-circle (θ) replication. The inherited, old RC-driven replication is also dependent on RNA polymerase and DnaA functions. It seems that DnaA licenses λ plasmid DNA for only one replication round, resembling the putative eukaryotic licensing factor in this respect. The AO binding to oriA does not seem to play any role in regulation of λ plasmid replication, and the Cro-autoregulatory loop may be deleted. The emerging picture shows λ plasmid circles with RCs bound to their ori, awaiting a signal triggering initiation of replication. The host DnaA initiator-regulated transcriptional activation of oriA seems to be coupled with RC assembly at the step of chaperone-mediated rearrangement of the pre-primosome. The once-assembled RC is inherited by one of two λ plasmid daughter copies at each round of circle-to-circle (θ) replication. The inherited, old RC-driven replication is also dependent on RNA polymerase and DnaA functions. It seems that DnaA licenses λ plasmid DNA for only one replication round, resembling the putative eukaryotic licensing factor in this respect. The AO binding to oriA does not seem to play any role in regulation of λ plasmid replication, and the Cro-autoregulatory loop may be deleted. The emerging picture shows λ plasmid circles with RCs bound to their ori, awaiting a signal triggering initiation of replication. The host DnaA initiator-regulated transcriptional activation of oriA may be involved in signal transmission. Inactivation of DnaA function blocks initiation of λ phage DNA replication, but the lambdoid prophage Rac compensates this defect and all parental phage DNA molecules, after one round of θ replication switch to the σ mode and produce progeny in high yield. We suspect that DnaA-regulated transcriptional activation is involved in installation and adequate positioning of two RCs, required for bidirectional replication, but in the Rac-promoted process only one RC may be installed, leading to unidirectional replication continued in the σ mode. In wild-type cells consumption of DnaA function by the rapidly replicating λ phage DNA may switch replication from bidirectional θ to unidirectional θ, and later to the σ mode; the λ circles produced earlier may play the role of Rac, which is required only when DnaA function has been inactivated prior to phage infection.

Keywords: Replication complex assembly; Regulation of initiation of replication; AO and DnaA initiators; Transcriptional activation of origin; Eukaryotic replication; Switch from θ to σ replication mode

1. Introduction

In the λ phage-infected cell of Escherichia coli, the phage double-stranded linear DNA becomes circular due to the action of bacterial ligase on its cohesive ends [1,2] (Fig. 1). Interaction with histone-like proteins partly compensates the superhelicity caused by gyrase. λ promoters are recognized by the host RNA polymerase and the immediate–early transcription starts. Already at this stage the transcription initiated at the pR promoter proceeds through the weak tR1 terminator into the λ replication region (Fig. 2). The λ early protein N in co-operation with...
A RNA and several host proteins alleviates the function of rR1 causing efficient (delayed-early) transcription of λ replication genes O and P. The initiator protein λO binds to the origin of λ DNA replication, oriλ, situated in the middle of the gene O (Fig. 2), initiating the assembly of the λ replication complex (RC). Bidirectional replication of the λ DNA circle is initiated, leading to duplication of λ circles in each round. The replication intermediates resemble the Greek θ (Fig. 1), hence this early circle-to-circle replication is called after this letter.

From 5 to 6 rounds of θ replication occur in the λ-infected cell which results in about 50 circles that code for phage proteins. The θ mode of replication cannot proceed further, probably due to exhaustion of one or more of required host proteins and due to the repression of the pR-initiated transcription by the dimeric Cro repressor protein. Hence the transcription required for the synthesis of λ replication proteins and for the activation of oriλ cannot occur. Around this time, in the middle of the eclipse period, in about 3 of 50 circles of λ phage DNA another mode of replication is initiated which results in structures resembling Greek σ (Fig. 1). This explains why the late mode of λ replication is called after this letter. It is assumed that this replication proceeds like the rolling-circle replication of φX174, but the lack of a specific nicking nuclease would require another mechanism of initiation here. Unsuccessful search for phage or host genes engaged in the switch from θ to σ mode of λ DNA replication leads to a presumption that a physiological change of phage-infected bacteria is responsible in this regulatory process. There is also a bypass pathway leading from λ circles to λ rolling circles through an ill-defined intermediate of λred recombination (Fig. 1). This bypass does not work in replication of λ gene vectors with the λred region deleted. Sigma replication produces concatemeric structures, several λ DNA units long. These units, cut out from concatemeric molecules with formation of cohesive ends, are packaged into proheads of progeny phages. The σ replication intermediates are substrates for the exonucleolytic activity of the bacterial RecBCD enzyme; however, one of phage λ early gene products, λGam, inhibits this activity (Fig. 1).

The region of λ DNA encompassing all genes and signals required for replication may be cut out; after circularization it may replicate autonomously in the bacterial cell as a plasmid. It contains λO and λP replication genes, oriλ (in the middle of λO), and the regulatory region composed of the promoter pR, operator oR, and the λcro gene. Since Cro repressor may bind to oR and block transcription of cro (and λ replication genes), this autoregulatory loop is believed to play a major role in the stable maintenance of λ plasmid in its host cell (Fig. 2). The first plasmid of this kind (called λdv) was discovered by Kenichi Matsubara in Dale Kaiser’s laboratory in 1968 in vivo from phage λvir DNA, probably by intramolecular recombination; dv stands for ‘defective virulent’ [3]. Of course, the ends of λdv plasmids were ill-defined, e.g. the left end sometimes contained the cl or even the N genes. In the era of restriction enzymes it was possible to cut out the fragment oRpR-cro-O-P from λ + DNA in a

![Fig. 1. General scheme of phage λ DNA replication. The linear DNA of the parental phage, after circularization, replicates according to the θ mode at early times after infection, later by the σ mode, producing progeny phage linear DNA. The λ plasmid replicates exclusively according to the θ mode. For details see Introduction.](https://academic.oup.com/femsre/article-abstract/17/1-2/109/632557)
Fig. 2. The generally accepted model of the regulation of \( \lambda \) plasmid replication. The structural genes are \( cro \) coding for the \( \lambda \) repressor, \( O \) coding for the unstable initiator protein binding to the origin of initiation \( ori \lambda \), and \( P \) coding for the ‘second initiator protein’ which interacts with \( An \). Transcription initiated at promoter \( PR \) (wavy line) proceeds through the weak terminator \( t 1 \), activating \( ori \lambda \). \( Cro \) repressor, active as a dimer, binds to the operator \( o \) blocking transcription. Main points of the model are: (i) due to rapid proteolysis of \( An \) initiator, the binding of newly synthesized \( An \) to \( ori \lambda \) is a rate-limiting step in \( \lambda \) plasmid replication; (ii) after each round of replication \( RC \) is disassembled and the next round starts from the naked \( ori \lambda \); (iii) replication of \( \lambda \) plasmid keeps pace with the host cell growth due to periodical \( Cro \)-depression caused by dilution of \( Cro \) in the increasing volume of the host cell. The short periods of de-repression (1) alternate the long periods of \( Cro \)-repression (0). Several reservations to this model have been presented in the text.

The \( \lambda \) plasmid replicates on average once per cell generation, in contrast to \( \lambda \) phage DNA which performs 5-6 rounds of replication in about half of this time [5]. This difference may be attributed to the fact that in a \( \lambda \) phage-infected cell the \( Cro \) repressor requires some time for reaching a concentration that makes possible the assembly of dimers, representing the active form of this protein. In \( \lambda \) plasmid-harbouring bacteria the concentration of \( Cro \) probably fluctuates around this value during the whole cell cycle. More difficult is to hypothesize, why \( \lambda \) plasmids do not initiate \( \sigma \) replication. In contrast to \( \lambda \) plasmid-harbouring cells, the \( \lambda \) phage-infected ones are under a physiological stress, and induction of stress protein(s), combined with exhaustion of host replication protein(s) by the rapidly replicating phage may be responsible for the switch from \( \theta \) to \( \sigma \) replication.

The \( \theta \) mode of \( \lambda \) DNA replication is reasonably well understood, mostly because \( \lambda \) plasmid DNA replication could be studied in vitro. The trials to study the \( \sigma \) replication in vitro were unsuccessful until now. The in vivo study is difficult because only 3 of 50 \( \lambda \) circles per cell initiate replication according to this mode and the switch from \( \theta \) to \( \sigma \) does not seem to be well synchronized [5]. The results of in vivo experiments performed recently in our laboratory provide new information to the model of \( RC \) assembly that has been constructed on the ground of in vitro research. Besides, they show that the once-assembled \( RC \) is inherited by one of two daughter plasmid circles after a replication round, and describe the phage and host functions required for the old \( RC \)-driven replication pathway. These studies contradict the generally accepted model of regulation of \( \lambda \) plasmid replication and suggest a new approach to this problem. The study of \( \lambda \) plasmid replication helped us to investigate the rolling-circle replication of \( \lambda \) phage DNA and to propose a new hypothesis concerning the switch from \( \theta \) to \( \sigma \) replication mode.

2. Assembly of the \( \lambda \) replication complex

The pathway of the assembly of \( \lambda \) preprimosomal complex has been reconstructed in vitro, using purified phage and bacterial proteins (Fig. 3) by the group of Roger McMacken in Baltimore [6] and the joint effort of laboratories of Maciej Żylcz in Gdańsk.
Protection of \( \lambda O \) protein from proteolysis

\[
\text{ori} \lambda \xrightarrow{\text{DnaB} \cdot \lambda P} \text{ori} \lambda \cdot \lambda P \cdot \text{DnaB} \\
\text{DnaA, RNA pol, DnaJ, DnaK, GrpE} \\
\text{Primase, DNA Pol III} \\
\text{ori} \lambda \cdot \lambda O \cdot \lambda P \cdot \text{DnaB} \\
\text{ori} \lambda \cdot \lambda O \cdot \lambda P \cdot \text{DnaB} \\
\text{ori} \lambda \cdot \lambda O \cdot \lambda P \cdot \text{DnaB} \cdot (\lambda P) \\
\text{ori} \lambda \cdot \text{RC}
\]

Fig. 3. Pathway of the \( \lambda \) replication complex (RC) assembly, based mostly on the in vitro experiments. The data from the in vitro studies on the protection of \( \lambda O \) initiator from proteolysis are from the authors' laboratory [30]. In contrast to other chaperone proteins, DnaJ and GrpE, the DnaK function is required for \( \lambda O \) stabilization, raising a possibility of an earlier access of DnaK to the pathway in vivo, than that shown above. The possible stage at which the DnaA-regulated transcriptional activation of \( \text{ori} \lambda \) occurs (DnaA, RNA pol) is suggested by the data from the in vivo studies performed in the authors' laboratory [22]. Further explanations can be found in the text.

and Costa Georgopoulos in Salt Lake City and Geneva [7]. The initial complex \( \text{ori} \lambda \cdot \lambda O \) [8] attracts the complex of \( \lambda P \) with bacterial helicase DnaB due to \( \lambda O \cdot \lambda P \) interaction [9] forming a pre-primosomal \( \lambda O \cdot \lambda P \cdot \text{DnaB} \) [8]. Since \( \lambda P \) is a strong inhibitor of DnaB helicase [10], it should leave this complex or be translocated in order to release DnaB's activity. The concerted action of three chaperone proteins, DnaJ, DnaK and GrpE results in such rearrangement that DnaB regains its helicase activity and is able to interact with bacterial primase DnaG [11]. First experiments, with omission of GrpE, suggested that \( \lambda P \) dissociates from the complex [12–15]; however, when the in vitro reaction contained all three chaperone proteins (a situation more closely resembling in vivo conditions), \( \lambda P \) was still found in the complex

Therefore, \( \text{ori} \lambda \cdot \lambda O \cdot \text{DnaB} \cdot (\lambda P) \) symbolizes the last pre-primosomal complex of the pathway. In comparison with the strong affinity of \( \lambda P \) to DnaB, the interaction of the mutant \( \lambda P \) protein with DnaB is so weak that the mutant DnaK756 protein, in cooperation with DnaJ and GrpE, unable to promote \( \lambda \) plasmid replication in vitro, does it when \( \lambda P \) is substituted for \( \lambda P \) [55]. In the generally accepted scheme DnaJ binds to the \( \lambda O \cdot \lambda P \cdot \text{DnaB} \) pre-primosome as the first of chaperone proteins and the DnaK–GrpE complex binds later [17]. The rearrangement of the pre-primosome by the DnaJ- and GrpE-stimulated ATPase activity of DnaK [18] may be connected with its entry between two complementary \( \lambda \) DNA strands. There are two nearly identical 14mers unique in the whole \( \lambda \) genome T-G-G-A-T-C-T-A-T-C/G-A-A-C/A-A symmetrically flanking \( \text{ori} \lambda \) immediately to the left of \( \lambda O \) (at the start of the \( \text{oop} \) RNA), and on the junction of \( \lambda O \) and \( \lambda P \) genes. The similarity of a part of their sequence to the left fragment of the AT-rich R-13mer G-A-T-C-T-C-T-A-T-A-A-G in the origin of \( E. coli \) DNA replication, \( \text{ori} C \), leads to speculation that they are entry sites for DnaB-AP and DnaB-DnaC, respectively. The importance of easily melting AT-rich sequences around replication origins is well known; there exists such a region to the right of \( \text{ori} \lambda \). Wrapping of \( \lambda \) DNA around a complex of several \( \lambda O \) molecules (O-some) produces a distortion of double-stranded DNA helpful for the entry of DnaB helicase, which should be later translocated to the DNA synthesis start sites. In contrast to the in vitro system of replication reconstituted from purified proteins [19], the transcriptional activation of \( \text{ori} \lambda \) is absolutely required for initiation in vivo [20], probably for opening the DNA duplex. Several observations reviewed in the next chapter, as well as our recent finding that elimination of \( \text{dnaA} \) function severely decreased the \( pR \)-initiated transcription [21] strongly suggest that this process is \( \text{dnaA} \)-regulated. We found that \( \text{dnaA} \) function blocks the assembly of RC at 43°C when \( \lambda P \) is substituted by \( \lambda P \), and suggested that the \( \text{dnaA} \)-regulated transcription is coupled with the chaperone-mediated rearrangement of the pre-primosome [22]. The DnaB helicase released from \( \lambda P \) inhibition is able to bind DnaG primase and to provide it with single-stranded DNA template by unwinding \( \lambda \) DNA. The final step in \( \lambda \)
replication complex assembly is the binding of the host DNA polymerase III holoenzyme [11,15].

In the in vitro replication of the oriC plasmid the DnaA protein, after having initiated the replication complex assembly, leaves it and may be even re-used in another initiation [23]; this fits to the slow-stop phenotype of dnaA mutants. In the in vitro studies on λ DNA replication one could not find λO protein in RC [14,15], and this was compatible with the well-known rapid proteolytic degradation of λO in vivo [24,25]. However, the in vivo experiments strongly support the idea that λO is a stable component of RC. In the phage λOts-infected bacteria λDNA replication stopped abruptly after a shift to a non-permissive temperature [26]. We have shown that a fraction of λO becomes resistant to proteolysis [27], and λ DNA replication that occurs in the absence of λO synthesis is still λO-dependent [28]. We concluded that λO remains in the assembled RC and is protected from proteases in this structure. Since the once-assembled λO-containing RC is inherited by one of two daughter λDNA circles [29], and λ phage DNA replication is λO-dependent also at later times after infection [26], we suggest that the λO-containing RC assembled for θ replication serves for the σ mode, too.

We have asked at which step of the pathway of RC assembly does the stabilization of λO occur. In accordance with the in vitro established order, we found that λP and DnaB functions are, but those of DnaJ and GrpE are not required for the protection of λO from proteolysis. Therefore, our results suggest that the first λO protecting structure of the pathway of RC assembly is the λO–λP–DnaB pre-primoosome. The next step of the pathway, the chaperone-mediated rearrangement of the pre-primoosome, is not essential for λO stabilization (Fig. 3). However, in contrast to other chaperones, the DnaK function was required for the protection of λO from proteolysis, suggesting an earlier access of DnaK to the pathway of RC assembly in vivo [30].

3. Regulation of λ plasmid replication

The generally accepted model of the regulation of λ plasmid replication (Fig. 2) emphasized the rapid decay of the newly synthesized λO initiator protein and the Cro-autoregulatory loop. The interplay of a positive (λO) and a negative (λCro) control would result in an elegant model of precise regulation of initiation [31]. Another positive control, the transcriptional activation of oriλ, was regarded as a process cooperating with the major event—the binding of λO protein to oriλ followed by the RC assembly. The rapid decay of λO would prevent overinitiation. The wave of pR-initiated transcription caused by Cro-depression would lead to only one initiation event. The binding of the unstable initiator to the origin of replication was regarded as a rate-limiting step in λ DNA replication and served as a model for other replicons. In this model it was tacitly assumed that after each round of λ plasmid replication the RC is disassembled and the next round starts from the naked oriλ. We present reservations to this model in the following paragraphs.

We have shown that in amino acid-starved λ plasmid-harboring E. coli relA cells, the λO protein is degraded as rapidly as in rich media and is not synthesized [27]; nevertheless, λO-dependent λ plasmid replication occurs for several hours [28]. We have solved this paradox by showing that (i) there is a fraction of λO which, as a component of RC, resists proteolysis [29]; and (ii) the once-assembled, λO-containing RC does not disassemble after a round of replication, but is inherited by one of two daughter copies [29]. Moreover, studying the round of replication before the onset of amino acid starvation, we found that only a half of plasmid circles is able to enter into the next replication round [29]. This finding and a strict coincidence of λ phage or plasmid DNA replication with the occurrence of the stable λO fraction in all conditions studied, including rich media, inclined us to suggest that in standard conditions a round of λ plasmid replication may be initiated by the inherited old RC or by the newly assembled RC (Fig. 4). In the specific conditions of amino acid-starved E. coli relA cells only the old RC-driven replication could occur. Studying this clean system we found that the replication driven by the inherited λO-containing RC was exempt from the λCro control [28,29]; nevertheless, it still required RNA polymerase and DnaA functions [32], presumably involved in the same regulatory process. It is amazing that in this system the λ plasmid seemed to replicate at regular intervals of the same
Fig. 4. Model of λ plasmid replication taking into account the inheritance of the once-assembled parental replication complex (RC) by one of two daughter copies. The assembly of RC on the naked oriλ may occur soon after termination of the replication round due to a transient Cro-derepression. In the long periods between replication rounds, the RC-oriλ complexes may await for a signal triggering initiation, as it has been suggested for ORC-ARS complexes in eukaryotic replication. The host DnaA initiator-regulated transcriptional activation of oriλ may be involved in the signal transmission. The RC assembly cannot occur in λ+ plasmid-harboring amino acid-starved relA cells due to lack of AO synthesis, or in λPts1 plasmid-harboring wild-type cells at a non-permissive temperature due to λPts1-dnaA+ incompatibility. In these conditions only the old RC-driven replication may occur as shown in the lower part of the figure.

Further studies revealed that this replication is independent of the functions of AP and DnaJ required in RC assembly, but DnaK and GrpE functions were still required [32]. Concerning AP function, its dispensability at later times after phage infection was already shown [26]. This is consistent with our suggestion that the RC assembled (with the help of λP) during early θ replication functions later in the σ replication. Elimination of λP function by a temperature shift in the replicating λPts1 plasmid eliminated the pathway starting from the naked oriλ, leaving the one driven by the inherited RC and permitting to study this pathway in rich media (our unpublished results).

The discovery of the ClpP/ClpX protease that is responsible for essentially all AO protein turnover in vivo [33–35] opened an opportunity to check if the rapid decay of AO has any meaning in the regulation of λ plasmid replication, including the pathway starting from the naked oriλ. The absence of this enzyme did not affect λ plasmid efficiency of transformation nor copy number [36], finally ruling out the hypothesis of AO as the limiting protein. The ClpP/ClpX protease would only degrade the surplus of the newly synthesized AO protein.

The emerging picture (Fig. 4) shows that in the long periods between replication rounds at least a half, if not all, of λ plasmid circles are provided with replication complexes bound at their origins of replication. This picture is reminiscent of the recent discoveries on the interaction of origin recognition complex (ORC) with origin, called autonomously replicating sequence (ARS) in the yeast Saccharomyces cerevisiae. ORC plays a fundamental role in the initiation of DNA replication from ARS. The strength of ORC's DNase I footprint in permeabilized unsynchronized cells suggests that it is probably bound to ARS through most of the cell cycle [37,38]. Therefore binding of ORC to an origin is not likely to signal initiation of replication. Instead, some other cell-cycle-related event(s) must trigger initiation. The same conclusion is valid for the initiation of λ plasmid DNA replication.

The Cro-autoregulatory loop is an intrinsic element of the generally accepted model of the regulation of λ plasmid replication (Fig. 2). In this model, the replication of λ plasmid keeps pace with the host cell growth due to periodical Cro-derepression of λ transcription caused by dilution of Cro in the increasing volume of the host cell. However, we found that autoregulation of Cro repressor synthesis is dispensable for the stable maintenance of a λ plasmid in its host [36]. The λR-pr–cro–tR1 region of a λ plasmid has been exchanged for the operator-promoter region of the lac operon, creating the plasmid pAS3 (Fig. 5). In this plasmid the transcription of the λ replication region λO-λP is under control of the chromosomal lactose operon repressor, LacI. The
Fig. 5. Cro-autoregulatory loop does not seem to play a decisive role in the regulation of \( \lambda \) plasmid replication. This has been demonstrated by construction of a \( \lambda \) plasmid devoid of the regulatory region \((\sigma_R\, p_R-cro-r_{m})\) of the classic \( \lambda \) plasmid. Transcription of the \( \lambda \) replication region occurs from the lactose operon promoter. However, when the \( \sigma_R\, p_R-cro \) fragment is present in the classic \( \lambda \) plasmid, Cro-autoregulation contributes to the overall regulation of \( \lambda \) plasmid replication, as proved by several authors.

incomplete repression is probably responsible for the maintenance of pAS3 in 10–20 copies per cell at 37°C. Curiously enough, induction by IPTG (1 mM) raises the copy number of this plasmid only to about 30 per cell, not affecting cell viability. We presume that the frequency of replication initiation cannot depend exclusively on the transcriptional initiation by RNA polymerase in this plasmid. Rather, it should be regulated by a factor cooperating with this enzyme. When the \( \sigma_R\, p_R-cro \) system is present in \( \lambda \) plasmid, it certainly influences the frequency of transcriptional initiation from \( p_R \) and, finally, the plasmid copy number, as proven by Matsubara [31]. The absence of this system, however, reveals the existence of another regulatory process, which will be presented further in this chapter.

We finally came to the conclusion that the generally accepted model of the regulation of \( \lambda \) plasmid replication (Fig. 2) should be corrected, since its main element, binding of \( \lambda O \) to \( ori\lambda \) is not involved in the triggering of initiation of replication, and the Cro-autoregulatory loop does not seem to play a decisive regulatory role. The last of the known elements of the model, the transcriptional activation of \( ori\lambda \), probably regulated by the DnaA function, remains as a major element of the rate-limiting step in \( \lambda \) DNA replication [39–41].

In \( E. coli \), the DnaA protein seems function in two processes important for the initiation of replication. The best known is its role in recognizing \( oriC \) and helping to assemble the replication complex [42]. The second, less investigated role, may consist in the control of \( oriC \) activation by regulation of transcription starting from the promoters in and around \( oriC \) [43]. The \( \lambda O \) initiator protein represents an equivalent of the host DnaA initiator only in the first respect, in origin recognition. We postulate, however, that DnaA’s second function, probably crucial in triggering initiation, the regulation of transcriptional origin activation, refers to both replicons, \( E. coli \) and \( \lambda \). The \( \lambda \) plasmid derives from a temperate phage which evolved to exploit host molecular mechanisms during evolution and one of them may concern the cell-cycle-related triggering of initiation of replication.

By binding to specific nucleotide sequences on DNA, called DnaA boxes, DnaA protein performs its functions: recognizes \( oriC \), inhibits or activates initiation of transcription or causes termination of this process. Walter Messer and his colleagues in Berlin defined the consensus sequence of a DnaA box that functions in vivo in the presence of DnaA as a transcriptional terminator [44]. In accord with the putative role of DnaA in \( \lambda \) DNA replication, there are no DnaA boxes in or around \( ori\lambda \), but we found five such sequences between \( p_R \) and the left end of \( \lambda O \) and one in \( \lambda O \), close to its left end. Three of these six DnaA boxes remained intact in pAS3. We postulate that DnaA’s activity in the transcriptional control is involved in regulation of initiation of \( \lambda \) plasmid replication.

Neither DnaA protein nor RNA polymerase is required for the proper assembly of RC in vitro; however, both these functions seem to be required in vivo. In a striking contrast to \( \lambda^+ \), the initiation of replication of \( \lambda Pts1 \) phage DNA at 43°C was completely blocked by the DnaA function [22]. Nevertheless, in these conditions \( \lambda Pts1 \) protein was able to participate in RC assembly at least to the step of the first \( \lambda O \)-protecting structure, most probably to the \( ori\lambda \)-bound \( \lambda O-\lambda Pts1-DnaB \) preprimosome [30,45]. We presume that this is the site and time of action of the DnaA-regulated transcription, revealed by the incompatibility of DnaA and \( \lambda Pts1 \). Hence the coupling of DnaA-regulated transcription with RC assembly would occur at the step of chaperone-mediated rearrangement of the pre-primosome [22]. We have also shown that RNA polymerase and DnaA functions are required for initiation of replication of \( \lambda \) plasmid DNA by the inherited, old RC [32]. Our observations presented in the next section suggest that DnaA-regulated transcription is crucial for proper
orientation of two RCs required for bidirectional initiation. This regulatory process has not been reconstructed in vitro until now, and in the systems reconstituted in vitro from purified proteins the replication of λ plasmid proceeds in one (to the right) direction. We propose that the installation and proper orientation of the pre-primosome or the inherited RC is the final result of a DnaA-regulated pathway called transcriptional activation of ori λ.

There are several observations suggesting mutual interactions of DnaA, DnaB, AP and RNA polymerase. The first was the suppression of cold sensitivity of E. coli dnaAcos by λP + and some of AP mutations [46] that prevented overinitiation of chromosomal replication in this peculiar bacterial mutant [47]. This suggested an interaction of AP with DnaA, probably through DnaB; physical interaction of DnaB with AP and DnaA is well documented [10,48]. Also an interaction of RNA polymerase with AP and DnaB in λ replication was suggested [49]. Interaction of the AP-DnaB complex with DnaA, as well as of DnaA with RNA polymerase, was proposed in order to explain another set of experiments [4]. The study on dnaA-dependence of λ plasmid replication revealed that only one of two λ plasmid daughter copies was able to enter a round of replication when the dnaA function has been inactivated. The replication, rifampicin-sensitive, performed in the absence of dnaA function was restricted to one round only [4]. Hence, the role of DnaA would be to license the λ plasmid DNA for only one replication round; in this respect, DnaA would correspond to the putative licensing factor of eukaryotic DNA replication [50].

Experiments on the early embryonic cell cycle of frogs (Xenopus laevis) performed mainly in Ron Laskey's laboratory in Cambridge and supported later by the studies on yeast led to the idea of a cytoplasmic replication factor that would bind to the chromosomal origins at mitosis, when the nuclear membrane is broken down and license DNA to initiate replication at the appropriate time in the next S phase. Licensing factor would be inactivated or degraded in this respect, DnaA would correspond to the putative licensing factor of eukaryotic DNA replication [50].

4. Switch from θ to σ mode of phage λ replication

We found that, after elimination of DnaA function, one round of λ plasmid θ replication may occur [4], and suggested that in bacteria devoid of this function the infecting λ phage DNA may enter into a unique round of θ and then switch to the presumably dnaA-independent σ mode. This hypothesis appeared to be generally true under one condition: the bacteria should contain the lambdoid prophage Rac in the chromosome or on a F' plasmid, or harbor a plasmid with Rac right half cloned [22]. This condition was totally unexpected, and we discovered it by chance. We do not know whether the role of Rac is active (by the action of rac genes), or passive (serving as a DNA partner in the formation of a transient heteroduplex), but our lack of success in cloning a putative important rac gene, and the existence of strong homology with λ DNA in the right half of rac [52] incline us to the second alternative. In dnaAts Δrac cells initiation of phage λ DNA replication was blocked at 43°C, revealing its dnaA dependence. However, when only rac was provided, all parental DNA molecules entered into one round of θ replication continued in the σ mode and the phage progeny was efficiently produced. The σ mode of replication has been verified by genetic and density shift experiments, and by electron microscopy. The above-presented observations suggest that the rac-promoted process substitutes, at least in part, for the dnaA-regulated transcription [22].

Hatch Echols, Sue Wickner, Roger McMacken and their colleagues put forward a hypothesis concerning the mechanism of initiation of σ replication of phage λ DNA [53]. It was postulated that σ may be preceded by one round of unidirectional θ replication initiated at ori λ, followed by the displacement of the 5' end of the newly synthesized leading strand by its 3' end. We would like to supplement this hypothesis: the RC once-assembled at ori λ, after one round of unidirectional θ replication, is not disassembled but may continue its function in the σ mode later on. We propose, that in the absence of dnaA-regulated transcription involved in bidirectional initiation, the less specific rac-promoted process (transient heteroduplex formation?) results in unidirectional initiation of replication. In λ + -infected
wild-type E. coli cells, the switch from bidirectional \( \theta \) to unidirectional \( \theta \), and later to \( \sigma \) replication would result due to the consumption of \( dnaA \) function by the rapidly replicating phage DNA. In \( dnaA^+ \) cells the \( \lambda \) DNA circles produced earlier in \( \theta \) replication may substitute for \( rac \), which is required only when \( dnaA \) function has been inactivated prior to phage infection [22].

Our findings concerning \( \lambda^+ \) phage DNA replication resulted from our studies on an unorthodox replication of \( \lambda Pts1 \) mutant at 43°C. Contrary to \( \lambda^+ \), in \( dnaA^+ \Delta rac \) cells at 43°C some of the parental \( \lambda Pts1 \) phage DNA molecules initiated one round of \( \theta \) replication. Probably the weak binding of Pts1 protein to DnaB helicase demonstrated in vitro [55] was responsible for this effect. When \( rac \) has been provided, all parental \( \lambda Pts1 \) phage DNA molecules behaved as \( \lambda^+ \), switching to \( \sigma \) after one \( \theta \) round and producing progeny in high yield at 43°C [22]. At first the phage progeny production by a \( Pts \) mutant at a non-permissive temperature was taken by us as a piece of evidence of our (wrong) hypothesis of dispensability of \( \lambda P \) function in \( \lambda \) phage replication in the absence of \( dnaA \) function. Now we conclude that sometimes wild ideas may produce interesting results.

5. Conclusions

Involvement of DnaA in the origin transcriptional activation may be studied easier for \( oriA \) than for \( oriC \), because this protein has additionally to promote replication complex assembly at \( oriC \). Due to a good knowledge of molecular genetics of \( \lambda \) and \( E. coli \) both these models may be of great value for those who study analogous problems in eukaryotes. Concerning the still mysterious switch from \( \theta \) to \( \sigma \) mode, it seems that, due to synchrony of initiation and efficiency of replication, a further study of the described experimental system is promising.

Twenty years ago Hatch Echols wrote a paper: "some unsolved general problems of phage \( \lambda \) development", in an issue dedicated to the late Denise Luzzati, and the first chapter was entitled "regulation of DNA replication" [54]. This opinion of our unforgettable colleague and friend seems to be still valid, in spite of a substantial progress in this field.

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