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DnaA- and PriA-DEPENDENT PRIMOSOMES: TWO DISTINCT REPLICATION COMPLEXES FOR REPLICATION OF *Escherichia coli* CHROMOSOME

Hisao Masai[1] and Ken-ichi Arai.

Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo, 108, Japan

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1. SUMMARY

Enzymatic analyses of primosome assembly at chromosomal and plasmid origins as well as that at single-stranded replication origins revealed the presence of two distinct primosomes in *Escherichia coli* for primer RNA synthesis and duplex unwinding. A DnaA-dependent primosome is assembled at *oriC*, the chromosomal origin of *Escherichia coli*, as well as at the A site, a single-stranded DNA hairpin containing a dnaA box sequence within its stem. In contrast, PriA protein recognizes a hairpin, called n'-*pas* (primosome assembly site), and initiates assembly of the ϕX174-type PriA-dependent primosome in conjunction with other prepriming proteins. Genetic analyses of the prepriming proteins required specifically for the latter primosome strongly suggested that it is responsible for RecA-dependent, DnaA/*oriC*-independent replication of the *Escherichia coli* chromosome. Furthermore, primosome assembly in replication of various plasmids may also be classified into either DnaA-dependent or PriA-dependent type. We propose that *Escherichia coli* possesses two distinct, mutually exclusive primosomes which are differentially utilized by the chromosome as well as by the plasmids. PriA protein
appears to be conserved in a wide range of prokaryotic species, and we will also discuss possible biological function of the PriA-dependent primosome in the process of responses to DNA damages.

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[1] To whom correspondence should be addressed at: Dr. Hisao Masai, Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo, 108, Japan. Tel #: 81-3-5449-5661; Fax #: 81-3-5449-5424.E-mail: hisao@ims.u-tokyo.ac.jp

2. INTRODUCTION

The process of DNA replication requires assembly of a replication complex, primosome, at the origin. The primosome is believed to be responsible for primer RNA synthesis of lagging strand and for duplex DNA unwinding, which are mediated by primase and DNA helicase, respectively, present in the protein complex. In conjunction with twin DNA polymerases and a swiverase which relieves torsional tension accumulating in advance of a replication fork, a primosome may contribute to efficient concurrent replication of leading and lagging strands at a replication fork. The molecular architecture of the primosomes have been studied in most detail in *E. coli*, leading to discovery of two structurally distinct primosomes. Molecular genetic analyses of protein components of the primosomes revealed their biological function of these protein complexes in replication of the *E. coli* chromosome as well as in that of plasmid replicons.

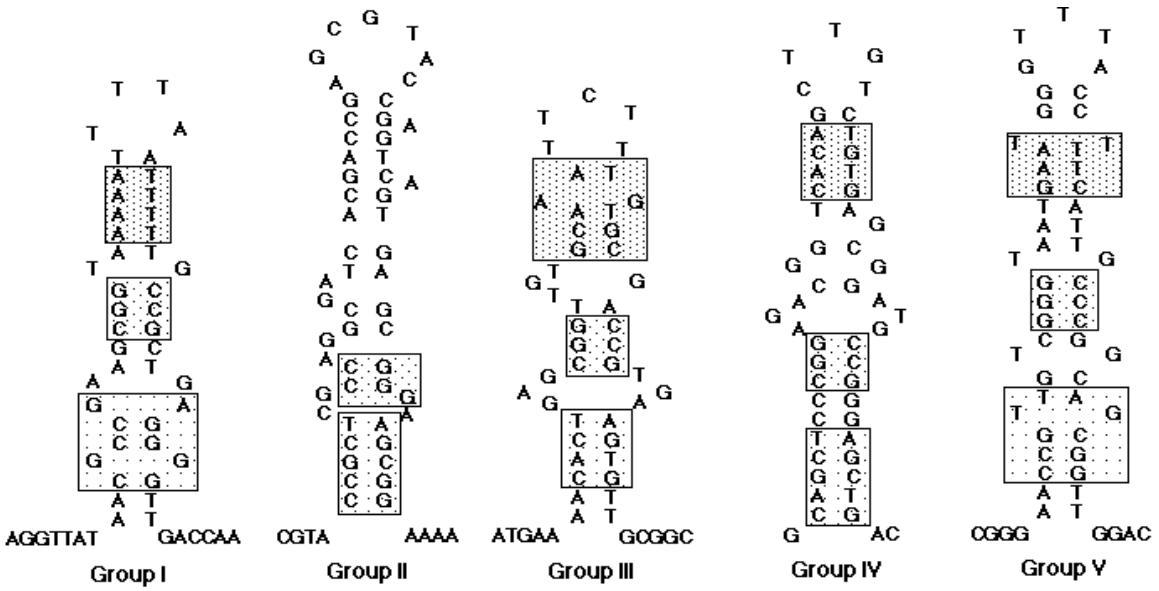

Figure 1: Nucleotide sequences and possible stem-loop structures of n'-*pas* isolated from various plasmid DNAs. Eight newly isolated n'-*pas* in addition to previously reported ones were classified into five groups on the basis of their primary structures. n'-*pas* of ϕX174 phage, R100 plasmid and F•f2b belong to group I, n'-*pas* on the lagging strand of ColE1-type plasmids (*ssi*A) and n' sites of F•f5, F•f7 and ColE2 plasmid to group II, n'-*pas* of the F•f2a to group III, n'-*pas* on the leading strand of ColE1-type plasmids (*ssi*B) to group IV, and n'-*pas* from Rts1 plasmid to group V. Each group is represented by the sequences of the first listing described above. The sequences indicated by boxes show some sequence similarities, although none of them are conserved among all the groups. The group I and group II were described before (15).

3. ϕX174-TYPE (PRIA-DEPENDENT) PRIMOSOME

3.1 Primosomal proteins for assembly of the ϕX174-type primosome (table 1)

In *E. coli*, a primosome was first discovered from the study of SS to RF replication of single-stranded DNA phage ϕX174 (1, 2). Assembly of this primosome is initiated by recognition of a specific hairpin structure (called n' site or n'-*pas* [primosome assembly site]) present on the ϕX174 genome by protein n' (later redesignated as PriA); (3, 4, 5), which is followed by assembly of a preprepriming complex through association of proteins n (PriB), n'' (PriC), and i (DnaT). A heterohexmeric DnaB-DnaC protein joins the complex to form a preprimosome, which moves along a DNA strand while periodically associating with primase to generate primer RNAs (1, 2, 6, 7). The preprimosome can be physically isolated by gel filtration, which can support efficient primer RNA synthesis and DNA chain elongation upon addition of primase and DNA polymerase III holoenzyme (5, 6).

3.2 n'-*pas* isolated from varoius plasmids

n'-*pas* on the ϕX174 DNA can adopt a stable secondary structure which is resistant to exonuclease VII digestion and can activate ATPase activity of PriA protein (3, 8). On ColE1 plasmid, sequences which could support efficient SS to RF conversion of an origin-defective M13 phage were discovered (9). The sequences were discovered on a related plasmid, pBR322, which could stimulate ATPase activity of PriA protein when converted to single-strand (10).

Later, it was demonstrated that these sequences could support assembly of the ϕX174-type primosome *in vitro* (11, 12). We have screened the genomes of various plasmids for the presence of n'-*pas* by utilizing a derivative of single-stranded M13 phage DNA which lacks the complementary strand origin (13). This defective phage generates only small, turbid plaques by itself. However, upon cloning of a sequence capable of directing efficient primer RNA synthesis, it can form big, clear plaques (14). Enzymatic analyses of primer RNA synthesis revealed that many of these so called *ssi* (single-strand initiation sequences) were functionally equivalent to n'-*pas* (15). Comparison of phage- and plasmid-derived n'-*pas* lead to classification of them into five groups on the basis of their primary structures (15); (Figure 1). n'-*pas* sequences in each group are capable of forming a stable secondary structure. The significance of these secondary structures was suggested by the conservation of the base paring in the stems in spite of divergence of primary sequences within the members of each group.

In contrast to *oriC* plasmid replication *in vitro*, which requires ATP-form of DnaA protein, the ABC primosome can be assembled on A site with ADP-form of DnaA protein and the DnaA-A site complex can be isolated in the absence of ATP. However, formation of an isolatable preprimosome complex requires the presence of ATP or ATPgS, which stabilizes heterohexamers formed with DnaB and DnaC proteins. DnaB protein is delivered to the DnaA-A site complex by virtue of its association with DnaC, which may interact with DnaA protein. The preprimosome can translocate on SSB-coated single-stranded DNA with energy supplied by hydrolysis of ATP, dATP or dCTP (21). The hydrolysis of the nucleotide is required also to facilitate the release of DnaC protein from DnaB-DnaC complex (22).

DnaB protein hydrolyzes ATP, CTP and GTP but not deoexynucleotides (23, 24). In contrast, the isolated ABC preprimosome hydrolyzes dATP, dCTP and to some extent dGTP, in consistent with its ability to utilize these nucleotides for helicase activity (21). Helicase activity of the ABC preprimosome is maximally activated by 100 to 200 µM ATP, whereas more than 1 mM of ATP is required for maximum activation of the helicase activity by DnaB protein alone (24). The preprimosome contains DnaA and DnaB proteins but most likely not DnaC protein. Among the proteins required for the primosome

4. DnaA-DEPENDENT PRIMOSOME

4.1 *oriC* primosome (table 1)

The 4700 kb *E. coli* chromosome is replicated from *oriC*, located at 83 min on the chromosome, and initiation at *oriC* strictly depends on DnaA protein (16). ATP-bound DnaA protein binds to the *oriC*, and changes the conformation of the origin sequences (17, 18). Localized melting of the three repeats of the 13mer sequences permits loading of DnaB/DnaC helicase. The *oriC* preprimosome, generated at the *oriC* sequence with DnaA, DnaB and DnaC proteins, can be isolated by sucrose gradient and addition of primase and DNA polymerase holoenzyme III together with SSB and gyrase to the isolated preprimosome can sustain DNA synthesis of the entire plasmid (19).

4.2 ABC primosome (table 1)

A *ssi* cloned from R6K plasmid did not support assembly of the ϕX174-type primosome *in vitro*, nor did it support primer RNA synthesis with any known priming enzymes. We discovered that SS to RF replication of a single-stranded phage containing this *ssi* was dependent on DnaA protein. We were able to reconstitute the replication with purified proteins including DnaA, DnaB, DnaC, primase, SSB and DNA polymerase III holoenzyme (20). The *ssi* is capable of forming a secondary structure, and its stem contains a dnaA box sequence. DnaA protein specifically recognizes the stem and forms a complex which is isolatable by gel filtration. Hence, we have named this *ssi* A site and the complex ABC primosome. assembly, only DnaB protein is capable of hydrolyzing nucleotides. These results indicate that assembly of the ABC preprimosome somehow leads to functional and/or structural alteration of DnaB helicase, which enables it to utilize low concentration of ATP as well as deoxyribonucleotides. dATP can not only support helicase activity but also support priming and replication by the ABC primosome, since the A site-dependent replication, which has been suppressed by the presence of ATPgS, can be reactivated by dATP in the absence of any ribonucleotides (21).

4.3 ABC primosome can support progression of replication forks

pBR322 plasmid contains an n'-*pas* on the lagging strand template, and efficient lagging strand synthesis depends on the assembly of the ϕX174-type primosome at this n'-*pas*, in the absence of which replication intermediates containing only the nascent leading strand are accumulated *in vitro* (25, 26). Furthermore, lagging strand synthesis *in vitro* is completely suppressed in the presence of anti-DnaT protein antibody, which inhibits assembly of the ϕX174-type primosome. Replacement of the n'-*pas* with A site on pBR322 restored the activity to synthesize lagging strand in the absence of DnaT protein (20). The same plasmid is capable of replication in the *priA1::kan* strain, in which the wild-type pBR322 cannot be replicated due to lack of the ϕX174-type primosome assembly (27). These results demonstrate that ABC primosome can replace the ϕX174-type primosome in replication of pBR322 for lagging strand synthesis and duplex unwinding at the replication fork.

5. DnaA/*oric*-INDEPENDENT REPLICATION OF THE *E. COLI* CHROMOSOME

The *E. coli* chromosome can be replicated in a manner independent of DnaA and *oriC* under certain conditions (Table 2). These altered modes of the chromosomal replication are called stable DNA replication (SDR), since they can continue stably in the absence of protein synthesis (28, 29). In the presence of DNA damaging agents or any treatment which temporarily halts the progression of replication forks, inducible SDR (iSDR) is observed. iSDR depends on RecA as well as on RecB and RecC. It is proposed that iSDR is initiated from a double-strand break (DSB) which is introduced in or near *oriM* (origin of SDR) (29). The linear end is converted to

Table 1 Components of the two primosomes for *E. coli* chromosomal replication

Site of primosome assembly	*oriC*, A-site	n'-*pas*, D-loop, R-loop
Recognition	DnaA protein	PriA protein
Auxiliary proteins for loading of helicase	(HU for *oriC*)	PriB PriC DnaT
Helicase	DnaB/DnaC	
Priming	Primase	

With both primosomses, DNA chains are elongated by DNA polymerase III holoenzyme.

single-strand by exonuclease activity of RecBCD complex, which is assimilated into a D-loop by action of RecA protein (30). On the other hand, in an *E. coli* mutant which contains reduced activity of RNaseH encoded by *rnhA*, constitutive SDR (cSDR) is observed (31). cSDR is proposed to be initiated from R-loops, which are efficiently removed by *rnhA*-encoded RNaseH activity in the wild-type strain but persist in *rnhA* mutants. *E. coli* cells can survive lack of DnaA and/or *oriC*, when *rnhA* is mutated, indicating that growth of *E. coli* can be supported solely by cSDR. Then, what is the nature of the protein complex responsible for replication initiated from D-loops or R-loops?

6. GENETIC STUDY OF PRIMOSOMAL PROTEINS REQUIRED FOR PRIA-DEPENDENT PRIMOSOME

6.1 dnaT (protein i)

In order to examine the physiological function of the ϕX174-type primosome in replication of the *E. coli* chromosome, we undertook molecular genetic analyses of primosomal proteins for the ϕX174-type primosome. First, we isolated the gene for protein i, which was mapped next to *dnaC* (32, 33). Further genetic analyses indicated that protein i is encoded by *dnaT*. *dnaT* and *dnaC* constitute an operon and are cotranscribed together with the p18 gene downstream of *dnaC* from a promoter present upstream of *dnaT* (33). *dnaT* was originally isolated as being defective in induction of iSDR (34) . This result suggests for the first time that the ϕX174-type primosome may function in iSDR.

6.2 PriA (protein n')

n' protein recognizes the n'-*pas* and triggers assembly of the ϕX174-type primosome. We isolated the gene for this protein and designated it *priA* (4, 35). *priA* is a previously unknown gene located at 88.7 min on the *E. coli* chromosome. PriA protein belongs to the DEXH-type RNA/DNA helicase family (Figure 2A) and it does have intrinsic ATPase and DNA helicase activities which are specifically stimulated by n'-*pas*.

An additional feature of the structure of PriA protein is the four repeats of CXXC (C, cysteine) which are inserted between the helicase conserved motifs. This region is likely to constitute a Zinc finger-like structure. Mutagenesis study indicated the cysteine residues are essential for priA function (36). Search of data base lead to identification of likely homologues of priA from two species, one from *Haemophilus influenzae* and the other from a photosynthetic bacteria, *Rhodospirillum rubrum* (Figure 2B). Identity (I) and similarity (S) are as follows:

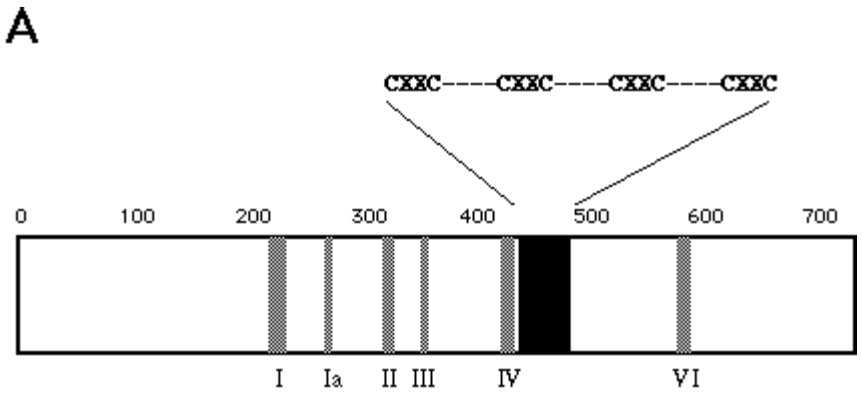

Figure 2A: Schematic representation of the structure of *E. coli* PriA protein and comparison of its primary structure with possible homologues form other bacterial species. A: Six conserved sequence motifs for DNA/RNA helicases and the clusters of cysteine residues for a possible Zinc-finger structure are shown by gray and black boxes, respectively. The numbers indicate the amino acid number from the N terminus.

```
                        1                                                  50
Escherichia coli        ......... ......... .MpvahVALP VPLPRtFDYl lPeGMt.vka
Haemophilus Influenzae  ......... ......... .MkIVrVALa VPLPRlFDYf VPddvs.lqi
Rhodospirillum rubrum   maglpalppg srelfpedah AepVVaVlLP lPLagayDYk VPaGMarpav

                        51                                                 100
Escherichia coli        GcRVRVPFGk QqERIGIVvs vsdASElPln eLKAvvevLD sePvFThsvW
Haemophilus Influenzae  GmRVlVPFGt Qk.RvaIVad fptkSdvPed kLKAilqpLD laPlFTpiyW
Rhodospirillum rubrum   GtlVRVPlGr reE.IGvVwg .agAgEtPpe rLKplig.fp ecPplpaplr

                        101                                                150
Escherichia coli        rlLlWAAdYY hhPiGDVLFh ALPilLRqGr pAa.NApmWy wfateQgqAv
Haemophilus Influenzae  dwLhWAAnYY qaglGDVLFq ALPVkLRnGe sAvkNdrtfw RitdagknAl
Rhodospirillum rubrum   afidWvAaYt vqPpGaVLrm ALsV..paal eApppAlgWr RpsagQraAg

                        151                                                200
Escherichia coli        d........l nsLKRSPKQQ qALAaLrqgk iwr....Dqv RtleFndAal
Haemophilus Influenzae  k........q GeLKRSkKQa eALqyLsetd lek....gnn ...dFssAiw
Rhodospirillum rubrum   qraegqgplp GgarlSPgrQ rvLAvLddhp glpfagaDla ReaavgpAvv

                        201                                                250
Escherichia coli        qALrKKGLcd ...lasetpe fsdWrtNyav ..sgeRLrLN teQAtAvGai
Haemophilus Influenzae  sALkaKGfiE eiTiqtnpls wqqrlgNnPi vnaenRLtLN kQQAlAfsql
Rhodospirillum rubrum   aAmaKaGLlE avT......r sneWspqaPd adrpgpLlsa dQQAaAdGlr

                        251                                                300
Escherichia coli        hsaadtFSaW LLaGVTGSGK TEVYLsvlEn vLaqGKQALV mVPEIGLTPQ
Haemophilus Influenzae  lfhsg.FnvW LLdGVTGSGK TEiYLqyIEE iLksGKQvLV LVPEIGLTPQ
Rhodospirillum rubrum   taldqgFSgl LLeGVTGSGK TEVYfeaIaE tLrrGrQALV LlPEIaLaaQ
                                                 ---------------
                                                        I
                        301                                                350
Escherichia coli        TiaRFreRFN ApveVLHSgL nDseRLsAWl kAknGeAAIV IGTRSALFTP
Haemophilus Influenzae  TvqRFkvRFN veidVLHSnL tDtqRLyvWd rArsGqsAIV IGTRSALFTq
Rhodospirillum rubrum   wprRFadRFg AapvqwHSqm gaaaRrrAWr avalGrApvV vGaRSALFlP
                                    --------
                                       Ia
                        351                                                400
Escherichia coli        FkNLGvIviD EEHDSSYKQQ EGWRYHARDL AVyRAhseqI PiiLGSATPa
Haemophilus Influenzae  FsNLGaIIlD EEHDSSYKQQ dsWRYHARDL AiVlAqklnI svlmGSATPS
Rhodospirillum rubrum   ypdLGlIIvD EEHDSafkQe EGvpYnARDm AVVRArlggf PavLaSATPS
                                 -----------------                 --------
                                        II                              III
                        401                                                450
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Escherichia coli       LETlcNVQQk KYRlLrLtRR AGNArpaiqh VLDLKgQkvQ ..........
Haemophilus Influenzae LEsInNVQnG KYqHLVLskR AGNstalrhf ViDLKnQniQ ..........
Rhodospirillum rubrum  LETIeNarQG rYRHLVLpRR hGgAempeit lLDLrrappQ kwlptdfagp

                       451                                                500
Escherichia coli       .......... .......... .......... .......... .....aGLaP
Haemophilus Influenzae .......... .......... .......... .......... .....nGLSk
Rhodospirillum rubrum  ggseglaapg gandeaeeqk apppsptasp sptaspspma rrarlgwLSP

                       501                                                550
Escherichia coli       aLITRMrqHL qAdNQViLFL NRRGFAPaLL CHdCGWIAeC PRCdhyYTlH
Haemophilus Influenzae PLleRMkaHL ekGNQVLLFL NRRGFAPvLL CHeCGWIAqC PhCekpYTyH
Rhodospirillum rubrum  PLITaveetL aAGeQVLLFL NRRGyAPltL CrsCGhrlkC PRCtawlveH
                                  ========                 ==========
                                     IV
                       551                                                600
Escherichia coli       QaQhhLRCHH CdsQrPvPRQ CPsCGSTH.L VPvGLGTEQL EqTLApLFPg
Haemophilus Influenzae QhQnvLRCHH CGaQktIPRQ CgdCGSTH.L VttGLGTEQL EETLktLFPh
Rhodospirillum rubrum  rrdgrLRCHH CGyQqPIPet CPaCGvadsL aPcGpGvErL aEeaAhrFPk
                       ==========================

                       601                                                650
Escherichia coli       vpisRIDRDT TSRKGaLEqq LaevhrGgar ILIGTQMLAK GHHFPdVTLV
Haemophilus Influenzae ysvaRIDRDs TSRKGkLEgy LedIqqGksq ILIGTQMLAK GHHFPnVTLV
Rhodospirillum rubrum  armdvaasDT vtgpkeaaal atrIanhdid liIGTQimAK GHHFPliTLV

                       651                                                700
Escherichia coli       ALlDVDgALF SaDFRsaERf AQLYtQVAGR AGRAgKQGEV VLQTHhPeHP
Haemophilus Influenzae ALVnVDsALF SlDFRAeERl AQLYiQVAGR AGRAdKQGEV VLQTHyPdHP
Rhodospirillum rubrum  gvVDgDlgLt ggDlRAsERt hQLlhQVAGR AGRAerpGrV liQTvdPgHP
                                              ====================
                                                     VI
                       701                                                750
Escherichia coli       LLqTLLykGY dAFAEqrLAE RrmMqLPPwT shVivrAedh nnqhAplfLQ
Haemophilus Influenzae LLtTLLanGY qAFAketLql RhsMgLPPFT fqalikAqar hSdlAencLs
Rhodospirillum rubrum  vmeaLasgdp alFlEveaAE RqalamPPFg rlValvisge dSar....vQ

                       751                                                800
Escherichia coli       QlrnlilSsp ladekLwVLG PVPAlapKrg GRwRWQiLLQ HPSRVrLQhi
Haemophilus Influenzae QiAdffqSkq itG..LqmLG PmPAPfsKka GqyRWQLLLQ HPSRmtLQka
Rhodospirillum rubrum  avAaalgraa pmGpgLdVLG PVPAPlamlr GRhRhrLLLk aargVkvQpv

                        801                          830
  Escherichia coli      ingtLaLint iPdsrkVKwv LDVDPIegz.
  Haemophilus Influenzae lReyqq..ae leknsqVrli LDVDPqdlsz
  Rhodospirillum rubrum  vRhwLsLvsi pPg...VKvq vDVDPIsfl.
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Figure 2B: The amino acid sequences of the *E. coli* PriA and those of two candidate homologues from *Haemophilus influenzae* and *Rhodospirillum rubrum* are aligned for maximum identity. The amino acid residues identical in at least two genes are shown with capital letters. Conserved motifs for helicases (as indicated in A) and the cysteine-rich region are underlined by gray and black lines, respectively. Sequences from *Haemophilus* (Fleischmann *et al.*, *Science* 269, 496 [1995]) and *Rhodospirillum* (Falk *et al.*, *Biochem. J.* 228, 391 [1985]) are under the accession number HIU32718 and X02499, respectively, in Genbank. Homology with the *Rhodospirillum rubrum* gene was previously reported (3).

• *E. coli* (733 aa) vs. *Haemophilus* (710 aa) : I, 52%; S, 71 %
• *E. coli* (733 aa) vs. *Rhodospirillum* (812 aa): I, 39%; S, 58%

The conserved motifs for helicases and CXXC sequences are precisely conserved among all the three proteins, indicating the importance of those sequences for priA function. Inactivation of the chromosomal copy of the priA gene by insertion of a kanamycine resistance gene (*priA1::kan*) can be tolerated for viability of the *E. coli* cells (37, 38), but *priA1::kan* cells grow extremely poorly and do not grow on rich medium (27). They are highly sensitive to DNA damaging agents such as UV and mytomycin C and exhibit filamentous morphology (39). Requirement of protein i for iSDR prompted us to examine involvement of PriA protein for iSDR. We

measured iSDR in the *priA1::kan* strain, and discovered that iSDR was completely lost in the mutant strain (27).

It was recovered by introduction of a plasmid carrying the wild-type priA gene. We constructed a double-mutant of *rnhA224* and *priA1::kan* and measured cSDR. cSDR was also completely wiped out in this strain (27). Furthermore, cSDR-dependent growth of *rnhA224* and *dnaA*(ts) double mutant at 42°C was also lost by *priA1::kan* mutation.These results demonstrate that PriA protein is essential for both iSDR and cSDR. They, in conjunction with requirement of *dnaT* for iSDR, strongly suggest that the ϕX174-type primosome is responsible for replication forks during the course of SDR. A possible additional role of PriA protein in chromosomal replication could be "reloading" of replication complexes which may fall off the forks during the course of their propagation along the DNA template more than 2000 kb in length, although this possibility has not been experimentally tested.

7. TWO PRIMOSOMES FOR REPLICATION OF THE *E. COLI* CHROMOSOME AND VARIOUS PLASMID REPLICONS

Replication of the *E. coli* chromosome under normal growth condition appears to be conducted solely by the DnaA-dependent primosome and dispensability of PriA protein for viability indicates that the ϕX174-type primosome is not involved in DnaA/*oriC*-dependent replication (4, 35). On the other hand, PriA-dependent SDR does not require *dnaA* function. Thus, DnaA-dependent and PriA-dependent primosomes function independently of each other. The *E. coli* cells can be replicated with either protein complex, depending on the environment or genetic background. Replication of various plasmids generally depends on host replication proteins except for plasmid-encoded initiators that specifically recognize cognate replication origins. Many replication origins contain one or more DnaA box sequences within the minimum replication origin sequences and their replication does require DnaA protein (40). Replication of these DnaA-dependent replicons, such as F, pSC101, R6K, RK2 or Rts1, does not require priA function, whereas replication of pBR322, RSF1030, and ColE2, which is independent of dnaA function, strictly requires PriA protein (27). These indicate that replication of plasmid replicons also can be classified into either DnaA-type or PriA-type (Table 2). In the former class, DnaA protein may help assembly of a replication complex similar to *oriC*-type or ABC primosome by binding to the DnaA box present within the replication origin, while, in the latter class, the ϕX174-type primosome may be assembled at an n'-*pas* on the lagging strand template. We propose that *E. coli* cells possess two functionally similar, independent primosomes, which are differentially utilized by the chromosome as well as by various plasmids (Table 2).

8. PriA-DEPENDENT PRIMOSOME FOR REPLICATION UNDER HARD LIFE

PriA-dependent replication of the *E. coli* chromosome (iSDR and cSDR) occurs only under specialized conditions. Especially, iSDR is specifically induced by DNA damages, and requires RecA, RecB and RecC proteins (41, 42, 43). It is hyperactivated in a *recD* mutant (43). These genetic requirements are extremely similar to those of adaptive (or induced) mutatgenesis which also requires the same set of recombination proteins (44, 45), which lead us to propose that iSDR is the mode of chromosomal replication, which is induced under

Table 2 DnaA- and PriA-type primosomes in replication of *E. coli* chromosome and plasmids

	DnaA-type	PriA-type
Chromosome	*oriC*	*oriMs* (iSDR) ← DNA damages *oriKs* (cSDR) ← *rnhA1*⁻
Plasmids	F pSC101 R6K RK2 Rts1 etc.	ColE1 pBR322 RSF1030 ColE2

*oriM*s and *oriK*s, which are present at multiple locations on the *E. coli* chromosome, are replication origins for iSDR and cSDR, respectively.

hard environmental condition to help *E. coli* cells better adapt with the environment by increased mutation rate. It was previously reported that iSDR is error-prone (41). It will be important to determine whether priA is required for induced mutagenesis.

priA1::kan cells are extremely sensitive to DNA damaging agents such as UV and mytomycin C, strongly indicating that PriA protein is involved in repair of DNA damages, especially in recombination-dependent repair of double-strand breaks (DSB). Furthermore, the *priA* mutant exhibits decreased Hfr conjugation and decreased P1 transduction efficiency, indicating that priA is required for general homologous recombination processes (39). We have concluded that the ϕX174-type primosome generally functions in DNA replication which is associated with recombination.

Our results indicate that the *E. coli* chromosome which is normally replicated by the DnaA-*oriC* pathway switches its replication mode to the PriA-dependent one in response to the environment (Figure 3). This temporal switch of the replication mode may permit the *E. coli* cells to adjust to "hard life" condition in two important ways. In individual cells within the population, it will enable them to repair lesions on DNA through a recombination-dependent manner, which requires PriA-dependent DNA synthesis. It will also increase the chance of survival as a species by enhanced mutation rate caused by PriA-dependent replication. In life-cycle of bacterial cells, this sort of switch of replication modes may occur more frequently than anticipated in response to changes of the environment and similar response may also occur in eukaryotic cells (see below).

9. FUTURE PERSPECTIVES

How does PriA protein initiate DNA replication from D-loops or R-loops? The cloned *oriM* sequences do not have n'-*pas* (46, 47), and there may not be any functional n'-*pas* on the *E. coli* chromosome, since extensive screening using an origin-defective M13 phage vector failed to detect any defined sequences with n'-*pas* activity (N. Nomura *et al.*, unpublished results). Therefore, it is unlikely that n'-*pas* on D-loops or on R-loops are recognized by PriA protein. We consider a possibility that PriA protein recognizes a part of the structural features of D-loops and R-loops. Absolute requirement of PriA protein for replication of pBR322 lacking n'-*pas* in the wild-type and in *rnhA* mutants supports this idea, since replication of this plasmid presents a model for D-loop or R-loop dependent replication.

Interestingly, we noticed that the nucleotide binding domain of PriA protein possesses a low but significant homology to that of RecG protein, which is an RNA helicase specific for R-loops (data not shown; 48). Our preliminary data suggests that PriA protein interacts with an R-loop structure, although ATPase is not activated by this interaction (H. Masai *et al.*, unpublished result). The presence of PriA homologues in two other distantly related bacteria suggests that PriA-dependent replication from D-loops and R-loops may be conserved throughout the eubacteria species. It will be essential to develop an *in vitro* replication system in which replication is specifically initiated from a D-loop or from an R-loop.

Multiple modes of primosome assembly, which we have demonstrated in *E. coli*, may operate in replication of the eukaryotic chromosomes as well. Chromosomal replication in eukaryotic cells is strictly regulated so that it is coordinated with various cell cycle events. At the same time, choice of replication origins and sequence specificity in origin recognition can vary depending on developmental stages (49) or state of transcription in the vicinity of the origin sequences (50, 51). In early cleaving embryo, the S phases are completed in a matter of several minutes. This extraordinary rate of DNA replication is achieved by increasing the numbers of replication origins fired during the short S phase. The replication origin in the intergenic space of b-globin region is fired early in S phase in blood cells where the gene is actively transcribed, whereas it is replicated late in the cells which do not express globins (52, 53). Flexibility in origin selection was also indicated by genetic studies of yeast *Saccharomyces cerevisiae*, in which it was shown that deletion of an actively firing origin from a chromosome can be generally torelated, and that in some cases normally inactive replication origins can be activated to compensate for the loss of active origins (54). It is not known whether these variations in origin usage reflect the switch of replication modes or changes in the chromatin structures in the origin region. We also know very little about the origin selection and mode of initiation during premeiotic DNA synthesis, which could be different from those of mitotic DNA replication. It is of interest whether a recombination-dependent replication pathway similar to the one discovered in *E. coli* exists in eukaryotic cells. If PriA protein is an "initiator" for DNA replication from D-loops and R-loops, which are commonly found in eukaryotic cells as well, it may well be conserved in eukaryotes.

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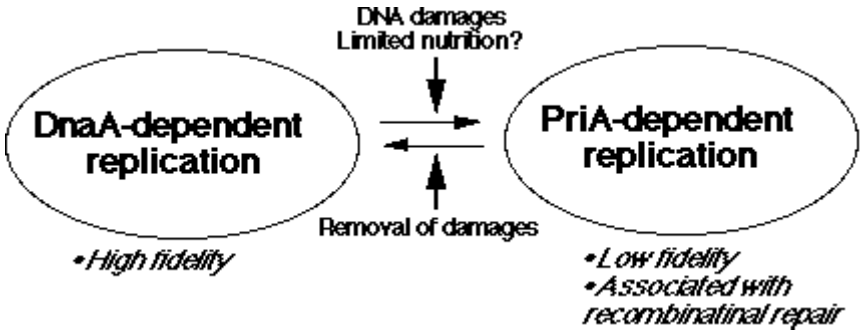

Figure 3: Switch of replication modes in response to environmental conditions in *E. coli*. In *E. coli*, the chromosome is normally replicated by the high fidelity DnaA-dependent machinery. However, upon encountering "harsh" environment such as the presence of DNA damage-inducing agents or possibly the absence of sufficient nutrition, it is temporally switched to PriA-dependent one, which may have lower fidelity and is associated with recombination-dependent repair of DNA lesions. See text for details.

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