Mechanism of chromosome DNA replication initiation by highly conserved initiator proteins among bacterial species

吉田, 竜星

(様式５)

氏　　名　：吉田　竜星

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(細菌種間に高度に保存された開始因子による染色体 DNA 複製開始の仕組み)

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論　文　内　容　の　要　旨

Bacterial genomic DNA has the replication origin *oriC*. *oriC* consists of the Duplex Unwinding Element (DUE) and the initiator ATP-DnaA-Oligomerization Region (DOR) which includes multiple sites of DnaA (DnaA box), an asymmetric 9-mer sequence. DnaA forms a complex at *oriC* resulting in the DUE unwinding. The DnaA complex then recruits a pair of DnaB helicases to the unwound region bidirectionally. In contrast to the highly evolutional conservation of DnaA and DnaB, the DnaA box arrangements in *oriC* have great varieties among *oriC*s of bacterial species. Because of these structural varieties, it is difficult to understand the common mechanical principle in bacterial genomic DNA replication initiation. To reveal the hidden principles, I investigated the genomic DNA replication initiation mechanism of *Escherichia coli* (*E. coli*) *oriC* underlying the DUE unwinding and the successive DnaB loading to the unwound region.

The *E. coli oriC* has two DnaA box clusters in opposite directions (Left-DOR and Right-DOR), which are frameworks of the Left-and Right-DnaA subcomplexes, respectively (Figure A). In the Left-DOR, ATP-DnaA forms a pentamer by binding to R1, R5M, and three other DnaA boxes. The DNA bending protein IHF binds sequence-specifically to the interspace between R1 and R5M boxes, promoting DUE unwinding, which is sustained predominantly by binding of R1/R5M-bound DnaAs to the single-stranded DUE (ssDUE) (Figure B *upper left*). Chapter I describes DUE unwinding mechanisms promoted by DnaA and IHF structural homolog HU, a ubiquitous protein in eubacterial species that binds DNA sequence-non-specifically, preferring bent DNA. Similar to IHF, HU promoted DUE unwinding dependent on ssDUE binding of R1/R5M-bound DnaAs. Unlike IHF, HU strictly required R1/R5M-bound DnaAs and interactions between the two DnaAs. Notably, the HU site-specifically bound to the R1-R5M interspace in a manner stimulated by ATP-DnaA and ssDUE. These findings suggest a model that interactions between the two DnaAs trigger DNA bending within the R1/R5M-interspace and initial DUE unwinding, which promotes site-specific HU binding that stabilizes the overall complex and DUE unwinding (Figure B

lower left). Moreover, HU site-specifically bound to the replication origin of the ancestral bacterium *Thermotoga maritima* depending on the cognate ATP-DnaA. The ssDUE recruitment mechanism could be evolutionarily conserved in eubacteria.

In Chapter II, to understand the structural principles and flexibility of *oriC* in DnaB loading to *oriC* ssDNA, I analyzed using *in vitro* reconstituted systems mutant *E. coli oriC* with various changes of Right-DOR arrangement. I found that under certain restrictions, the directions and positions of Right-DOR were changeable with substantial activities of the DnaB loading to *oriC* ssDNA. Further analyses suggested that the DnaB loading to the unwound region by the Left-DnaA subcomplex was a prerequisite for that by the Right-DnaA subcomplex. These observations are consistent with the model that the Left-DnaA subcomplex loads DnaB to *oriC* ssDNA at first and the Right-DnaA subcomplex loads DnaB to the opposite strand. Due to the structural flexibility of Right-DOR, the DnaB loading principle similar to the *E. coli oriC* system could operate in other bacterial *oriC* systems (Figure B *right*).

In conclusion, I suggest the chromosomal DNA replication initiation mechanism in *E. coli oriC*. The essential schemes are shown below. First, DnaA forms two functionally differentiated complexes. The one connects to DUE and includes a bending structure to unwind DUE stably. The other permits flexible arrangements as long as the resultant DnaA subcomplex is spatially accessible to an extended unwound region. Each complex recruits and loads a DnaB to each strand of the unwound DUE. This DnaB loading principle seems to be feasible in other bacterial *oriC*s. Moreover, these rules in the arrangements of initiator protein binding sites could apply to replication initiation origins of plasmid DNA, archaeal chromosomal DNA, and yeast DNA, which deepens the evolutional insights. Further research in this field will contribute to solving clinical problems, such as antibiotic resistance.