Analyses for mechanisms in formation of the replication initiation complex and for functional characteristics of the DNA unwinding region at the replication origin of hyperthermophilic bacterium Thermotoga maritima

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（高度好熱性真正細菌 *Thermotoga maritima* の複製起点における開始複合体の形成メカニズムと開裂部位の特性の解析）

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論　文　内　容　の　要　旨

Chromosomal DNA replication is a central process in all cellular organisms. To initiate DNA replication, a specific nucleoprotein complex called the initiation complex is constructed at the origin of replication, promoting recruitment of the replisome components, DNA helicase, primase, DNA polymerase, etc.. If the regulation of replication initiation is disturbed, it can lead to various cellular abnormalities, including abnormal chromosomes, inhibition of cell division, and the proliferation of abnormal cells. To avoid these problems, the processes of the initiation complex formation are highly organized in order to ensure the replication of chromosomal DNA to occur only once at a specific time during the cell cycle progression. Thus, the study addressing the common principles underlying initiation complexes plays a fundamental role in the fields of pharmaceutical biochemistry and molecular biology.

In well-characterized *Escherichia coli*, the main components of the initiation complex are the replication origin *oriC*, the initiator protein DnaA and the DNA bending protein IHF. In most eubacteria, the *oriC* contains the DNA unwinding element (DUE) and DnaA oligomerization region (DOR) bearing multiple DnaA box sequences to which the initiator protein DnaA binds specifically. DnaA is ubiquitous in eubacterial domain and the canonical DnaA box consists of an asymmetric 9-mer consensus sequence, TTA[T/A]NCACA. DnaA consists of four domains and the C-terminal domain IV binds to the DnaA box sequence specifically. The central DnaA domain III containing AAA+ (ATPases associated with various cellular activities) motifs, promoting head-to-tail oligomerization, which underlies the formation of an initiation complex by the ATP form of DnaA (ATP-DnaA). Domains III and IV are connected by a short linker. Domain I contains a specific binding stie for helicase and domain II is a flexible linker. In *Escherichia coli oriC*, the head-to-tail ATP-DnaA oligomers constructed on the IHF-bound DOR promote unwinding of DUE and concomitantly bind the single-stranded DUE to stabilize the unwound form, enabling loading

of helicases to the single-stranded region. Despite the significant sequence homologies among DnaA proteins, bacterial *oriC* sequences are highly diverse. In particular, the number of DnaA boxes and their spatial arrangements are differentiated substantially among bacterial *oriCs*. Moreover, the functional motifs within bacterial DUEs have been insufficiently determined due to the lack of in-depth characterization using *in vitro* reconstituted systems.

In order to elucidate the basic mechanisms underlying DNA unwinding at DUE and the mechanistic mode of DnaA oligomerization at the origin of replication, I focused on the hyperthermophilic eubacterium *Thermotoga maritima* as a model organism. *T. maritima* is placed at a deep branch in the evolutional tree of life. The 149-bp minimal *oriC* (*tma-oriC*) region of this bacterium, which contains a 24-bp AT-rich DUE (*tma*DUE) and a flanking DOR (*tma*DOR) with five *tma*DnaA boxes, is required for open complex formation by the cognate DnaA initiator (*tma*DnaA). However, the unwinding motifs within *tma*DUE and the mode of *tma*DnaA oligomerization remained poorly characterized.

In this study, I first investigated the *tma*DUE sequence motifs crucial for open complex formation. Using the electron mobility shift assays in combination with mutant *tma*DUE analyses, I found that *tma*DUE was comprised of two distinct functional modules, an unwinding module and a *tma*DnaA-binding module. Three direct repeats of the trinucleotide TAG within *tma*DUE were essential for both unwinding and single-stranded *tma*DUE binding by *tma*DnaA complexes constructed on the DnaA boxes. Its surrounding AT-rich sequences stimulated only duplex unwinding.

Moreover, I show biochemical evidence that head-to-tail oligomers of ATP-bound *tma*DnaA were constructed within *tma-oriC*, irrespective of the directions of the *tma*DnaA boxes. This binding mode was considered to be induced by flexible swiveling of DnaA domains III and IV, which were responsible for DnaA-DnaA interactions and DnaA box binding, respectively. The flexible nature of the linker between *tma*DnaA domains III and IV would allow considerable swiveling of the domains. Phasing of specific *tma*DnaA boxes in *tma-oriC* DNA was also responsible for unwinding. These findings indicate that a single-stranded DUE recruitment mechanism was responsible for unwinding and would enhance understanding of the fundamental molecular nature of the origin sequences present in evolutionarily divergent bacteria, including numerous pathogenic bacteria. Consequently, this research has the potential to contribute to the development of innovative antibiotics against pathogenic bacteria in the future.