Subcellular dynamics of a chromosome partition factor CrfC protein in Escherichia coli

谷口, 紗輝

（様式５）

氏　　名　：　谷口　紗輝

論文題名　：Subcellular dynamics of a chromosome partition factor CrfC protein in *Escherichia coli*　（大腸菌の染色体分配制御因子 CrfC の細胞内動態）

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論　文　内　容　の　要　旨

Precise regulation of the chromosome partitioning is essential for the proliferation of all living cells. For the accurate transmission of all the genetic information from one generation to the next, chromosomal DNA should be correctly replicated and equally segregated into the daughter cells. Errors in these processes occasionally cause an abnormal number of chromosomes in a cell, which can be a cause of various diseases including cancer. The chromosome partitioning is strictly regulated in both prokaryotes and eukaryotes. In this study, I used a model bacteria *Escherichia coli*, which is a relatively simple organism.

The bacterial chromosome is organized into a condensed structure called the nucleoid. Specific nucleoid conformation and its dynamic changes are important for various cell-growth processes including partitioning of the nucleoids. A large number of proteins are involved in the nucleoid dynamics, and many of those proteins localize to the specific subcellular positions. However, coupling between the nucleoid partitioning and protein localization dynamics remain largely elusive.

The nucleoid-associated proteins (NAPs) of *Escherichia coli*, such as HU, IHF, H-NS, and Dps, bind DNA and play important and specific roles in the nucleoid dynamics. *E. coli* MukB, a SMC (structural maintenance of chromosomes) protein, also plays an important role in nucleoid organization and partition. Also, the *E. coli* chromosome is segmented into four macrodomains and two non-structure regions. The Ter macrodomain, which contains the replication terminus *terC*, is organized by the MatP protein. MatP binds the specific sites present in this macrodomain, resulting in the folding of this macrodomain. These macrodomains are important to regulate the structural changes and positioning of the nucleoid in chromosome partitioning. In addition, SlmA, a NAP interacts with the essential division factor FtsZ, and supports the coupling of chromosome partitioning and cell division by inhibiting division machinery formation at the nucleoid position.

In *Escherichia coli*, the sister chromosomes bidirectionally migrate in parallel with chromosomal DNA replication. Chromosomal DNA replication is initiated at the unique replication origin *oriC*, and the resulting sister replication forks as well as the nascent DNA regions co-localize transiently at mid-cell. The β clamp for DNA polymerase III holoenzyme remains on the nascent DNA strands during DNA synthesis. Ozaki et al. demonstrated that a novel β clamp-binding protein CrfC (colocalization of replication fork DNA by clamp) is a regulator of nucleoid positioning and partitioning. CrfC is a homolog of dynamin protein and has a dynamin-like GTPase domain and a β clamp–binding motif at the N-terminus. Like dynamin, CrfC forms homomultimers from dimers to higher-order oligomers, that bind multiple β□clamp molecules. At the mid-cell, CrfC colocalizes with nascent DNA regions in a manner dependent on β clamp-binding motif, promoting colocalization of the sister replication forks, which

ensures the nucleoid equipartition. In addition, CrfC localizes at the cell pole-proximal loci throughout the cell cycle via an unknown mechanism.

To reveal subcellular dynamics of CrfC, I analyzed the subcellular localization of cell pole-proximal CrfC intensively by fluorescent microscopy. To investigate the subcellular localization of CrfC, I used fluorescently labeled CrfC protein by replacing the *crfC* gene on the chromosome with *crfC-venus*. Similarly, to observe the nucleoid, I used fluorescently labeled HU protein. The HU protein is abundant in a cell and binds to the entire nucleoid. As the result, I revealed that the cell pole-proximal CrfC localizes to the distinct subnucleoid regions, which was termed the nucleoid poles (i.e., the cell pole-proximal nucleoid-edges) in this study. To investigate whether this CrfC localization depends on the nucleoid rather than the cell poles, I used *dnaA46* (Ts) cells, which is a temperature-sensitive mutant gene of the replication initiator DnaA. When incubated at the restrictive high (42°C) temperature, the *dnaA46* cells inhibit new rounds of replication initiation but allow progression of the pre-existing replication forks and protein synthesis, which results in elongation of cells with a single nucleoid at mid-cell. CrfC formed foci and localized at the nucleoid poles even in the elongated *dnaA46* cells, suggesting that the localization of CrfC at the nucleoid pole was independent of the distance between the nucleoid and the cell poles.

Next, I hypothesized that the specific nucleoid substructures could be an important determinant of CrfC localization. To investigate this hypothesis, I analyzed the CrfC localization in the deletion mutants of various NAPs. In H-NS-depleted cells, the expression of CrfC protein was increased. As a result, a part of CrfC was distributed on nucleoids. This suggests that H-NS down-regulates CrfC expression, which ensures the CrfC foci formation. In deletion mutants of the *slmA* gene or the *hupB*, a gene of a subunit of HU, CrfC formed foci in cells, although polar CrfC location was broadened in the region proximal to the cell pole. This suggests that HU and SlmA are required for regulation of the CrfC foci localization. Moreover, the number of CrfC foci was decreased in Δ*hupB* cells, suggesting that HU is also important for CrfC foci formation. In addition, *dnaA46* cells bearing the Δ*hupB* or Δ*slmA* exhibited diffused CrfC localization throughout the cytoplasmic region, although CrfC basically formed foci in each single mutant cells as described above. In contrast, IHF, MatP, Dps and MukB little or only slightly affected CrfC localization. These results suggest that CrfC localization is regulated not only by specific NAPs such as HU and SlmA but also by chromosomal replication indirectly.

Finally, I tried to determine a functional region of CrfC for localization of the nucleoid pole by using a serious of the truncation mutants. I found that a C-terminal region of CrfC might be required for localization of CrfC at the nucleoid pole.

Taken together these results, I propose a novel mechanistic model that CrfC specifically localizes to the nucleoid poles in two steps: assembly of CrfC and recruitment. In this model, assembly of CrfC depends on the specific substructure of the nucleoid that organized by HU and SlmA. DNA replication and H-NS assists in this step directly or indirectly. Assembled CrfC are next recruited to the nucleoid poles depending on specific structure of the nucleoid poles, which is organized by HU and SlmA. In this step, the C-terminal region of CrfC interacts with the structure. As no other nucleoid partition factors are reported to show the localization similar to CrfC, CrfC at the nucleoid poles is suggested to play novel and unique roles in nucleoid regulation. This study could contribute to revealing the spatiotemporal coupling between chromosome and protein localization dynamics. Also, this study could contribute to revealing the more detail mechanisms of chromosome partition, improving the treatment of diseases caused by the chromosome partition defects.