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学 位 論 文 題 目	A study of boundary regions of the Ter domain, which is organized at the replication terminus region of *E.coli* chromosome
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論文内容の要旨

A circular chromosome of prokaryote has a single replication origin, *oriC*, at which bidirectional replication is initiated. Bidirectionally progressive replication forks meet at the opposite chromosomal region of *oriC* and the replication terminates. In general, this chromosomal region has been referred to the replication terminus. The large chromosomal region including the replication terminus is organized as a domain. It is called the Ter domain. In addition to the main function of the replication terminus, this chromosomal region plays a crucial role for physical separation of replicated daughter chromosomes. Recombination between circular daughter chromosomes can produce a jointed molecule of them, or dimer. The dimer of daughter chromosomes cannot physically segregate to each daughter cell. A mechanism of site-specific recombination is provided to circular bacterial chromosomes to resolve the dimer to monomers. In *E.coli*, an unique sequence for the dimer resolution (*dif*) is located at the center of the replication terminus. Interestingly, chromosomal position of *dif* is on just the opposite site of *oriC* and function of the dimer resolution. When *dif* is translocated out of the replication terminus, chromosomal dimers are accumulated at high frequently. To reveal positional effect of *dif*, chromosome arrangement of the terminus region has been studied using deletion mutants in the replication terminus. In consequence, it is thought that organization of the replication terminus contributes for effective function of the dimer resolution at *dif*.

In addition, it is known that a membrane protein FtsK can help to resolve dimers. FtsK is integrated into inner membrane only at septum when cells divide. Thus, the chromosomal dimer can be effectively resolved according to the cell division cycle. An enzymatic function of FtsK is known as DNA translocase, which directionally pulls a DNA strand depending of specific sequences (KOPS). Consensus sequences of KOPS are scattered at the entire chromosome. They

are not at random, but polarized distribution in the *E.coli* genome. KOPS is symmetrically distributed around the axis between *oriC* and *dif* on the *E.coli* circular chromosome so that the *dif* sites tend to be stayed at the middle of cells at which FtsK is positioned. Recently, it was found that the replication terminus is organized as folded structure by a DNA binding protein, MatP. The binding sites of MatP (*matS*) are distributed only within the replication terminus. Depending on growth conditions, cells with a mutation of *matP* are defective in chromosome segregation. However, the biological function of the MatP-*matS* DNA binding system is not clear. Thus, a complex system is involved in accurate segregation of circular chromosomes at the replication terminus. Probably the unique chromosomal configuration at the replication terminus promotes physical separation of daughter chromosomes. How and what chromosomal configuration of the replication terminus can contribute for chromosome segregation? In this study, a series of experiments using inversion mutants between the *oriC* and *dif* were carried out to investigate configuration of the terminus region for proper chromosome segregation.

The analysis of chromosomes using inversion mutations, as well as deletion analysis of chromosome, is helpful to consider effects of chromosomal configuration on accurate chromosome segregation. In fact, it has been known that the inversion between two chromosomal positions, the 84.054 min locus near the *oriC* (84.6 min) and the 33.092 min locus near the *dif* (34.6 min), Inv(84.054-33.092), causes a defect in chromosome segregation so that anucleate cells are produced at high frequency (29% of total cells). It seems likely that the inversion, Inv(84.054-33.092), splits the replication terminus into two parts. To confirm it, localization of MatP fused with fluorescent protein mCherry was observed. While one or two discrete foci were seen in the wild type, the number of MatP-mCherry foci increased in the Inv(84.054-33.092) mutant, indicating that the replication terminus was split. In contrast, Inv(84.054-22.483) mutation, in which one inversion position is far from *dif* as compared with the Inv(84.054-33.092)

and entire the replication terminus was inverted without splitting, hardly affects chromosome segregation. These results suggest that distance of the inversion position from *dif* is of critical importance for accurate chromosome segregation and splitting of replication terminus compromise it.

To know why the splitting of terminus in the Inv(84.054-33.092) causes a defect in accurate chromosome segregation, effect of the genetic system was examined. Either mutation of *ftsK* or *matP* was introduced into the inversion mutants and frequency of production of anucleate cells was tested. The *ftsK* mutant that lacks its ATPase activity for DNA translocation remarkably reduced production of anucleate cells in the Inv(84.054-33.092). The *matP* null mutation also remarkably suppressed production of anucleate cells. Thus, both FtsK and MatP were closely related to the deficiency of accurate chromosome segregation in Inv(84.054-33.092).

The results of the inversion mutants also suggest existence of the core of the terminus region required for accurate chromosome segregation. To identify the core of the terminus region, frequencies of producing anucleate cells were measured in a nested series of inversion mutants. The frequencies of producing anucleate cells increased gradually between 27.859 min and 28.469 min, and steeply between 28.469 min and 28.473 min. Thus, it is concluded that the boundary is located between 28.469 min and 28.473 min (268 kb far from *dif*). In this boundary, *matS4*, a single binding sequence of MatP, is located. Similar analysis identified the other boundary at 4.9 min (248 kb) far from *dif* in the opposite direction, where *matS21* is located. Therefore, the core of the terminus region is located between 27.859 min and 39.592 min (544 kb). This region is referred to cTer.

The 544 kb cTer region is thought to form folded structure by binding to MatP and this structure or organization is required for accurate chromosome segregation. This region also has *dif* that plays a curial role for dimer resolution and the dimer resolution requires a specialized

mechanism to promote the directional reaction. Perhaps the specialized mechanism might be the chromosomal organization that is composed of cTer and might contribute to accurate chromosome segregation. Further analysis of cTer will reveal an exact mechanism for coupling the chromosome segregation and cytokinesis at septum, because FtsK localizes at septum when cells divide and brings the *dif* sites to the middle of the cells.

博士論文の審査結果の要旨

大腸菌染色体は環状で、その複製は唯一の開始点である *oriC* から始まり両方向に進行し複製終結領域で出会い完了する。その間 *oriC* 領域が複製された姉妹染色分体を引っ張るように菌の両極に移動し、中央に残された複製終結領域が最後に分離してそれぞれ娘細胞に分配され細胞分裂へと向かう。細菌の場合、この一連の染色体の複製、分配に異常が生じると核様体（真核細胞の核に相当）のない無核細胞を生じる場合がある。染色体の複製、分配には *oriC* の移動や複製終結領域での分離が重要な働きを担っていると考えられるが、複製終結領域の役割については不明な点が多い。

出願者である田口氏は、大腸菌環状染色体の複製終結領域の分配時における機能を明らかにするため、相同組換えを利用し染色体の一部を逆転（inversion）させた株を多数作製し、それらが染色体分配に与える影響を無核細胞の発生率で調べた。複製終結領域の中心には、染色体の分離に働く *dif* と呼ばれる配列が存在する。この *dif* 配列の近傍を *oriC* 付近と入れ代わると、無核細胞発生率が上昇することが判明した。そこで田口氏はさらに多くの複製終結領域の逆転株を作成し無核細胞発生率と逆転部位の関係を調べたところ、*dif* を中心とした 544 kb の領域が *oriC* 付近と入れ代ると、急激に無核細胞発生率が上昇することを発見した。その領域の両端には明らかな境界（boundary）が存在する事から、田口氏はそこが、染色体分離に関係するコア領域と予想し “cTer” と名づけた。その領域の機能を探るため、田口氏は様々な染色体分離に関わる変異株で同様の無核細胞検出実験を行い、２つの遺伝子（*matP*、*ftsK*）に変異が生じると cTer の逆転による無核細胞発生率が減少することを見いだした。さらに田口氏は cTer の欠損解析を行い、*dif* からの距離依存的にその機能が規定されていることを突き止めた。以上をふまえて、cTer の機能を説明する仮説（モデル）として MatP タンパク質が cTer 近傍に複数存在する *matS* 配列に結合し、さらに MatP 同士が会合することにより cTer が高次構造を形成し、それが染色体に一カ所のみ存在することが DNA を移動させるモータータンパク質 FtsK の正常な機能に必要なのではないかと考えている。

田口氏の規定したcTerはこれまで発表された複製終結領域の中心に位置し、その境界も明確であることから、染色体の分離に働く最小機能領域である可能性がある。さらに田口氏の提唱するモデルは、今までにない新しいものであり、これまで報告されている結果とも矛盾しない。以上の成果を総合的に判断して、本論文は今後のこの分野の研究発展に大きく貢献するものと考えられる。