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Article

A Replicase Clamp-Binding Dynamin-like Protein Promotes Colocalization of Nascent DNA Strands and Equipartitioning of Chromosomes in *E. coli*

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SUMMARY

In *Escherichia coli,* bidirectional chromosomal replication is accompanied by the colocalization of sister replication forks. However, the biological significance of this mechanism and the key factors involved are still largely unknown. In this study, we found that a protein, termed CrfC, helps sustain the colocalization of nascent DNA regions of sister replisomes and promote chromosome equipartitioning. CrfC formed homomultimers that bound to multiple molecules of the clamp, a replisome subunit that encircles DNA, and colocalized with nascent DNA regions in a clamp-binding-dependent manner in living cells. CrfC is a dynamin homolog; however, it lacks the typical membrane-binding moiety and instead possesses a clamp-binding motif. Given that clamps remain bound to DNA after Okazaki fragment synthesis, we suggest that CrfC sustains the colocalization of sister replication forks in a unique manner by linking together the clamp-loaded nascent DNA strands, thereby laying the basis for subsequent chromosome equipartitioning.

INTRODUCTION

In *Escherichia coli*, equipartitioning of sister chromosomes is promoted during chromosomal replication. The process involves a series of dynamic molecular events including transient colocalization and active translocation of nascent DNA strands (Sunako et al., 2001; Molina and Skarstad, 2004; Bates and Kleckner 2005; Reyes-Lamothe et al., 2008b; Joshi et al., 2011; Wang et al., 2013). After initiation of replication at the replication origin *oriC*, the sister *oriC* regions and the nascent DNA regions of the sister replisomes colocalize transiently (Figure 1A) (Sunako et al., 2001; Molina and Skarstad, 2004; Bates and Kleckner, 2005; Fossum et al., 2007; Reyes-Lamothe et al., 2008a). The *oriC* regions are then rapidly bidirectionally segregated via a specific translocation system functioning at a centromere-like site *migS* near *oriC* (Figure 1A) (Yamaichi and Niki, 2004). Segregation of the main body of the chromosome outside of the *migS-oriC* region requires the MukBEF complex (Hiraga, 2000), which condenses DNA strands like eukaryotic condensins (Cui et al., 2008) (Figure 1A). MukB, a SMC (structural maintenance of chromosomes)-fold protein (Nasmyth and Haering, 2005; Hirano 2006; Bloom and Joglekar, 2010), is located at quarter-cell positions during chromosomal segregation (Hiraga, 2000; Badrinarayanan et al., 2012).

Even in *E. coli,* only a few factors are known to support colocalization of nascent DNA regions following sister replisomes. One of these factors is the hemimethylated DNA-binding protein SeqA (Molina and Skarstad, 2004; Fossum et al., 2007). SeqA forms multimers on nascent DNA strands (Brendler et al., 2000; Waldminghaus et al., 2012) and is thought to link the strands temporarily for colocalization. However, the duration of the hemimethylated state of the nascent DNA region is only 1–10 min (Lu et al., 1994), whereas the colocalization of nascent DNA regions can persist for 5 min to more than 40 min (Sunako et al., 2001; Fossum et al., 2007; Adachi et al., 2008; Reyes-Lamothe et al., 2008a). Thus, SeqA binding cannot fully account for the entire duration of nascent strand colocalization. Another mechanism, catenation of DNA, has also been suggested to assist nascent strand colocalization; however, the results of experiments with topoisomerase IV (Topo IV), which dissociates catenated DNA strands, suggest that catenation is effective only for the colocalization of sister *oriC* regions, not for the colocalization of nascent DNA strands outside of *oriC* (Wang et al., 2008). Therefore, the biological significance and molecular mechanisms responsible for the colocalization of nascent DNA regions still remains largely elusive.

Replisomes contain DNA polymerase III holoenzyme consisting of the polymerase core-DnaX complex and the clamp

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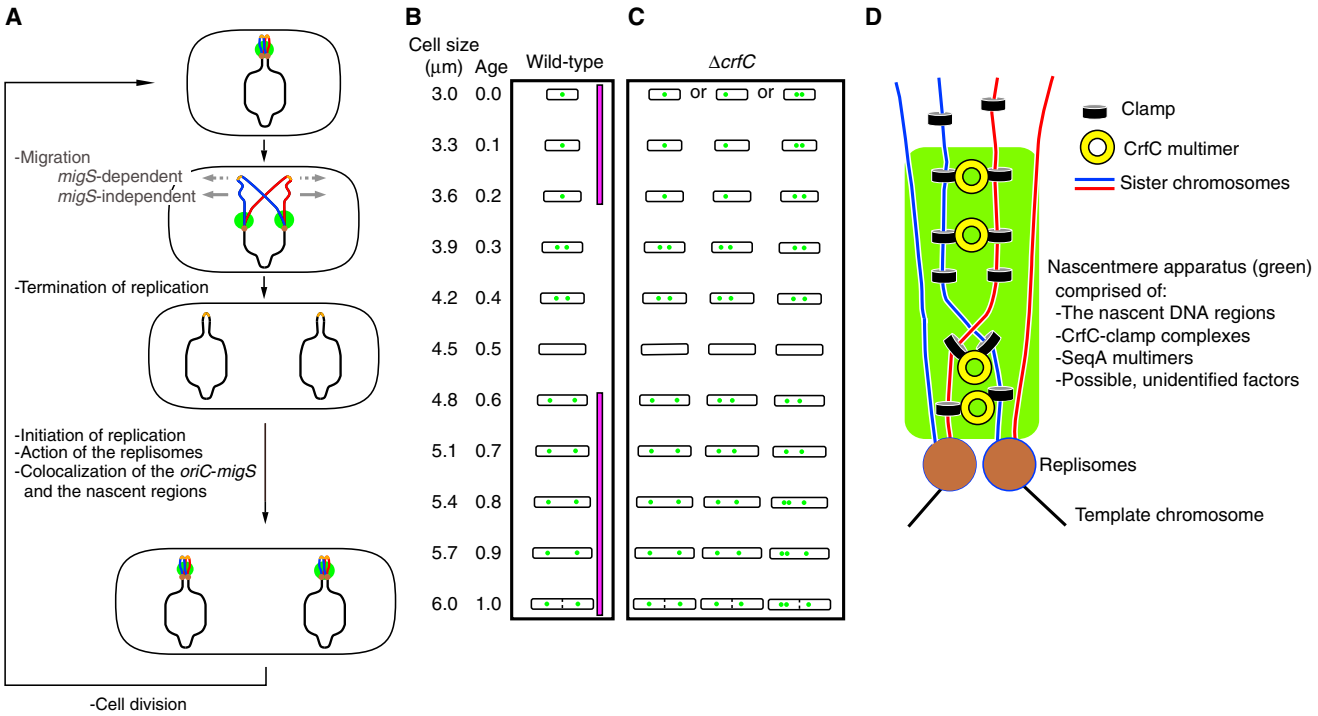

Figure 1. Colocalization of Sister Replication Forks and Chromosome Segregation

(A) Replication intermediates are illustrated. The Ori macrodomain contains *oriC* and *migS* (Yamaichi and Niki, 2004), whereas the Ter macrodomain contains the replication termination region (Mercier et al., 2008). These macrodomains span approximately 20% of the chromosome and are more tightly folded than the intervening macrodomains (Dame et al., 2011). The sister replication forks are transiently colocalized after the initiation of replication. The sister chromosomal regions (cyan and red) are then translocated to specific quarter-cell positions during further progression of replication forks, which results in the formation of a pair of foci. The *oriC-migS* region (yellow) migration system (broken gray arrows) is different from that of other chromosomal regions (gray arrows). The replisomes and the foci of the replication forks are indicated by brown circles and green circles, respectively.

(B and C) Schematic illustration of the subcellular dynamics of the foci of the replication forks in wild-type (B) and Δ*crfC* mutant (C) cells containing two to four origins. Defects in equipositioning or premature separation of the foci can occur in Δ*crfC* cells. Cell size and the relative cell age (where 0 represents the newborn time and 1.0 represents the division time) were deduced from microscopic and flow cytometry data. The green circles indicate the foci of the replication forks. The magenta bar indicates the duration of colocalization of the sister replication forks.

(D) Model for colocalization of the nascent DNA regions. Immediately after replication, the nascent DNA strands are hemimethylated and bind SeqA multimers. The DNA-loaded clamps remain bound to lagging mode nascent DNA strands and form complexes with CrfC multimers. These nucleoprotein complexes stabilize the colocalization of the nascent strands. The nascent DNA region bearing such dynamic higher-order complexes is termed the "nascentmere." Unidentified proteins also may participate in the nascentmere complexes. Catenation of DNA is not shown because the catenation of sister DNA strands following a replisome is released by the transient dissociation of the lagging strand polymerase (Kurth et al., 2013).

(DnaN) (Johnson and O'Donnell, 2005). The clamp has a ring-like structure that encircles DNA (Georgescu et al., 2008). After the synthesis of an Okazaki fragment on the lagging strand, the polymerase core-DnaX complex dissociates from the clamp, but the clamp remains temporarily attached to the nascent DNA (Yuzhakov et al., 1996; Onogi et al., 2002; Wang et al., 2011; Su'etsugu and Errington, 2011), where it interacts with various proteins to regulate replication cycle and DNA repair (Katayama et al., 1998, 2010; Johnson and O'Donnell, 2005).

Here, we identify a clamp-interacting protein that supports both nascent DNA colocalization after sister replisome passage and equipartitioning of chromosomes. This protein, named CrfC (colocalization of the *r*eplication *f*ork DNA by the clamp), structurally resembles eukaryotic dynamin, which forms spiral homomultimers that directly bind to the cell membrane for the promotion of membrane fission during endocytosis and mitochondrial division (Ferguson and De Camilli, 2012). The function of dynamin homologs in prokaryotes is largely unknown except for their membrane-binding properties (Low et al., 2009; Bramkamp, 2012). CrfC is a unique dynamin homolog that lacks the membrane-binding moiety but instead carries a clamp-binding motif. We propose that CrfC functions as a molecular anchor for the tethering of clamp-loaded sister lagging strands, thereby sustaining colocalization of nascent DNA regions after replication fork passage.

RESULTS

CrfC Is a Clamp-Binding Homolog of Dynamin

The clamp has diverse functions in various cellular events. To uncover as-yet-unidentified clamp functions, we searched for clamp-binding factors in *E. coli* using a clamp-conjugated affinity column. Peptide-mass fingerprint analysis identified 65 candidate clamp-binding proteins (Table S1). This list included known