

## Crystal Structure Analysis of Septum Site-determining Protein MinD from *Pyrococcus horikoshii* OT3

Bacterial cell division requires the formation of a septum at midcell, circumferential invagination of the cytoplasmic membrane, and synthesis of a peptidoglycan layer. The key step in septum formation is the polymerization of essential cell division protein FtsZ at the potential division site. FtsZ recruits several other essential proteins to form mature cell division machinery and the cell division process then progresses. Rod-shaped bacteria such as *Escherichia coli* have three potential division sites in a cell. One of them is at the midcell position, while the others are adjacent to the cell poles. Thus the precise placement of the FtsZ ring at the cell center is a prerequisite for the accurate cell division of bacteria. In *E. coli*, the cell division site is determined by the cooperative activity of *min* operon products MinC, MinD, and MinE. MinC is a nonspecific inhibitor of the septum protein FtsZ, and MinE is the suppressor of MinC. MinD plays a multifunctional role. It is a membrane-associated ATPase and is a septum site-determining factor through the activation and regulation of MinC and MinE (Fig. 1). MinD is also known to undergo a rapid pole-to-pole oscillation movement *in vivo* as observed in fluorescent microscopy [1].

We studied recombinant MinD from *Pyrococcus horikoshii* OT3 (PH0612) expressed in *E. coli*, and determined the three-dimensional structure at 2.3 Å resolution by X-ray crystallography using the Se-Met MAD method at beamline BL41XU [2]. The crystal structure consists of a  $\beta$ -sheet with seven parallel and one antiparallel strands and eleven peripheral  $\alpha$ -helices (Fig. 2). Although we made no attempt to add ATP or ADP molecules in the purification or crystallization step, the electron density clearly shows that MinD from *P. horikoshii* contains bound ADP and a magnesium ion at the

pocket close to the edge of the  $\beta$ -sheet on the surface of the MinD molecule (Fig. 3). It has been shown that the ADP molecule was bound during the growth of the *E. coli* cells, and MinD from *E. coli* has ATPase activity in the presence of  $Mg^{2+}$  ion. Therefore, the observed coordination of ADP in the present crystal is considered to be the product of the molecule's ATPase activity.

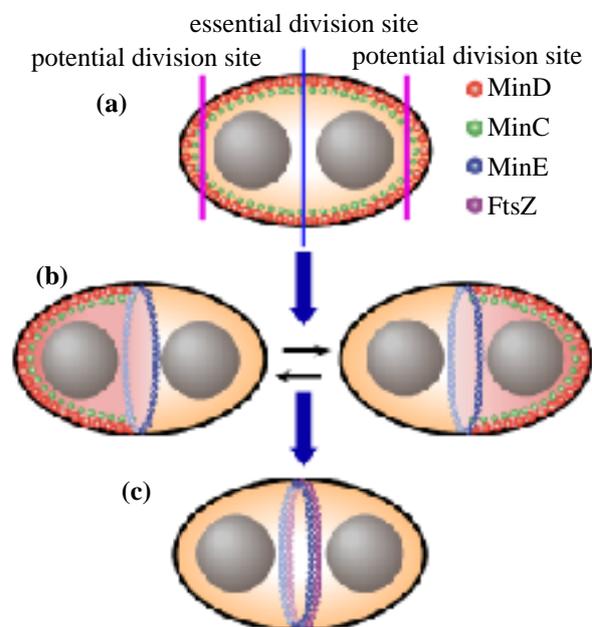
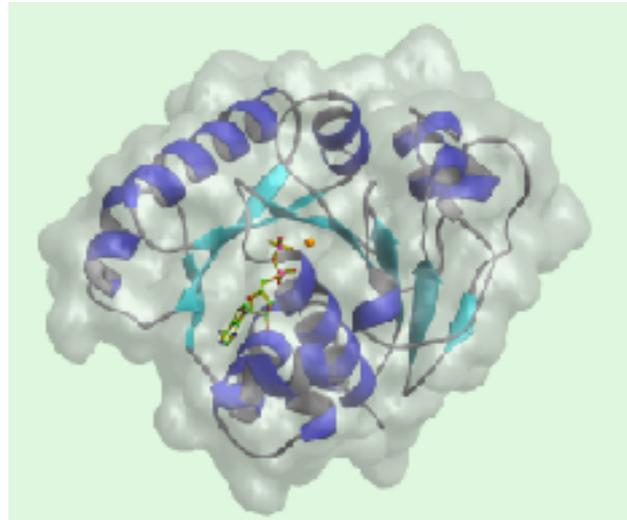


Fig. 1. The schematic diagram of the bacterial cell division machinery. (a) The rod-shaped bacteria have three potential division sites in the cell. The MinCD complex inhibits division at all potential division sites in the cell. (b) The MinE ring is formed, and the activity of cell division inhibition of MinCD is suppressed by MinE. The MinCD complex undergoes a rapid pole-to-pole oscillation movement. (c) MinE recruits the cell division proteins as FtsZ at midcell division sites. The circumferential invagination with squeeze of FtsZ ring starts.

Structure analysis shows that MinD is most similar to nitrogenase iron protein which is a member of the family of the P-loop containing the nucleotide triphosphate hydrolase superfamily of proteins. Unlike nitrogenase or other member proteins that normally work as a dimer, MinD was present as a monomer in the crystal. MinD is also known to behave like a motor protein in *E. coli* cells. The present analysis has shown that MinD has a limited structural similarity with family of motor proteins. Although the tertiary structure of ATPase activity site is similar in these proteins, the overall topology is different. Thus, they are distantly related if at all. Both the  $^{31}\text{P}$  NMR and Malachite Green method exhibited relatively low levels of ATPase activity. These facts suggest that there are some additional factor(s) for MinD to exhibit ATPase activity in the cell and MinD may work as a molecular switch in the multiprotein complex in bacterial cell division.



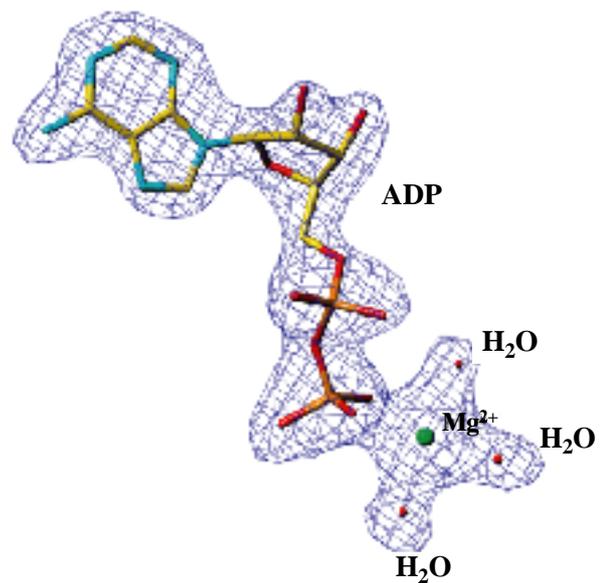
*Fig. 2. The ribbon diagram of the crystal structure of MinD from *P. horikoshii* with transparent molecular surface. Bound ADP is shown as ball and stick and magnesium ion is shown as an orange ball. The nucleotide binding pocket is close to the edge of the  $\beta$ -sheet. This pocket is exposed to solvent region.*

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### References

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[2] Naoki Sakai, Min Yao, Hiroshi Itou, Nobuhisa Watanabe, Fumiaki Yumoto, Masaru Tanokura, and Isao Tanaka, Structure **9** (2001) 817.



*Fig. 3. Electron density of Mg-ADP region. The sigma-weighted omit map is calculated at 2.3 Å resolution and contoured to  $3.0\sigma$ .*