Effects of Phospholipid Composition on MinD-Membrane Interactions in Vitro and in Vivo*

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The peripheral membrane ATPase MinD is a component of the Min system responsible for correct placement of the division site in Escherichia coli cells. By rapidly migrating from one cell pole to the other, MinD helps to block unwanted septation events at the poles. MinD is an amphitropic protein that is localized to the membrane in its ATP-bound form. A C-terminal domain essential for membrane localization is predicted to be an amphipathic α-helix with hydrophobic residues interacting with lipid acyl chains and cationic residues on the opposite face of the helix interacting with the head groups of anionic phospholipids (Szeto, T. H., Rowland, S. L., Rothfield, L. I., and King, G. F. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 15695–15699). To investigate whether E. coli MinD displays a preference for anionic phospholipids, we first examined the localization dynamics of a green fluorescent protein-tagged derivative of MinD expressed in a mutant of E. coli that lacks phosphatidylethanolamine. In these cells, which contain only anionic phospholipids (phosphatidylglycerol and cardiolipin), green fluorescent protein-MinD assembled into dynamic focal clusters instead of the broad zones typical of cells with normal phospholipid content. In experiments with liposomes composed of only zwitterionic, only anionic, or a mixture of anionic and zwitterionic phospholipids, purified MinD bound to these liposomes in the presence of ATP with positive cooperativity with respect to the protein concentration and exhibited Hill coefficients of about 2. Oligomerization of MinD on the liposome surface also was detected by fluorescence resonance energy transfer between MinD molecules labeled with different fluorescent probes. The affinity of MinD-ATP for anionic liposomes as well as liposomes composed of both anionic and zwitterionic phospholipids increased 9- and 2-fold, respectively, relative to zwitterionic liposomes. The degree of acyl chain unsaturation contributed positively to binding strength. These results suggest that MinD has a preference for anionic phospholipids and that MinD oscillation behavior, and therefore cell division site selection, may be regulated by membrane phospholipid composition.

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MinD protein, along with MinC and MinE, is required for selection of the correct placement of the division site in bacterial cells (1). During vegetative growth, cell division in rod-shaped bacteria occurs at the cell center. The earliest event in this process is the polymerization of the tubulin-like protein FtsZ at mid-cell into an annular structure called the Z-ring (2–4). In the absence of the min system, Z-rings form at mid-cell as well as cell poles, resulting in the production of minicells (5). The ATP-bound form of MinD, an amphitropic peripheral membrane protein, is localized to the membrane. The binding of MinE to MinD induces hydrolysis of ATP and the release of MinD into the cytoplasm (6). The ATP binding cycle induced by MinE results in the rapid movement of MinD from one cell pole to the opposite cell pole, forming alternating broad polar zones (7). MinC is a specific inhibitor of Z-ring formation (8, 9). Because MinC binds to MinD, the movement of MinC from pole to pole with relatively long polar dwell times and a short transit time blocks the formation of polar Z-rings but not medial rings (10, 11). Therefore, the ATPase activity of MinD is presumed to provide the driving force for the pole-to-pole oscillation of the MinC division inhibitor.

The mechanism of MinD binding to the membrane recently has been elucidated (12, 13). The C-terminal region of MinD contains a highly conserved motif that is essential for membrane localization. This motif is unstructured in crystals of MinD (14, 15). On the other hand, the motif is predicted to be an amphipathic α-helix with one side of the helix containing mainly hydrophobic amino acids and the other side containing mainly positively charged amino acids (12). According to the model (12), ATP binding to MinD induces a conformational change in the protein that results in a release of the C-terminal motif and exposure of its hydrophobic residues followed by binding to the phospholipid bilayer and helix formation. Such amphipathic helices usually align parallel to the membrane surface so that the hydrophobic residues interact directly with lipid acyl chains, whereas the cationic residues on the opposite face of the helix interact with the head groups of anionic phospholipids (for review, see Ref. 16). In the present study we use both in vivo and in vitro systems to examine directly whether the Escherichia coli MinD protein has a preference for anionic over zwitterionic phospholipids.

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Experimental Procedures

Strains and Growth Conditions—To construct a phosphatidylethanolamine (PE)−-deficient strain expressing a green fluorescent protein

* The abbreviations used are: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; PC, phosphatidylcholine; GFP, green fluorescent protein; FRET, fluorescence resonance energy transfer; MIANS, 2-(4′-maleimidylphenyl)napththalene-6-sulfonic acid; LY, lucifer yellow iodoacetamide.
MinD Binding to Anionic Phospholipids

(GFP) derivative of MinD, plasmid pWM1255 (17) was introduced into strain AD90(pss93::kanR- recA) carrying plasmid pDD72 (pssA::cmR), which has a thermosensitive origin of replication derived from plasmid pSC101 (18). Plasmid pWM1255 (ampR ColE1 origin) synthesizes MinE and a translational fusion of GFP to the N terminus of MinD, both from the isopropyl-1-thio-β-D-galactopyranoside-inducible Ptrc90 promoter (19). Strain AD90/pDD72/pWM1255 (PE-containing) exhibits wild-type phospholipid composition. Curating of plasmid pDD72 as described previously (18) resulted in strain AD90/pWM1255, which lacks PE (contains only phosphatidylglycerol (PG) and cardiolipin (CL)) but maintains the same membrane protein-to-phospholipid ratio as wild-type cells made up of solely PG and CL (18). Strains were grown on LB agar or liquid LB medium supplemented with 50 mM MgCl₂ and 50 μM ampicillin.

To construct a MinD-overproducing strain, the E. coli minD gene was cloned as an EcoRI-HindIII fragment into plasmid pET28a+ (Novagen), fusing minD to an N-terminal hexahistidine tag and placing the His-minD construct under the control of the T7 promoter. This plasmid, pET28a-MinD (kanR), then was introduced into strain BL21(DE3) (20) to make strain WM1682. For His-MinD purification, WM1682 cells were grown overnight in LB medium with kanamycin (50 μg/ml) at 37°C. The culture was diluted 100-fold in the same medium, and growth was continued to an A₆₀₀ of 0.6. To induce overproduction of their MinD, isopropyl-1-thio-β-D-galactopyranoside was added to 1 mM, and cells were grown for an additional 2–3 h. The culture was collected by centrifugation at ~90°C for 1 h, and the supernatant was centrifuged further at 120,000 × g for 30 min to make strain WM1682. For His-MinD purification, WM1682 cells were captured with a light-sensitive Photometrics CoolSnap FX cooled Olympus BX60 microscope fitted with a GFP filter cube, and the images were stored on a microscope slide, and was covered quickly with a cover glass. Fluorescence images were observed with a ×100 oil immersion objective on an Olympus BX60 microscope fitted with a GFP filter cube, and the images were captured with a light-sensitive Photometrics CoolSnap FX cooled charge-coupled device camera driven by QED image capturing software. Time-lapse images were taken every 10 s, each with 2–4 s exposure time, aiming to improve signal, and the images were saved as PICT files. Movies were made from these files with iMOVIE 2.2 software.

Preparation of Liposomes—Soybean phosphatidylcholine (PC), PG, and CL from heart, synthetic dioleoyl-PG, E. coli PE, and E. coli polar phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL). E. coli lipids were purified from E. coli polar phospholipids (21). Liposomes were prepared by water bath sonication for 1–2 h (6–12 times for 10 min each) at 0°C in a buffer containing 25 mM Tris-Cl, pH 7.5, and 50 mM KCl and were stored at ~80°C.

MinD Binding to Liposomes—The sedimentation assay (6) with minor modifications was used to study MinD binding to liposomes. MinD preparations first were centrifuged at 263,000 × g for 10 min to remove potential MinD aggregates. MinD (0.2–20 μM) was mixed with 1 mM ATP and ADP and liposomes (160 μM/mL) in 100 μL of buffer containing 25 mM Tris-HCl, pH 7.5, 50 mM KCl, and 5 mM MgCl₂ and was incubated for 10 min at 30°C. After incubation, samples were centrifuged at 263,000 × g at 30°C for 10 min. After centrifugation, the pellets were resuspended in 40 μL of sample buffer. Aliquots of 20 μL were subjected to 12% SDS-PAGE and stained with Bio-Safe Coomassie Blue (Bio-Rad). The bands were quantified using FluoroMax Multi-Imager with Quantity One software (Bio-Rad). The experimental data were compiled in KaleidaGraph 3.01. Lines on the graphs (Figs. 4 and 5) are curves calculated by the Hill equation that best fit the data provided. Hill coefficients (n, Hill coefficient and association constants (K₅0) were calculated from the graph in Fig. 5.

Labeling of MinD with Fluorescent Probes—MinD (0.6 mg/ml) in 100 μL of the storage buffer was incubated at 22°C in the dark with a 20-fold molar excess of 2’-4’-maleimidoxyanilinonaphthalene-6-sulfonic acid (MANS; Molecular Probes, M-8) or Lucifer yellow isothiocyanate (LY; Molecular Probes, L-1338) for 2 h. The conjugate was separated on Sephadex G-25 columns equilibrated with the same buffer. The level of modification, estimated from protein concentration and fluorescence of standard dye solutions, was close to 1:1 for both dyes. Steady-state fluorescence resonance energy transfer (FRET) experiments were conducted at 22°C on a PFTI QuantumMaster spectrofluorometer (Photon Technology International). Samples contained 1 μM total MinD protein in the same buffer that was used for the liposome sedimentation assay. Additional components were added as indicated. In a single scan, a sample was excited at 322 nm (slit width 4 nm), and emitted light was scanned from 350 to 600 nm (slit width 4 nm, scan rate 100 nm/min).

RESULTS

GFP-MinD in PE-lacking Cells—We demonstrated previously that E. coli cells lacking PE were filamentous because of inhibition of cell division. The lack of PE did not prevent localization of FtsZ, FtsA, and ZipA in these cells, but the proteins often formed aberrant spiral structures (22). We first examined the dynamic localization of GFP-MinD in the pss93 mutant (AD90/pWM1255) containing the covering pDD72 plasmid, which maintains wild-type phospholipid composition and cell morphology. In these cells, GFP-MinD localized into a typical horseshoe structure on the membrane at the poles of the cells and oscillated from pole to pole in large zones with cycle times similar to those reported previously (data not shown) (7). In contrast, GFP-MinD movement in cells lacking PE was markedly different as shown by a typical time-lapse series of GFP-MinD movements in a filamentous PE-lacking cell (Fig. 1). Although the change in phospholipid composition still allowed movement of GFP-MinD in the PE-lacking cells, fluorescence localized as a “zigzag” pattern of compact spots along the filamentous cells. No clear back-and-forth movement was observed. As can be seen by following individual spots in Fig. 1, the lifetime of most spots was ~30–50 s, about a factor of 2 longer than the shift-dwell-shift observed for GFP-MinD zones in PE-containing cells (7) (data not shown). Because of the apparent random localization of the spots, it was difficult to establish their lineage during the time course. However, the simultaneous appearance of a new spot and disappearance of an old spot nearby in most cases suggested that material from the old spot was migrating to the new spot. For example, fluorescence in spots B and C, visible in the first panel, appears to have been transferred to spots E and D, respectively. If so, this would represent a distance of ~0.5–1 μm, considerably less than the migration distances observed in filamentous cells with normal phospholipid composition (7). Interestingly, the spots appeared to move not along the long axis of the cell but also in the perpendicular direction (for example, see M–Q).

This disturbed pattern of movement suggested that the high level of PG and CL and/or the lack of PE in the membrane results in the alteration of the GFP-MinD assembly-disassembly process on the membrane surface. This effect might be a
consequence of the change in the affinity of MinD to the membrane composed of only anionic phospholipids. To test this proposal, we studied the interaction of MinD with liposomes of different phospholipid compositions in vitro.

Interaction of Purified MinD with Liposomes—It was demonstrated that MinD with a His tag at the N terminus is functionally active in E. coli (23). Therefore, we constructed and purified a His-tagged MinD protein for in vitro experiments (see “Experimental Procedures”). To study binding of MinD to liposomes, we used the method developed by Hu et al. (6) in which the protein is incubated in the presence of ATP and liposomes followed by high speed centrifugation (see “Experimental Procedures”). Fig. 2, A and B, represents an experiment in which MinD was bound to liposomes of different phospholipid compositions. As seen in Fig. 2, A and B, MinD at 6 μM can bind to PC liposomes in an ATP-dependent manner. Enrichment of PC liposomes with an anionic phospholipid (CL from heart) strongly enhanced both specific (ATP-dependent) and nonspecific (ADP-dependent or without any nucleotide) binding of MinD. At the same time, enrichment of PC liposomes with PE did not change the level of MinD bound to liposomes. Binding to liposomes made from E. coli total phospholipid containing about 20% of anionic phospholipid was also higher than binding to PC or PC-PE liposomes. All these data together indicate that MinD has a higher affinity to liposomes that are enriched with anionic phospholipids.

To investigate whether MinD binding to CL from heart is ATP-dependent, we decreased the concentration of MinD in the incubation mixture to 2.5 μM and determined the dependence of MinD binding on levels of CL by using PC-CL liposomes of varying CL concentrations (Fig. 3, A and B). The increase in the content of CL in PC liposomes enhanced ATP-dependent specific binding. However, nonspecific binding without nucleotides also was elevated in liposomes with higher CL concentrations.

To exclude the possibility that the higher affinity to anionic phospholipids is a property of the His-tagged protein but not the native MinD, the tag was removed by thrombin digestion. MinD without the His tag had the same high affinity to PC-CL liposomes as His-tagged MinD (data not shown). All experiments described below were carried out with His-tagged MinD, and in all in vitro experiments described herein, His-MinD is referred as MinD.

To study in more detail whether the affinity of MinD to the membrane depends on its phospholipid composition, we investigated the affinity of MinD to binding to liposomes of different phospholipid compositions as a function of MinD concentration. For each type of liposome, we measured the dependence of MinD binding on MinD concentration in the presence of ATP or ADP. Fig. 4 represents an example of such a titration experiment for PC liposomes enriched with E. coli PG. The amounts of MinD bound to liposomes are expressed in arbitrary units (A.U.). Every experimental point was obtained by quantification of the corresponding band after SDS-PAGE (see “Experimental Procedures”). Protein determination showed that in all experiments the amount of MinD bound to liposomes was no more than 15% of the total amount of MinD present (data not shown). Lines on the graphs represent the best fit to the experimental points as calculated by the Hill equation. The calculated association constant ($K_a$) and Hill coefficient ($n_H$) for the ATP form of MinD were 4.5 μM and 2, respectively. Fig. 5 summarizes the results of nine titration experiments with liposomes of the indicated composition. Data points in Fig. 5 represent the amount of MinD bound to liposomes in the presence of ATP. To compare results of all experiments, data points for each experimental set were normalized by dividing by the theoretical maximal binding calculated from graphs similar to
Incubated with liposomes (160 μg/ml E. coli) from liposome preparation were PC from soybean, CL from bovine heart, PE (H9262) 1mM ATP in the presence of liposomes (160 μg/ml) without (−) nucleotides (1 mM ATP or ADP). The phospholipids (PL) used for liposome preparation were PC from soybean, CL from bovine heart, PE from E. coli, and total E. coli phospholipid extract (E. coli). PC-CL liposomes contained 25 mol % CL, and PC-PE liposomes contained 40 mol % PE. Liposomes from E. coli phospholipids had the same phospholipid composition as E. coli cells. After a 10-min incubation at 30 °C, the samples were centrifuged, and the pellets were analyzed by SDS-PAGE. Bands were quantified using a FluorS-Max MultiImager. Bound MinD is expressed in arbitrary units (A.U.). For further details, see “Experimental Procedures.”

Fig. 2. Analysis of MinD binding to liposomes of different phospholipid compositions. A, MinD, at a concentration of 6 μM, was incubated with liposomes (160 μg/ml phospholipid) with (+) or without (−) nucleotides (1 mM ATP or ADP). The phospholipids (PL) used for liposome preparation were PC from soybean, CL from bovine heart, PE from E. coli, and total E. coli phospholipid extract (E. coli). PC-CL liposomes contained 25 mol % CL, and PC-PE liposomes contained 40 mol % PE. Liposomes from E. coli phospholipids had the same phospholipid composition as E. coli cells. After a 10-min incubation at 30 °C, the samples were centrifuged, and the pellets were analyzed by SDS-PAGE. Bands were quantified using a FluorS-Max MultiImager. Bound MinD is expressed in arbitrary units (A.U.). For further details, see “Experimental Procedures.”

Fig. 3. Analysis of MinD binding to PC-CL liposomes with varying CL concentrations. 2.5 μM MinD was incubated with (+) or without (−) 1 mM ATP in the presence of liposomes (160 μg/ml phospholipid) composed of soybean PC and the indicated mol % CL (heart). In panel A, samples were processed as in Fig. 2A. In panel B, samples were quantified as in Fig. 2B. A.U., arbitrary units.

The one presented in Fig. 4. The values of the association constants and Hill coefficients are summarized in Table I. As shown in Figs. 5 and Table I, the affinity of MinD-ATP to liposomes is twice as high for the liposomes containing a fraction of anionic phospholipids and 9-fold higher for the entirely anionic liposomes than for the pure PC liposomes. The higher affinity of MinD to liposomes composed of PC and CL from heart compared with PC and CL from E. coli might be due to the difference in fatty acid composition of these two species of CL. CL from bovine heart is highly unsaturated and contains four polyunsaturated acyl chains, whereas acyl chains of CL from E. coli are both saturated and monounsaturated (24). This finding is consistent with results (25) showing that the affinity of DnaA, another protein with amphipathic α-helices, to phospholipids depends on both the negative charge of head groups and the unsaturation of acyl chains.

As shown in Figs. 4 and 5 and Table I, the binding of MinD to liposomes in the presence of ATP displays a considerable positive cooperativity with respect to the protein concentration. The Hill coefficient of ~2 is characteristic of MinD-ATP binding to all types of liposomes used in the experiments. This result suggests that MinD-ATP oligomerizes at least to the level of dimers on the liposome surface. In contrast, binding of MinD-ADP to liposomes (Fig. 4 and data not shown) was characterized by a Hill coefficient close to 1, which may reflect nonspecific binding without oligomerization on the surface of liposomes.
Dimerization of MinD Probed by FRET—One very useful method for the analysis of protein-protein interactions is fluorescence resonance energy transfer. The specific utility of FRET rests in its ability to provide direct information on molecular proximity. In general, FRET measurements are made by observing the transfer of excited state energy from one chromophore (the donor) to another (the acceptor). In the most favorable instances, FRET can be used to determine accurately molecular distances over a range of 20–90 Å (26–28). To address the question of MinD dimerization, we chose MIANS and LY as a donor-acceptor pair because of the very good overlap of their emission and absorption spectra (322/417 and 426/531 nm excitation/emission maxima, respectively). MinD from E. coli contains two cysteines, Cys-52 and Cys-119; the former appears to be accessible for derivatization by these thiol-reactive dyes. Fig. 6 shows that the fluorescent spectrum of the equimolar mixture of MinD fluorescent derivatives in solution (thin continuous line) is identical to the sum of their individual spectra (wide gray line), indicative of the absence of any FRET between them. In contrast, incubation of this mixture with PC liposomes containing 20 mol % CL (heart) caused a decrease of the donor fluorescence and a proportional increase of the fluorescence from the acceptor, which is typical for FRET (Fig. 6, bold continuous line). Moreover, the addition of a 5-fold excess of unlabeled MinD had no further effect on the shape of this spectrum (data not shown).

**DISCUSSION**

In this study, we investigated the role of anionic phospholipids in the interaction of MinD with the membrane. Our data demonstrate a preferential interaction of MinD with anionic phospholipids accompanied by oligomerization of MinD-ATP on the liposome surface. The observed cooperativity of MinD binding to liposomes, thus indicating oligomerization, is supported by our FRET measurements. The absence of FRET in the mixture of MinD carrying different fluorescent labels (Fig. 6) demonstrates that the protein does not form dimers in solution at least at 1 μM concentration. Because only 10–15% of the total amount of MinD was bound to liposomes (see above), the extent of FRET observed in the presence of anionic liposomes is significant. The appearance of FRET could be simply the result of an increase of the local concentration of the protein on the membrane surface and the consequent increased collision probability. However, when an excess of unlabeled MinD was added to mixtures containing labeled MinD, FRET was unchanged. This suggests that MinD formed stable dimers or oligomers on the liposomes. Our data are consistent with previous results demonstrating self-enhanced binding of MinD to liposomes (23), formation of MinD filament bundles in solution (29), and cryoelectron microscopy images of the MinD tubes (6).

We have found that both the negative charge of the phospholipids and the degree of acyl chain unsaturation contribute to binding affinity. These findings are consistent with the model of Szeto et al. (12), in which MinD interacts with the phospholipid membrane by partitioning its C-terminal amphipathic α-helix into the membrane bilayer. The helix orientation is likely to be such that hydrophobic residues interact directly with lipid acyl chains, whereas the cationic residues on the opposite face of the helix interact with the head groups of anionic phospholipids. An explanation for the higher affinity of MinD to CL from heart might be that membranes with higher unsaturated acyl chains of phospholipids are packed more loosely and the distance between head groups is greater. This factor, in combination with the higher degree of disorder in unsaturated phospholipid bilayers, might create more favorable conditions for insertion of a short amphipathic α-helix oriented parallel to the membrane surface (16, 30).

Orientation of amphipathic α-helices in the membrane can be predicted by a hydrophobic moment plot analysis (31, 32). We calculated the sequence hydrophobic moment according to Eisenberg et al. (31) for the putative C-terminal α-helix of E. coli MinD based on the putative membrane-targeting sequence of this motif (12, 13). The calculated mean of the sequence hydrophobic moment per residue is about 0.6, and the hydrophobicity per residue is about 0.07. On a conventional plot of hydrophobic moment versus hydrophobicity (32), the values place this putative C-terminal α-helix of E. coli MinD on the border between a surface-active and a globular peptide. The helix also falls within the area delineating parallel orientation to the plane of the membrane but also is very near the border delineating an oblique orientation. This is consistent with the localization of the C-terminal α-helix in the plane parallel to the membrane surface. As such, the hydrophobic residues in-

**TABLE I**

| Phospholipid composition | $K_a$ | $n_H$ |
|--------------------------|-------|-------|
| PC                      | 9.1   | 2.1   |
| Total E. coli PL         | 4.8   | 2.1   |
| PC with 20 mol % CL      | 4.7   | 2.5   |
| PC with 40 mol % PG      | 4.5   | 2.0   |
| PC with 20 mol % CL      | 2.0   | 1.8   |
| PG                       | 1.9   | 1.5   |
| DOPG                     | 1.4   | 2.1   |
| PG with 25 mol % CL      | 1.2   | 2.1   |
| DOPG with 35 mol % CL    | 1.0   | 2.1   |

$^a$ $K_a$ and $n_H$ have been calculated from Fig. 5.

$^b$ PL, phospholipids.

$^c$ DOPG, dioleoyl-PG.
teract directly with lipid acyl chains, whereas the cationic resi-
dues on the opposite face of the helix interact with the head
groups of anionic phospholipids. However, a possibility of some
oblique orientation is not excluded. This may explain the signif-
ificant input of hydrophobic interactions into the binding of MinD
to liposomes (Ref. 13 and this study), i.e. the significant affinity of
MinD for PC liposomes lacking anionic phospholipids.

MinD appears to be associated with a growing set of proteins
that are classified as amphiphilic and share the same property of
reversible binding to membrane lipids. This process regu-
lates their function, and the binding affinity is subject to reg-
ulation. Regulatory switches that control membrane affinity
include modification of the protein itself, for example, by ligand
binding, and modulation of membrane lipid composition. These
proteins use an amphipathic α-helix to sense the membrane
lipid composition. Increases in the proportion of a particular
phospholipid in the membrane can trigger protein-membrane
binding through the generation of an electrostatic pull to the
membrane surface, where the protein searches for intercala-
tion sites (for review, see Ref. 16).

If the membrane binding affinity of MinD depends on lipid
composition, changing the protein retention time on the mem-
brane should modulate both spatial and temporal oscillatory
characteristics of the protein. The observed aberrant behavior
of GFP-MinD in cells lacking PE is consistent with such
changes. It has been proposed that the MinD protein forms a
lattice on the membrane surface (1). Alternatively, MinD may
assemble into two-stranded filaments of limited size, which
are dispersed over one-half of the cell membrane in wild-type
E. coli (29). We have shown that in PE-lacking cells, in which
negative charge density on the membrane surface is very high,
MinD polymers are not dispersed over the membrane surface
but rather organized in compact clusters. Why might MinD
associate in compact areas instead of forming the broad zones
observed in PE-containing cells? One possibility is that the in-
creased concentration of anionic phospholipids increases the
cooperativity of MinD assembly at the membrane, causing as-
sembly to occur at a higher local MinD concentration and
reduces repulsion of the helix. CL domains in the
membrane are the best candidates for such a role because they
create a low pH environment at the membrane surface that
facilitate the membrane insertion of a cluster of glutamic acid
residues at one end of the MinD C-terminal motif (12, 42).
Alternatively, the formation of MinD polymers on the mem-
brane surface may induce the formation of anionic phospho-
lipid microdomain(s). The mutual effect of MinD and anionic
phospholipids on MinD polymerization and phospholipid do-
main formation in the E. coli membrane is under investigation.

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