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FtsZ polymers bound to lipid bilayers through ZipA form dynamic two dimensional networks

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ABSTRACT

Bacteria divide by forming a contractile ring around their midcell region. FtsZ, a cytoskeletal soluble protein structurally related to tubulin, is the main component of this division machinery. It forms filaments that bundle at the inner side of the cytoplasmic membrane. These FtsZ bundles do not attach to bare lipid surfaces. In *Escherichia coli* they remain near the membrane surface by attaching to the membrane protein ZipA and FtsA. In order to study the structure and dynamics of the ZipA–FtsZ bundles formed on a lipid surface, we have oriented a soluble form of ZipA (sZipA), with its transmembrane domain substituted by a histidine tag, on supported lipid membranes. Atomic force microscopy has been used to visualize the polymers formed on top of this biomimetic surface. In the presence of GTP, when sZipA is present, FtsZ polymers restructure forming higher order structures. The lipid composition of the underlying membrane affects the aggregation kinetics and the shape of the structures formed. On the negatively charged *E. coli* lipid membranes, filaments condense from initially disperse material to form a network that is more dynamic and flexible than the one formed on phosphatidyl choline bilayers. These FtsZ–ZipA filament bundles are interconnected, retain their capacity to dynamically restructure, to fragment, to anneal and to condense laterally.

1. Introduction

Bacteria divide through the action of a complex molecular machine (the divisome) that assembles at midcell. About a dozen proteins organize into a dynamic ring (the division-ring) that generates the contractile force required for cell division. FtsZ, a GTPase tubulin prokaryotic homologue [1], is the first protein to polymerize forming a ring anchored to the inner face of the cytoplasmic membrane through ZipA and FtsA. Subsequently, the rest of the divisome proteins (FtsK, FtsQ, FtsB, FtsL, FtsW, FtsI, FtsN) forming the periplasmic connector and the peptidoglycan factory incorporate into the divisome (reviewed in [2,3])

The first stage in the assembly of the division ring is the formation of the so called proto-ring by the concerted assembly of FtsZ, FtsA and ZipA [4]. These three proteins assemble some time before the rest of the division proteins are incorporated [5]. Assembly of FtsA and ZipA into the ring is dependent solely on FtsZ [6], while Z-ring formation requires only one of the other two. ZipA binds to the membrane through its N-terminal transmembrane domain [7] while FtsA associates through a conserved short C-terminal amphipathic helix [8]. Both proteins have been found to stabilize the Z-ring, probably by a direct interaction with the carboxy-terminal tail of FtsZ [9,10]. This suggests that one role of these proteins is to stabilize and anchor the FtsZ polymers to the membrane [4,11].

The FtsZ ring is a membrane bound dynamic structure. *In vivo* experiments in *Escherichia coli* [12], have shown that in the functional divisome complex, both proteins, FtsZ and ZipA, can exchange in and out of the ring within seconds. It is not known yet how this protein exchange takes place and how it affects the stability of the ring.

E. coli ZipA is a 36.4 kDa membrane-anchored protein with a short N-terminal transmembrane domain (aa 1–25) followed by a basic region (~23 aa), an acidic region (~17 aa), and a long proline rich region, the P/Q domain (residues 86–188), that stands between the membrane and the C-terminal globular domain binding FtsZ [7]. Evidence obtained by electron microscopy showed that this P/Q domain is flexible and may stretch over a length from 8 to 20 nm [11]. Different *in*

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vivo and *in vitro* assays have indicated that the interaction between ZipA and FtsZ is mediated by the C-terminal region of the proteins [13,14] and that the C-terminal domain of ZipA (residues 176–328) is required and sufficient to bind FtsZ. X-ray crystallography [10] and NMR [15] have both provided high resolution information on the interaction region, but little is known about the role played by the rest of the ZipA domains. It has been speculated [16] that the flexible tether constituted by the P/Q domain might be important for defining the FtsZ functional orientation on the membrane surface.

This flexible tether stands in the proximity of the membrane, therefore the surface characteristics determined by the membrane phospholipid head groups could be relevant for defining the functional configuration. Recent experiments have shown that the distribution of phospholipids in bacterial membranes is heterogeneous and that this is relevant in cell division [17]. It is not known however how the charge and lateral organization of the main glycerophospholipids found in bacteria (phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin) [18] affect the protein interactions at the interface.

In this work we perform a high resolution structural and dynamic characterization of the FtsZ polymers formed in the presence of sZipA anchored on lipid membrane surfaces using atomic force microscopy. To mimic the initial stages of the divisome formation, we use a simplified reconstituted system of controlled composition using only three elements: FtsZ, ZipA and the lipid bilayer. We visualize at single-molecule level FtsZ self-assembly occurring on the surface of a supported lipid bilayer containing sZipA with a histidine-tag replacing its membrane region and oriented on the surface by including nickel chelating lipids in the bilayer.

We have examined the ability of FtsZ bound to the artificially anchored sZipA to assemble dynamically and form higher order structures. In addition we have investigated the effect of different lipids, *i.e.* egg phosphatidyl choline or *E. coli* lipids on the behavior of the assembled molecules.

2. Materials and methods

2.1. Reagents

GTP was acquired from Sigma. Other analytical grade chemicals were from Merck or Sigma. *E. coli* lipid polar extract (EcPL) and 1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1carboxypentyl)iminodiaceticacid)succinyl] (DGS-NTA (Ni)), L-α-phosphatidylcholine from chicken egg (EPC) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were purchased from Avanti polar lipids.

2.2. Protein purification

E. coli FtsZ was purified by the calcium-induced precipitation method as described in [19]. The soluble mutant of ZipA was constructed by eliminating the hydrophobic N-terminal domain (aa 1–25) of the full length protein, as described elsewhere [20]. sZipA fractions were pooled and stored at −80 °C. The purity of sZipA was >95% according to SDS-PAGE.

2.3. Liposome preparation

Liposomes were prepared from mixtures of lipids (EcPL, EPC or DOPC) and DGS-NTA (Avanti Polar Lipids, Alabaster), in a molar ratio of 10:1.

Lipids were dissolved in 1:1 chloroform:methanol solution, mixed in the desired amount, dried under a stream of nitrogen and resuspended at 4 mg/ml lipid concentration in MiliQ water. The resulting multilamellar vesicles were extruded through a polycarbonate membrane with 200-nm nominal pore diameter (Avanti Mini extruder) resulting in large unilamellar vesicles (LUVs).

2.4. Planar lipid bilayer formation

40 μl of 0.1 g/l LUV solution in Tris–HCl 10 mM, 100 mM NaCl, and 2 mM $CaCl_2$ (pH 7.5) were incubated on a freshly cleaved mica surface for 1 h resulting in the formation of a Supported Lipid Bilayer as described in [21]. The solution containing the non-fused lipid vesicles was removed and replaced by working buffer (Tris 50 mM, KCl 0.5 M, $MgCl_2$, 5 mM, pH 7.5). Incomplete lipid bilayers were formed by reducing to a few minutes the time the mica surface is exposed to solution containing the lipid vesicles.

2.5. Quartz crystal microbalance (QCM)

A quartz crystal microbalance, capable of measuring frequency and dissipation changes (KSV QCM-Z500) was used to quantify the amount of protein adsorbed to the membrane containing linker lipid. A lipid bilayer containing 9:1 molar ratio DOPC:DGS-NTA was formed on a SiO_2 quartz crystal sensor (qsense QSX 303) upon exposure to a solution containing lipid vesicles at 0.1 g/l. After bilayer formation, the liposome containing solution in the chamber was replaced by a solution containing 3 μM sZipA in working buffer (Tris 50 mM, KCl 0.5 M, $MgCl_2$ 5 mM, pH 7.5). After replacing the ZipA solution by buffer, the binding of FtsZ was registered after injection of FtsZ in the same buffer to reach a 10 μM concentration.

2.6. Atomic force microscopy

Atomic force microscopy (AFM) images were taken with a microscope from Nanotec Electrónica, Madrid, Spain operated in the jump mode [22]. The scanning piezo was calibrated using silicon calibrating-grating (NT-MDT Moscow, Russia). Silicon nitride tips (Olympus) with a force constant of 0.05 N/m were used. To observe ZipA–FtsZ bundles on mica, FtsZ filaments were polymerized in solution in the presence of sZipA and adsorbed on mica immersed in buffer containing 1 mM GTP. To observe the ZipA–FtsZ bundles on the lipid bilayers, sZipA (3 μM) and FtsZ (12.5 μM) were incubated for an hour in Tris 50 mM, 0.5 M KCl, and 5 mM $MgCl_2$, pH 7.5 buffer over the lipid bilayer previously fused on the mica surface. Excess protein was removed from solution and GTP was added before imaging. This protocol resulted in reproducible attachment of the ZipA–FtsZ complex to the lipid bilayer leaving the FtsZ protein responsive to the presence of GTP.

2.7. Transmission electron microscopy (TEM)

A drop of the solution of FtsZ polymers (protein concentration 0.5 g/l (12.5 μM)), formed in the absence or in the presence of sZipA (0.25 g/l (7 μM)) in Tris 50 mM, 0.5 M KCl, 5 mM $MgCl_2$, and 1 mM GTP, pH 7.5 was placed in the microscope grid after one minute incubation with GTP to be visualized by electron microscopy after negative staining with 2% uranyl acetate, using a JEOL-1200 electron microscope.

3. Results

3.1. sZipA–FtsZ bundles on mica

Fig. 1 shows a schematic of the different components used in the sample preparations and a reasonable prediction on how they may assemble on the mica surface before imaging with the AFM. sZipA incubated with FtsZ in solution in the presence of GTP forms individual filaments similar to the ones observed in the absence of sZipA, as shown in the TEM images (Fig. 2A and B). The behavior observed under our experimental conditions (Tris 50 mM, 0.5 M KCl, 5 mM $MgCl_2$, pH 7.5) (Fig. 1 B) contrasts with previous observations in which the presence of ZipA induced the formation of FtsZ bundles

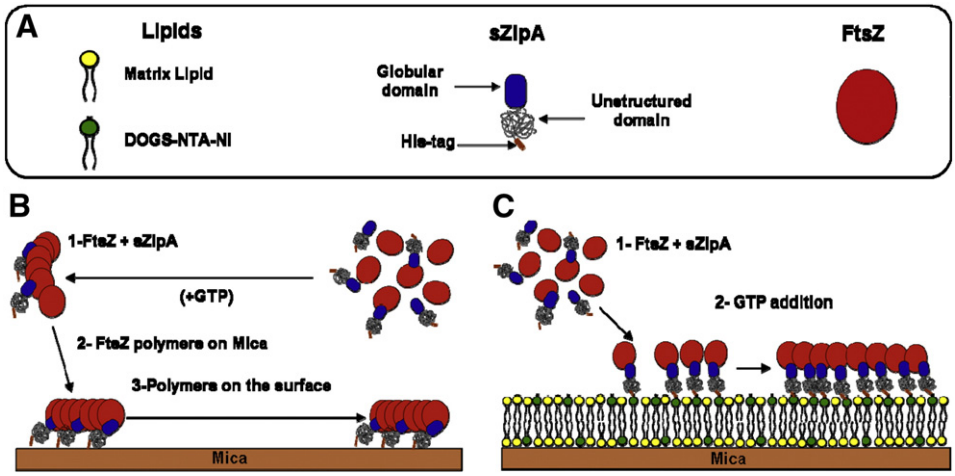

Fig. 1. Schematic prediction of the reconstituted system. Panel (A) describes the elements used. Panel (B) illustrates the procedure followed to form the sZipA–FtsZ bundles on mica and panel (C) the procedure followed to form the bundles on lipid surfaces.

[13]. The fact that those experiments were carried out at pH 5.8, conditions under which GTPase activity is expected to be at least 5 fold lower [23], could explain this difference. However, when these sZipA–FtsZ filaments are adsorbed on mica in the presence of GTP, they undergo a substantial rearrangement over the surface (Fig. 2C). They are about 5 nm in height above the mica, indicating that they remain only one filament thick, but they form longer and wider structures with widths varying from 30 to 60 nm, compatible with a

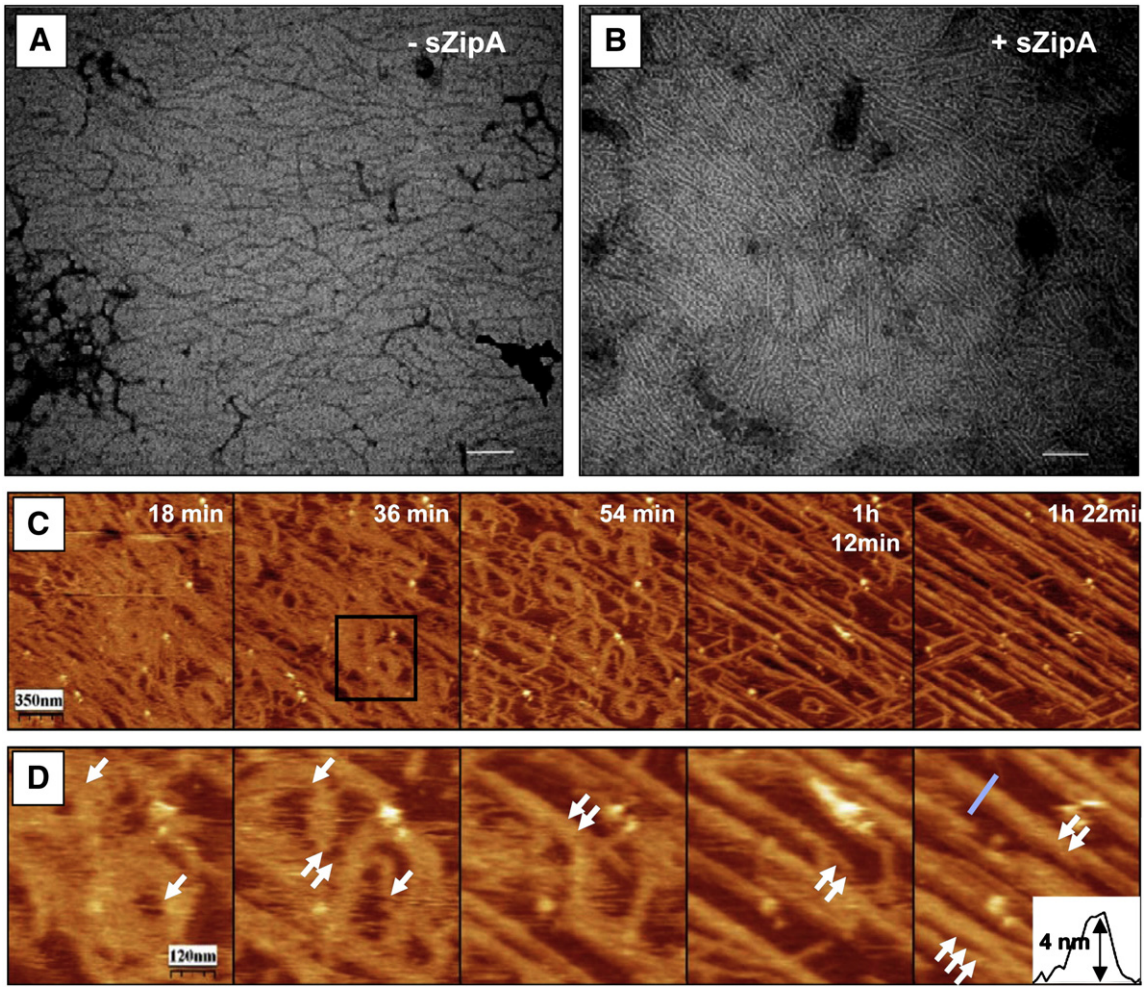

Fig. 2. TEM images of FtsZ filaments formed in the absence (A) (Scale bar is 100 nm) and presence (B) of sZipA. (C) Reorganization of FtsZ filaments by sZipA on mica. Snapshots of FtsZ filaments polymerized in solution in the presence of sZipA and adsorbed on mica immersed in buffer containing 1 mM GTP over a period of 1 h and 22 min. Panel (D), enlarged region shown in C (subsequent images about 10 min apart), illustrates the rearrangement that precedes the formation of the straight bundles (opening, reannealing and lateral aggregation events are marked with single, double and triple arrows respectively). The inset shows the height of the filaments.

parallel arrangement of 6 to 15 FtsZ protofilaments (see below). A zoom of a smaller area (Fig. 2D) illustrates that the reorganization over the surface takes place by fragmentation, reannealing, and lateral condensation, as was previously described for individual FtsZ filaments [24]. These straight and thick filament aggregates are not found in the absence of sZipA.

In this situation in which sZipA is free to move and not attached to the surface, polymers on the surface reorganize if GTP is available in the solution, indicating that sZipA bound to FtsZ filaments does not restrict their dynamic behavior, similarly to the one previously described for filaments assembled in solution [25] and on mica [24] (see movie S3 in Supplementary material). When GTP is not added to the imaging buffer, the initially formed filaments disappeared without further reorganization. We were unable to identify the position of sZipA within the bundles to obtain direct information about the way it crosslinks the filaments. Indirect evidence of its location comes from measuring the height of the bundles (see inset in Fig. 2D), corresponding to the height of the FtsZ filaments, which suggests that sZipA is not sitting between the filaments and the mica but is most likely intercalating between the filaments and modulating lateral contacts to induce the formation of thicker bundles. We can conclude from the observed behavior on mica that, as long as GTP is present, the sZipA–FtsZ bundles remain active to restructure on a surface.

3.2. sZipA–FtsZ on lipid bilayers

We first checked the formation of the lipid–protein modified surface using a QCM. The formation of a lipid bilayer on a solid surface and the subsequent sZipA and FtsZ binding to the lipids was monitored by following the frequency changes of the sensor after the different incubations (Fig. 3).The 35 Hz frequency change registered when the surface is exposed to a 0.1 mg/ml liposome solution (9:1 molar ratio of DOPC:DGS-NTA), confirms the formation of a full bilayer [26]. The additional frequency change observed after exposure of this lipid surface to a 3 μM sZipA solution indicates the formation of a dense monolayer of protein. The estimated amount, obtained from converting the frequency change into mass using the Sauerbrey relation [27], was 710 ng/cm^2 (~21.2 pmol/cm^2). The average area occupied by each ZipA molecule is then approximately 8 nm^2, which corresponds to a dense protein monolayer. Higher amounts of sZipA in solution gave the same result, indicating that this concentration, comparable to the one used in the preparation of the samples characterized with AFM, was enough to saturate the lipid surface. To test the FtsZ binding capacity of this sZipA monolayer, a 10 μM FtsZ solution, in the absence of GTP, was added. FtsZ binding to the ZipA was confirmed by the observed decrease in the resonance frequency (Fig. 3A). No sZipA or FtsZ bound to the bilayer in the absence of nickel chelating neither lipids nor FtsZ (Fig. 3B) in the absence of sZipA. The addition of both sZipA and FtsZ to the solution, a protocol that gave reproducible results and FtsZ polymerization on the lipid surface after GTP addition, gave a comparable mass increment as that obtained after sequential addition of the proteins.

The sZipA could be detached from the membrane upon addition of 200 mM imidazol [28] (Fig. 3C) indicating that the interaction of the protein with the membrane is governed by the N-terminal His-tag binding to the NTA lipid.

After confirming with the QCM that sZipA bound to the membrane containing NTA lipids through the histidine tag and that this oriented sZipA was competent to bind FtsZ, we proceeded to image the modified lipid surfaces with the atomic force microscope.

In order to explore the effect of the type of lipid environment on the formation of sZipA–FtsZ bundles, lipid bilayers of two different compositions were used, one containing egg phosphatidyl choline (EPC), a zwiterionic lipid not found in *E. coli* membranes, and another one containing only polar *E. coli* lipids, rich in negatively charged phospholipids. Both were prepared at a 9:1 molar ratio of phospholipid:DOGS-NTA in order to contain the same amount of linker lipid.

Samples were prepared as depicted in Fig. 1C. On egg phosphatidylcholine (EPC) lipid bilayers, filaments formed immediately upon addition of GTP (Fig. 4A, t = 0 min). If GTP is maintained in the imaging buffer, these structures evolve into straight long arrangements of parallel filaments.

Intermediate stages show the formation of transient wiggly condensates aligned perpendicular to the filaments' longitudinal axis (see movie S4 in Supplementary material documenting events at approximately 4 minute intervals). In the absence of GTP during imaging (movie S5), the filaments initially formed in solution in the

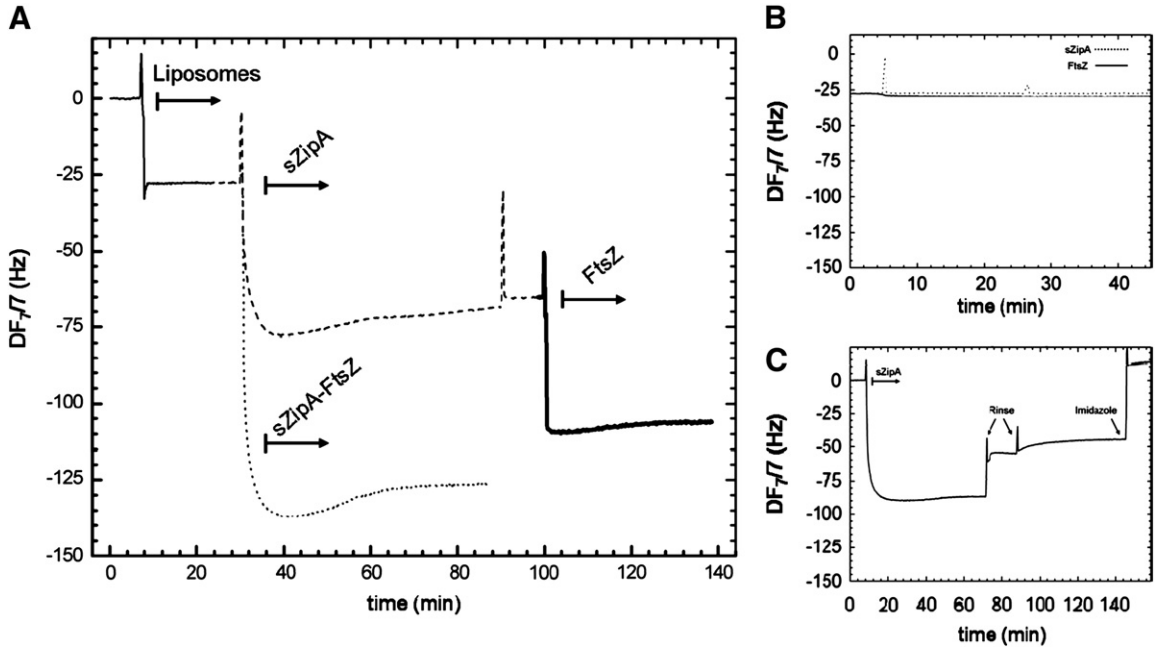

Fig. 3. QCM monitoring of lipid surface modification. (A) Frequency change upon addition of liposomes (thin line), sZipA (dashed line), FtsZ (bold line) and sZipA–FtsZ complex (dotted line). (B) Non specific binding of sZipA and FtsZ on a DOPC bilayer without NTA. (C) Elution of sZipA bound to the DOPC-NTA bilayer after addition of imidazole 500 mM.

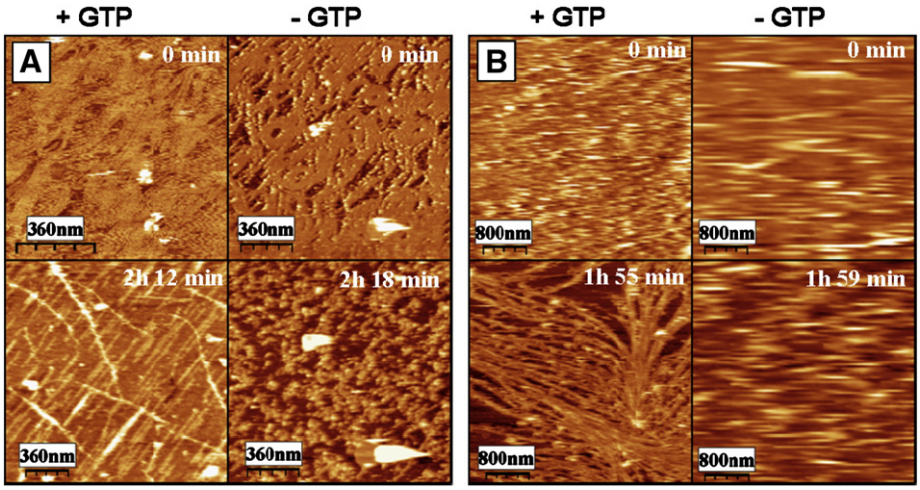

Fig. 4. Evolution of the filaments formed on lipid-sZipA. The upper panel shows the initial structure of FtsZ anchored to a lipid surface through sZipA in EPC (A) and EcPL (B) after addition of 1 mM GTP. The lower panel shows the final structures formed on the surface after approximately 2 h in the presence and absence of GTP in the imaging buffer.

presence of GTP depolymerize on the surface without undergoing any further rearrangement.

The same behavior observed on EPC bilayers was observed on DOPC lipid membranes, a phospholipid sharing the same headgroup as the EPC but differing in the fatty acid composition (results not shown). This suggests that the relevant parameter affecting the behavior of the polymers is the nature of the lipid head group and not the nature of the fatty acids.

sZipA–FtsZ polymers formed on the bilayer prepared with *E. coli* polar lipid extract produce a substantially different image. In this case no FtsZ filaments are initially observed after GTP addition (Fig. 4B), in contrast to what was observed on the EPC bilayer. The

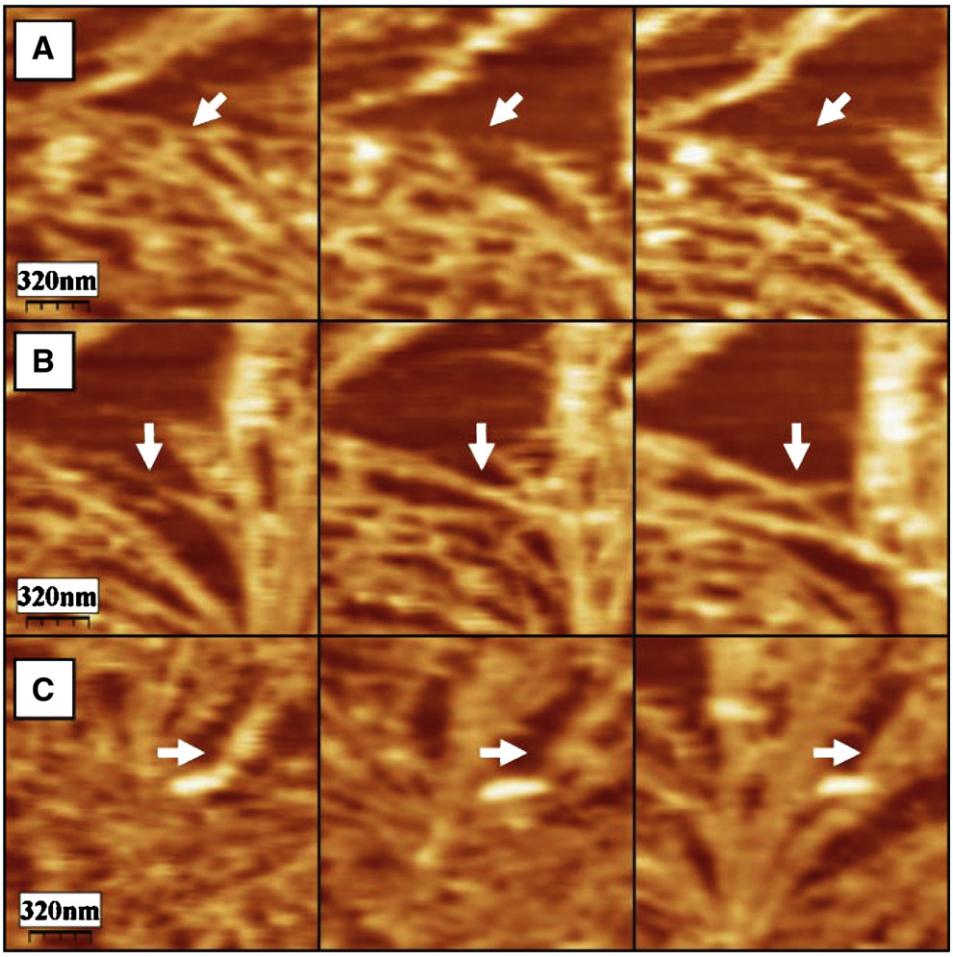

Fig. 5. Dynamics of FtsZ network on EcPL bilayer. Network shown in Fig. 4B was observed over the following 30 min. Upper row shows rupture, central sequence shows branching and annealing and lower sequence lateral condensation.

fuzziness of the images indicates that an amorphous protein layer covers the surface, even when GTP is present in the solution. It takes several minutes to condense and develop into well defined filamentous structures (see movie S6a in Supplementary material documenting the process at approximately 4 minute intervals). GTP in the solution is required for the transformation of the unstructured surface into well defined polymers.

The condensation into curved flexible thicker structures occurs without prior formation of individual filaments as the ones formed on bare mica or on EPC. These aggregates are also extensively branched, in contrast to the straight structures observed on EPC. The assemblies display an active dynamic behavior that retains the same features previously described for individual filaments: fragmentation (Fig. 5A), annealing, crosslinking (Fig. 5B), as well as lateral condensation (Fig. 5C). It is interesting to note that these short range local transient structural modifications do not destabilize the micrometer range features of the network (Supplementary material, movie S6b).

The proximity of the FtsZ polymers to the membrane surface and their plasticity are affected by the lipid composition. Fig. 6 shows the height profiles of the polymers formed on mica, EPC and on EcPL lipid surfaces. The polymer bundles formed on mica and on EPC lipids stand away from the surface a short distance corresponding to one protein monolayer (Fig. 6 A,B,D). In contrast, bundles formed on *E. coli* lipids stand further away from the surface and curve, both on and off plane, forming a highly interconnected network (Fig. 6 C, E). The bundle widths range from 100 to 150 nm and their average height over the surface is around 18 nm. The structures can also curve away from the surface, as is shown in Fig. 6E.

Straight and stiff bundles observed on EPC bilayers are similar to the ones observed on mica, whereas the sZipA–FtsZ bundles on EcPL bilayers are curved. The unstructured ZipA domain, including the charged N-terminal part [7], is expected to have nearly 8 nm end-to-end distance when relaxed, as measured in solution by FRET [29]. Considering that the size of the FtsZ monomer is approximately

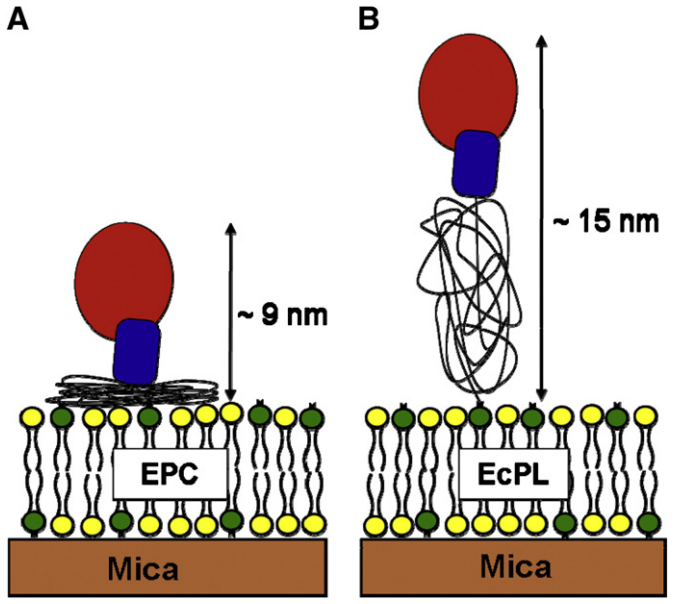

Fig. 7. Schematic of the lipid effect on the sZipA nonstructured domain. (A) EPC bilayer with no net charge does not repel the highly charged non-structured domain of sZipA, which then lies closer to the lipid surface. (B) The negatively charged EcPL bilayer could repel the sZipA charged region and expanding it, causing the increased height of the protein complexes.

4 nm in diameter [30] and the size of the carboxi globular domain of the ZipA [10] is roughly 3 nm, the height measured on EcPL can be interpreted as indicating that the sZipA non-structured domain is located between the FtsZ filaments and the membrane, whereas on the EPC lipid surface, it is probably lying parallel to the membrane surface intercalated within the longitudinally aligned FtsZ filaments, which is similar to what is observed on mica. Fig. 7 depicts a

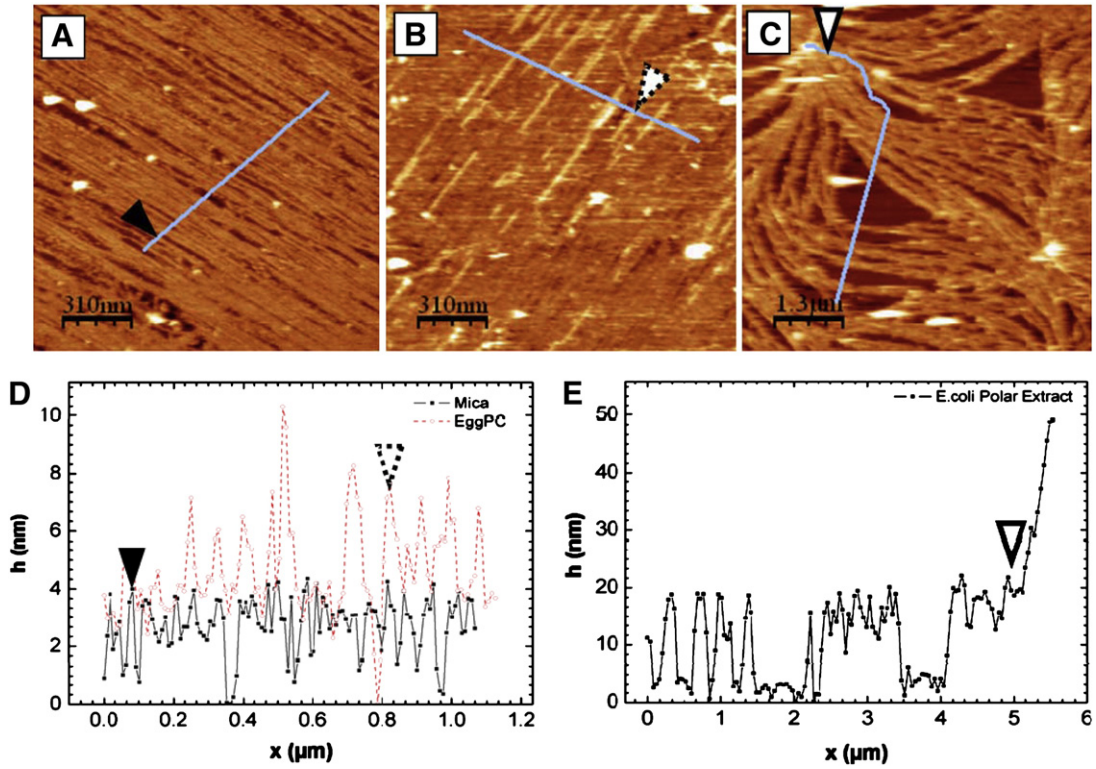

Fig. 6. Height profile of FtsZ polymers on different surfaces. sZipA–FtsZ bundles on mica (A), on EPC bilayer (B), and on EcPL (C) bilayer. D and E show the height profiles of filament bundles on the different surfaces.

schematic of this interpretation. Figs. S1 and S2 in Supplementary material show that the height difference of the protein complexes above the two lipid membranes is present before GTP addition. This suggests that the effect is mainly due to the sZipA interaction with the lipid surface and not to FtsZ polymerization state. A different degree of stretching of the non-structured ZipA P/Q domain induced by the lipid head charges could account for this effect.

4. Discussion

The goal of our work was to study the effect of ZipA on FtsZ polymerization on lipid membranes. In order to do this, we tethered the His-tagged sZipA to nickel chelating lipids incorporated to the membrane [31]. This experimental setup shares two important features with the situation found *in vivo*: sZipA is oriented on the membrane with the carboxi end exposed to the solution, and the protein is free to diffuse laterally on the fluid underlying lipid bilayer.

We found, unexpectedly, that the surface reorganization process and the shape of the filament aggregates were very sensitive to the type of lipid present in the bilayer. EPC and EcPL lipids greatly differ in their surface charge. Whereas PC is a zwitterionic lipid, the phospholipid headgroups of the *E. coli* lipid extract include 30% of the negatively charged lipids cardiolipin and phosphatidylglycerol [18]. The non-structured domain of ZipA is highly charged. The 20 residues (aa 28–48) following the hystidine tag have a net charge of +8 followed by an acidic region (49–65) with negative charge (−11) followed by another basic region of net positive charge of +4 (66–75). The positive region closest to the membrane is likely to induce lipid segregation to recruit negatively charged lipid heads, which could then repel the neighboring negatively charged region causing the observed extension away from the membrane. Charged lipid segregation in fluid membranes has been proposed to play an important role in the subtle interplay between the various entropic and energetic contributions that determine the binding free energy of flexible charged polymers to mixed fluid membranes [32,33].

The surface distribution of membrane lipids in the bacterial membrane is known to change during cell division and CL is enriched in the septal region [17]. This lipid segregation could play an important role in modulating the flexible and charged region of ZipA to optimize FtsZ orientation and function on the surface [16]. FtsZ, having an isoelectric point of 4.9 [34], could be charged under our experimental conditions. However the QCM results clearly indicate that at the pH and salt concentrations used in the experiments, in the absence of sZipA, there is no significant electrostatic interaction between FtsZ and the membrane.

There are two intriguing differences in the behavior of the polymers dependent on the underlying lipid composition: the shape of the final structures formed and their formation kinetics. On EPC, as well as on mica, the filament bundles evolve into predominantly straight structures whereas on EcPL lipids they adopt curved shapes. They also form immediately after GTP additions, indicating that any longitudinal interactions needed to form filaments occur immediately. On EcPL, however, this polymerization step takes several minutes. Both effects could be a consequence of the distance away from the surface at which ZipA holds the FtsZ, as observed from the height measurements. Their orientation could be less restricted, increasing the time required for their alignment to form filaments, but allowing enough flexibility to form curved aggregates.

Both longitudinal and lateral interactions between FtsZ monomers [35,36] appear to participate in the formation of the bundles observed on the surfaces. GTP is required to maintain the longitudinal monomer–monomer association, and the lateral interactions modulated by sZipA induce the aggregation of individual filament into thicker bundles. Structures were observed to evolve with time on the different surfaces, indicating that diffusion on the plane of the membrane could contribute to tune the formation of the polymers on lipid surfaces. Our results provide strong evidence that ZipA acts not merely as a filament anchor but that its presence modulates the shape and aggregation state of the FtsZ filaments on the surface. This is also the first experimental evidence that ZipA acts as a flexible tether between the membrane and the C-terminal domain of FtsZ conferring a large plasticity to the membrane bound FtsZ polymers, as had been previously suggested [11,36]. Additionally, we observe that the presence of charged lipids on the underlying membrane is essential to increase the flexibility of the ZipA nonstructured domain.

Recently, theoretical calculations have proposed different ring formation and force generation mechanisms in bacteria [35–48]. They are based on different assumptions about FtsZ monomer interactions and filament attachment to the membrane. The results presented here address some of these assumptions. The increased flexibility, dynamism and interconnectivity of FtsZ bundles formed on EcPL bilayers, as well as the decreased on plane curvature and increased off plane curvature relative to the ones found on mica, are all compatible with recent models that pointed out that modulating these two parameters, on plane and off plane curvature, could favor the formation of rings on a cylindrical surface [36]. Our observations are also compatible with assumptions made by theoretical models based on the formation of cross-linked polymeric structures of FtsZ with “healing” ends after monomer removal [38], on condensation through the effect of lateral interactions [35,36,39] or on cross-linking and bundling between filaments to generate forces [40].

5. Conclusion

Oriented ZipA on lipid surfaces promoted the assembly of FtsZ polymers into higher order lateral condensed aggregates of individual filaments. Filaments on EPC bilayers formed immediately after GTP addition and aggregated into straight bundles, whereas on *E. coli* lipids, the polymers formed after a certain lag time, stayed further away from the membrane and were curved and dynamic. The presence of ZipA therefore affects differently the crosslinking, bundling, orientation, flexibility and curvature (on and off plane) of FtsZ polymers depending on the type of lipids present on the underlying membrane.

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