RESEARCH COMMUNICATION

The bacterial cell division protein FtsZ assembles into cytoplasmic rings in fission yeast

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During cytokinesis, most bacteria assemble a ring-like structure that is composed of the tubulin homolog FtsZ. The mechanisms regulating assembly and organization of FtsZ molecules into rings are not fully understood. Here, we express bacterial FtsZ in the fission yeast *Schizosaccharomyces pombe* and find that FtsZ filaments assemble into cytoplasmic rings. Investigation of the *Escherichia coli* FtsZ revealed that ring assembly occurred by a process of closure and/or spooling of linear bundles. We conclude that FtsZ rings can assemble in the absence of all other bacterial cytokinetic proteins and that the process might involve hydrolysis of FtsZ-bound GTP and lateral associations between FtsZ filaments.

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Cytokinesis in a majority of bacteria requires the function of the prokaryotic ancestor of tubulin, FtsZ (Lutkenhaus et al. 1980; Lowe and Amos 1998; Errington et al. 2003; Erickson 2007). FtsZ is the first protein to localize to the mid-cell site, where it plays a critical role in the recruitment of a dozen other proteins that are required for assembly of the division septum (Bi and Lutkenhaus 1991; Weiss 2004; Goehring and Beckwith 2005). FtsZ has been shown to be a GTPase capable of forming a variety of polymeric structures in vitro (de Boer et al. 1992; RayChaudhuri and Park 1992; Bramhill and Thompson 1994; Mukherjee and Lutkenhaus 1994; Erickson et al. 1996; Romberg and Levin 2003). In vivo it localizes to the division site to form a ring-like structure (termed the FtsZ-ring or the Z-ring) and remains at the leading edge of the septum throughout cytokinesis (Bi and Lutkenhaus 1991; Wang and Lutkenhaus 1993; Ma et al. 1996). FtsZ rings have been shown to be highly dynamic structures with half-lives as low as ~9 sec and have been pro-

posed to exist as short polymers of 25–30 subunits (Anderson et al. 2004). These observations have been supported recently by cryo-electron tomography images in *Caulobacter crescentus* (Li et al. 2007). Although a major understanding of the polymerization process of FtsZ has come from in vitro studies (Lu and Erickson 1998; Mukherjee and Lutkenhaus 1998; Romberg et al. 2001; Scheffers et al. 2002; Romberg and Levin 2003; Chen and Erickson 2005; Mingorance et al. 2005), studies over the past decade have identified several molecular regulators of FtsZ in vivo (Romberg and Levin 2003; Weiss 2004; Goehring and Beckwith 2005; Margolin 2005), and models for FtsZ ring positioning and assembly have been established. One model predicts a nucleation point at the mid-cell site for the initial assembly of FtsZ, from which FtsZ polymerizes into a ring-like structure, and has been called the membrane nucleation model (Bi and Lutkenhaus 1991; Addinall and Lutkenhaus 1996; Addinall et al. 1997). Another model relying on careful live cell imaging of *Escherichia coli* (Thanedar and Margolin 2004) and *Bacillus subtilis* (Peters et al. 2007) suggests that FtsZ spirals are remodeled to assemble the division ring. However, how FtsZ assembles into supramolecular ring-like structures in vivo still remains largely unknown.

We previously established the fission yeast as a cellular model for studying prokaryotic cytoskeletal proteins (Srinivasan et al. 2007). In this study, we express wild-type and mutant versions of *E. coli* FtsZ (*EcFtsZ*) in fission yeast. Surprisingly, we find that *EcFtsZ* assembles into ring-like structures in fission yeast in a mechanism involving closure and/or spooling of linear cables.

Results and Discussion

Previous studies have shown that *EcFtsZ* expressed in fission yeast assembles into spot-like structures and cables (Fig. 1A, panels i,ii; Srinivasan et al. 2007), which are largely abolished upon coexpression of SulA (Srinivasan et al. 2007), a known inhibitor of FtsZ polymerization in bacterial cells. These spot-like structures were very similar in appearance to the spots observed when FtsZ was expressed in mammalian cells lines by Cabral and coworkers (Yu et al. 1999). In addition, as in fission yeast cells, FtsZ expressed in mammalian cells was also observed to form a filamentous network interconnecting the dots (Yu et al. 1999). Fluorescence recovery after photobleaching experiments (FRAP) revealed that FtsZ turns over with a recovery half-time $t_{1/2}$ of ~11 sec ($n = 22$), which is very close to the FtsZ turnover rates determined in bacterial cells (Anderson et al. 2004). FRAP was carried out on both spot- and cable-like structures of *EcFtsZ*. Both the spots and cables showed similar turnover rates of 10.82 ± 4.5 sec ($n = 7$) and 10.88 ± 4.2 sec ($n = 15$), respectively (Fig. 1B,C; Supplemental Table 1). Based on these two criteria, we concluded that FtsZ polymerization in fission yeast occurred by mechanisms similar to those in bacterial cells.

A closer examination of the polymeric structures formed by *EcFtsZ* using 3D confocal microscopy revealed that the FtsZ patches were indeed ring-like structures with diameters of 0.51 ± 0.04 μm , $n = 100$ (Figs. 2A–C, 3H). *EcFtsZ* also assembled into linear cables, and in many instances, cables appeared to be connected with

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Figure 1. FtsZ structures are dynamic in fission yeast. (A) *E. coli* FtsZ formed cables (panel i) and spots or patches (panel ii). (B–G) Fluorescence recovery after photobleaching (FRAP) of the various FtsZ-GFP-containing structures. (B) *EcFtsZ* ring. (C) *EcFtsZ* cable. (D) *MtFtsZ* ring. (E) *MtFtsZ* cable. (F) *EcFtsZQ47K* ring. (G) *EcFtsZQ47K* cable. Fission yeast cultures expressing FtsZ-GFP were mounted onto agarose slides, and a Zeiss Meta 510 confocal microscope was used for the FRAP experiments. The FtsZ-GFP structures were bleached at 0 sec (using 100% laser power and 100 iterations), and the recovery of the fluorescence was followed by time-lapse imaging. Images were obtained at 4-sec intervals in the case of wild-type FtsZ and 30-sec intervals for the FtsZQ47K mutant. Quantification of the fluorescence recovery of a representative experiment is shown in the adjoining graphs. Normalized fluorescence intensities were used for graphical representations. The $t_{1/2}$ values mentioned in the text were obtained by fitting the fluorescence recovery curves to a single exponential curve $F(t) = C(1 - e^{-kt})$ and calculated from $t_{1/2} = \ln(2)/k$. (*EcFtsZ*) *E. coli* FtsZ; (*MtFtsZ*) *M. tuberculosis* FtsZ. Bar: 2 μ m (except in the case of F); 1 μ m.

rings, giving a lariat-like appearance (Fig. 2D). In order to see whether assembly of a FtsZ ring-like structure was peculiar to expression in *Schizosaccharomyces pombe*, we expressed *EcFtsZ* in another host, *Saccharomyces cerevisiae*. *EcFtsZ* formed cables and rings with a diameter of $0.48 \pm 0.042 \mu$ m, $n = 100$, in this yeast as well (Fig. 2E), showing that assembly into ring-like structures was an intrinsic property of FtsZ polymers. We then asked whether FtsZ from other bacterial species also showed similar behavior. To this end, we first expressed FtsZ from the Gram-positive human pathogen *Mycobacterium tuberculosis* (*MtFtsZ*). *MtFtsZ* assembled into spots, rings, and cables upon expression in fission yeast (Fig. 2F, panels i,ii, and insets therein), although the rings were less clear in these cases compared with those observed upon expression of *EcFtsZ* in fission yeast.

MtFtsZ has been reported to display a reduced GTPase activity (White et al. 2000) and a concomitant reduction in the rate of filament turnover in vivo (Chen et al. 2007). Consistently, rings and cables of *MtFtsZ* displayed a significant increase in $t_{1/2}$ in FRAP experiments (Fig. 1D,E; Supplemental Table 1), suggesting that *MtFtsZ* was potentially using similar mechanisms of assembly and polymerization in fission yeast. FtsZ from the Gram-negative plant pathogen *Xanthomonas oryzae* (*XoFtsZ*) was also able to assemble into rings and cables upon expression in fission yeast (Fig. 2G, panels i,ii). We conclude that FtsZ polymers have an intrinsic ability to assemble into cytoplasmic ring-like structures upon expression in the yeasts *S. pombe* and *S. cerevisiae*.

We then imaged fission yeast cells expressing *EcFtsZ* to determine the relationship, if any, between FtsZ rings and FtsZ cables. Time-lapse microscopy revealed that the rings were formed from the linear cables (Fig. 2H; Supplemental Movie 1). However, due to the excessive number of cables and rings and the dynamic nature of these structures, it was difficult to visualize the steps leading to assembly of FtsZ rings in fission yeast.

Since the time-lapse studies were typically performed by transfer of cells grown in liquid medium to agar containing solid pads, it was possible that the act of transferring or placement on the solid surface itself might initiate ring assembly. However, since FtsZ spots and

Figure 2. FtsZ assembles into rings in fission yeast. (A–C) *EcFtsZ*-GFP assembled into ring-like structures in fission yeast. The medium-strength *nmt42* promoter was used for the expression of FtsZ-GFP in *S. pombe*. Cultures were grown for 16–20 h at 30°C in the absence of thiamine to allow the expression of the proteins. Panel ii in A–C show the boxed region in panel i in A–C, respectively. (C, panel iii) The white ring labels the region used to measure the diameter of the rings and labels the center of the outer and inner diameters of the fluorescence of the FtsZ ring. (D) Assembly of *EcFtsZ* into cable-like structures. The inset shows a ring connected to a cable. (E) Assembly of rings and cables upon expression of *EcFtsZ*-GFP in budding yeast. Expression of FtsZ was achieved by the addition of 1% galactose for 3 h. (F, panels i,ii) Expression of *MtFtsZ*-GFP in fission yeast showing both rings (panel i) and cable (panel ii). The inset shows the assembly of FtsZ into a ring-like structure. (G, panels i,ii) Expression of *XoFtsZ*-GFP in fission yeast. Panel i shows cells with FtsZ rings and panel ii shows a cell with both FtsZ rings and cables. The inset shows the assembly of FtsZ into a ring-like structure. (H) Ring-like structures of FtsZ are formed from the FtsZ cables. Time-lapse images showing the formation of FtsZ ring-like structures from the cables. Cells were grown in minimal medium without thiamine for 16 h and were mounted onto agarose pads and imaged using a confocal microscope. Images are shown at 3-min intervals. Bars: (except for those in the insets), 2 μ m; insets, 1 μ m.

Figure 3. GTP binding and GTP hydrolysis are crucial for assembly of FtsZ into multiple rings in fission yeast. (A) A GTP-binding mutant (FtsZF182S) fails to assemble cables, spots, and rings in fission yeast and exhibits only diffuse cytoplasmic fluorescence. (B) FtsZG105S (*ftsZ84*), a well-characterized temperature sensitive allele of FtsZ forms only cable-like structures. (C,D) Assembly of FtsZQ47K-GFP into rings (C) and cables (D) in fission yeast. (E) Linear filaments seemed to connect with the FtsZQ47K-GFP ring structures in some cells. (F) Magnified images of the FtsZQ47K rings. (G) The white ring marks the region used to measure the diameter of the rings and marks the center of the outer and inner diameters of the fluorescence of the FtsZ ring. (H) Quantification of the ring diameters of FtsZ-GFP and FtsZQ47K-GFP expressed in fission and budding yeasts. The diameters of 100 rings were measured in each case. The error bars represent the standard deviation from the mean. Bars: F,G, 1 μ m; others, 2 μ m.

rings are observed in cells grown and fixed in liquid medium (data not shown), it is likely that assembly of FtsZ rings starting from cables might represent a property of the FtsZ polymer, rather than an artifact of the experimental procedure used.

We then expressed several previously characterized *EcFtsZ* mutants (Redick et al. 2005) in fission yeast and found that abolishing GTP binding (FtsZF182S) completely abrogated polymer formation of FtsZ and gave rise to only diffuse cytoplasmic fluorescence (Fig. 3A). This experiment suggested that GTP binding was important for assembly of FtsZ cables. FtsZG105S, the product of a well-characterized conditional lethal allele of FtsZ (*ftsZ84*) (Lutkenhaus et al. 1980; RayChaudhuri and Park 1992; Redick et al. 2005), was able to assemble into cables, but was unable to organize into rings (Fig. 3B).

Interestingly, expression in fission yeast of the FtsZQ47K mutant provided novel insight into the mechanism of FtsZ ring assembly. Previous studies have shown that *EcFtsZQ47K* mutant has a GTP-binding activity of 1.19 GTP/FtsZ compared with that of wild-type FtsZ, which has 1.05 GTP/FtsZ (Redick et al. 2005). However, the GTPase activity of *EcFtsZQ47K* is only 0.5/FtsZ per minute as compared with that of 14.5/FtsZ per minute of the wild-type FtsZ (Redick et al. 2005). FtsZQ47K expressed in fission yeast formed ring-like structures (Fig. 3C,E,F) that were, curiously, consistently larger than those formed by wild-type FtsZ. In addition, expression of FtsZQ47K in fission yeast led consistently to the formation of fewer FtsZQ47K rings per cell. FtsZQ47K also assembled into linear bundles and cables (Fig. 3D). Consistent with the idea that GTP hydrolysis regulates turnover of FtsZ filaments (Anderson et al. 2004; Chen and Erickson 2005), FRAP experiments with the slow-hydrolysis mutant FtsZQ47K revealed that the $t_{1/2}$ of FtsZQ47K cables and rings was significantly higher (Fig. 1F,G; Supplemental Table 1). It is noteworthy that minimal fluorescence recovery was detected upon photo bleach-

ing of FtsZQ47K rings (Fig. 1F,G; Supplemental Table S1). The diameters of the rings formed by the *EcFtsZQ47K* averaged $0.63 \pm 0.05 \mu$ m (Fig. 3H), with maximum diameters reaching 0.77μ m. *EcFtsZQ47K* when expressed in *S. cerevisiae* also assembled similar structures (Supplemental Fig. 1A,B) with an average diameter of $0.71 \pm 0.1 \mu$ m (Fig. 3H).

Interestingly, in many cells, ring-like structures appeared to be linked to a linear filament; again giving rise to a lariat-like appearance (Fig. 3E). Thus, we considered two scenarios. First, it was possible that FtsZ ring unraveling led to the presence of a cable of FtsZ connected to a ring structure. Alternatively, it was possible that a ring of FtsZ might assemble from cable-like FtsZ structures. To distinguish between these possibilities, we imaged cells containing cables and those containing rings. Fully formed rings of *EcFtsZQ47K* did not unravel into cables in 15 cells imaged, each followed for up to 2 h (data not shown). Strikingly, however, linear filaments of FtsZQ47K were found to organize into FtsZQ47K rings. As shown in Figure 4A and Supplemental Movie 2, the linear FtsZQ47K filaments formed lariat-like structures and subsequently organized into a ring structure in what appeared to be a spooling-like mechanism. Photobleaching experiments (Fig. 4B; Supplemental Fig. 1C; Supplemental Movies 3, 4) wherein a part of the ring was bleached and the recovery of fluorescence was followed by time-lapse microscopy suggested the assembly of the filament tails into the ring. Additional experiments in which a part of the linear bundle that was associated with the ring was photobleached showed that the bleach mark moved toward the ring (Fig. 4C), further providing evidence that the organization of the FtsZQ47K bundles into rings might involve a spooling-like mechanism.

All the studies performed with FtsZQ47K involved the only available reliably regulatable fission yeast promoter (*nmt1*), which requires ~ 16 h for full induction. As a result, we were unable to trace the events leading to the formation of FtsZQ47K bundles, although we were able to image the assembly of FtsZQ47K rings from linear bundles. Recently, Bahler and colleagues (Watt et al. 2008) have described a uracil-regulatable promoter (*urg1*), which allows a faster induction. We therefore made a plasmid under the *urg1* promoter control that allowed expression of FtsZQ47K. Upon induction of gene expression with uracil, FtsZQ47K assembled into multiple short filaments (Fig. 5A, marked by white arrowheads), which appeared to either become ligated or laterally bundled leading to the formation of a thick bundle (Fig. 5A,B; Supplemental Movies 5, 6). As shown in previous examples, spooling of these bundles led to the formation of FtsZQ47K rings (Fig. 5A; Supplemental Movie 5). We also found examples in which FtsZQ47K assembled short cables that underwent closure directly leading to the formation of FtsZQ47K rings (Fig. 5B; Supplemental Movie 6, marked by arrows). The intensity of GFP fluorescence in these FtsZQ47K rings increased with time. It is currently unclear if this increase in fluorescence intensity reflects direct association of shorter protofilaments of FtsZQ47K with the preformed ring or if it arises from spooling of weakly fluorescent tails. The fact that FtsZQ47K assembled first into cables and then into rings was also established by quantification over time of the various structures present in fission yeast cells expressing FtsZQ47K (Supplemental Fig. 2B).

Figure 4. FtsZQ47K assembles into ring-like structures from cables by a spooling-like mechanism. (A) Time-lapse images show the formation of FtsZQ47K-GFP rings from linear filaments by a spooling-like mechanism. Projections were made from Z-stacks of 0.3- μ m thickness and the images were acquired at 2-min intervals. The time-lapse series show the initial formation of a lariat-like structure (at 22 min) and the subsequent disappearance (probably by spooling) of the filament to assemble the ring structure. (B) Spooling-like behavior of the filament into the ring structure shown by fluorescence recovery after photobleaching (FRAP). The movement of the unbleached region of the ring is seen in the time-lapse images. The cultures were grown for 16–20 h at 24°C in minimal medium without thiamine and mounted onto agarose slides for time-lapse microscopy and FRAP experiments. (C) Apparent spooling of linear cable of FtsZ into the ring. A part of the filament attached to the ring was photobleached. The white rectangle shows the bleached region. The bleached region was seen to move toward the rings, implying a spooling-like behavior. Bars: B, 1 μ m; others, 2 μ m.

Our studies showed that FtsZQ47K rings assemble either by closure of linear cables or by apparent spooling of linear bundles. We reasoned that the compaction of a spiral (generated by closure and/or spooling of a long cable of FtsZ) might also involve lateral associations between FtsZ protofilaments. Earlier biochemical and in vivo studies have identified several lateral contact mutants that are known to be essential for FtsZ ring assembly (Lu et al. 2001; Stricker and Erickson 2003; Redick et al. 2005). We therefore created a double mutant in *EcFtsZ*, FtsZQ47KD86K (the positions of which are shown in Supplemental Fig. 2A), to test whether lateral contacts were required for FtsZ ring compaction. *EcFtsZQ47KD86K* formed long linear cables and occasionally large closed loops that covered the entire circumference of the fission

yeast cell (Fig. 4C, panels i,ii), but were never found to form rings of 0.6–0.7 μ m in diameter.

In summary, we show that *E. coli* FtsZ assembles into rings of diameter 0.51 ± 0.04 μ m in fission yeast cells. Although it is clear that the bacterial cell diameter establishes the diameter of the FtsZ rings, it is curious that the diameter of FtsZ rings expressed in fission yeast is roughly similar to the cell diameter of several bacterial species. Interestingly, several studies using electron microscopy and atomic force microscopy approaches have demonstrated the ability of FtsZ to assemble into rings (Chen et al. 2005; Gonzalez et al. 2005; Mingorance et al. 2005; Michie et al. 2006). These rings have been predominantly reported to be composed of a single protofilament. Although we conclusively established that FtsZ can assemble into cytosolic rings in fission yeast, the numbers of overlapping protofilaments that make up this ring structure are currently unknown.

Our studies also established that FtsZ can assemble into rings in the absence of all other known bacterial proteins implicated in cytokinesis. Our conclusions are consistent with the recent study by Osawa et al. 2008. These authors using purified FtsZ (with a membrane tether) and reconstituted liposomes have shown that FtsZ can organize into rings in the absence of all other bacterial cytoskeletal proteins (Osawa et al. 2008). Finally, our studies begin to provide a mechanism for assembly of FtsZ rings starting from protofilaments. As in the case of tubulin, GTP hydrolysis by FtsZ has been shown to generate curvature in FtsZ protofilaments (Lu et al. 2000). Thus, it is likely that FtsZ ring assembly might be mediated by nucleotide hydrolysis coupled with further lateral associations among the protofilaments. This idea

Figure 5. Assembly into linear cables or short filaments precedes the formation of FtsZQ47K rings, and such ring assembly might involve lateral interactions. (A,B) Time-lapse images of FtsZ-GFP after 15 min of induction with uracil. Short filaments of FtsZ are assembled early from multiple nucleation points in the cell. (A) 0 sec. (B) 1157 sec. (A) The short filaments assemble into long cables, which go on to form rings by a spooling-like mechanism. The arrowheads mark the appearance of short filaments of FtsZ polymers while the arrow represents a ring undergoing further spooling-like process. (B) Rings assembly by closure of linear filaments. The white arrows mark the events in which short filaments reorganize into ring structures. The arrowhead in magenta shows a breakage event in the linear cable due to cell division. (C, panels i,ii) FtsZQ47KD86K fails to assemble into rings. Introduction of a lateral contact mutation into FtsZQ47K abolished its assembly into 0.7- μ m rings. Expression of FtsZQ47KD86K-GFP was achieved by growing the fission yeast cultures for 16–20 h at 24°C in minimal medium without thiamine. Images were obtained by maximal projections of the Z-stacks (0.2- μ m-thick slices). Bars: A,B, 5 μ m; C, 2 μ m.

is in agreement with the recent structural studies, which have shown that lateral associations play an important role in the determination of the curvature of FtsZ protofilaments (Oliva et al. 2007; Horger et al. 2008). Our results are also consistent with imaging studies in *E. coli*, *B. subtilis*, and *Streptomyces coelicolor* (Addinall and Lutkenhaus 1996; Schwedock et al. 1997; Ben-Yehuda and Losick 2002; Thanedar and Margolin 2004; Grantcharova et al. 2005). In all these bacteria, FtsZ rings at the mid-cell site as well as at the cell ends, during sporulation, have been shown to involve compaction of spiral structures, although the biochemical basis of this compaction was unclear. Our studies might thus provide a biochemical framework to understand FtsZ ring assembly in bacteria. We also note that although FtsZ rings can assemble in fission yeast cells, we did not observe any of these to undergo constriction. Future studies should test whether coexpression of FtsZ with other known bacterial cytokinesis proteins can stimulate FtsZ ring constriction in fission yeast cells.

Materials and methods

Strains, plasmids, and constructs

Transformation of *S. pombe* strain MBY192 (*h⁺ leu1-32 ura4-D18*; lab collection) with the various plasmids was carried out using the lithium acetate method (Keeney and Boeke 1994). FtsZ-GFP and FtsZ mutant versions were expressed from the medium-strength *nmt42* promoter (Basi et al. 1993). FtsZQ47K was also expressed under control of the *urg1* promoter. The construction of the plasmids is described in the Supplemental Material.

Expression of GFP fusion proteins and fluorescence microscopy

FtsZ-GFP and its mutant versions (under the *nmt42* promoter) were expressed in *S. pombe* by growing the transformed cultures in the absence of thiamine for 16–20 h at 24°C or 32°C. Induction of expression of FtsZQ47K-GFP under the uracil-inducible *urg1* promoter was carried out by adding 0.5 mg/mL uracil. For time-lapse imaging, the cells were mounted onto agarose slides, and images were acquired at fixed time intervals. In the case of the *urg1* promoter, cultures were induced with uracil for 15 min and then mounted onto the agarose slides, which also contained uracil. A Zeiss Meta 510 confocal microscope, with an apochromat 100×/1.3 NA oil-immersion objective was used for acquiring images, and for time-lapse imaging and fluorescence recovery after photobleaching experiments. Photobleaching was carried out with 100% laser power and with 100–150 iterations. Z-stack images were acquired at fixed intervals with a Z-depth of 0.2 or 0.3 μ m. An unbleached region in the same field/cell was used to correct for acquisition bleed. Ring diameters and fluorescence recovery were analyzed using Zeiss 3D for LSM software. Other image manipulations were done using ImageJ (<http://rsb.info.nih.gov/ij/>) and Adobe Photoshop. The FRAP data were fit to a single exponential equation $I(t) = C(1 - e^{-kt})$ and recovery half-times ($t_{1/2}$) were calculated as $\ln(2)/k$ (Anderson et al. 2004; Sprague et al. 2004). MATLAB and the open source software R (<http://www.R-project.org/>) were used for curve fitting by the method of nonlinear least squares regression analysis.

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