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Fission Yeast Num1p Is a Cortical Factor Anchoring Dynein and Is Essential for the Horse-Tail Nuclear Movement During Meiotic Prophase

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ABSTRACT

During meiotic prophase in the fission yeast *Schizosaccharomyces pombe*, the nucleus oscillates between the two ends of a cell. This oscillatory nuclear movement is important to promote accurate pairing of homologous chromosomes and requires cytoplasmic dynein. Dynein accumulates at the points where microtubule plus ends contact the cell cortex and generate a force to drive nuclear oscillation. However, it remains poorly understood how dynein associates with the cell cortex. Here we show that *S. pombe* Num1p functions as a cortical-anchoring factor for dynein. Num1p is expressed in a meiosis-specific manner and localized to the cell cortex through its C-terminal PH domain. The *num1* deletion mutant shows microtubule dynamics comparable to that in the wild type. However, it lacks cortical accumulation of dynein and is defective in the nuclear oscillation as is the case for the dynein mutant. We also show that Num1p can recruit dynein independently of the CLIP-170 homolog Tip1p.

THE microtubular cytoskeleton has crucial roles in many cellular processes. Microtubules guide the motor-protein-mediated movement of organelles, including the nucleus. Precise regulation of the nuclear movement is essential in a number of contexts (Morris 2003).

In the fission yeast *Schizosaccharomyces pombe*, microtubules position the interphase nucleus at the middle of a cell (Drummond and Cross 2000; Tran *et al.* 2001). During meiotic prophase, striking nuclear migration occurs. The nucleus migrates back and forth between the two poles of a cell for some hours (Chikashige *et al.* 1994). This oscillatory nuclear movement, called "horse-tail" movement, is led by the spindle pole body (SPB; equivalent to the centrosome in higher organisms), to which telomeres are attached (Chikashige *et al.* 1994). The horse-tail movement in combination with telomere clustering is assumed to facilitate pairing of homologous chromosomes, which is a prerequisite for accurate chromosome segregation (Yamamoto *et al.* 1999; Niwa *et al.* 2000; Ding *et al.* 2004; Davis and Smith 2005). It has been shown that the horse-tail movement is driven by a cytoplasmic dynein–dynactin complex (Yamamoto *et al.* 1999; Miki *et al.* 2002; Niccoli *et al.* 2004). The dynein heavy chain (encoded by *dhc1*) and the Glued subunit of dynactin (encoded by *ssm4*) localize to the SPB and microtubules during the horse-tail period. Furthermore, dynein and dynactin are also detected on the point where microtubules contact the cell cortex (Yamamoto *et al.* 1999; Niccoli *et al.* 2004). It has been proposed that microtubules, formed from the SPB, generate a pulling force on the SPB by sliding along the cell cortex mediated by dynein anchored to it, which is likely also to regulate the disassembly of microtubules at the cell cortex (Yamamoto *et al.* 2001; Yamamoto and Hiraoka 2003). Thus, cortical accumulation of dynein appears crucial for the horse-tail movement. It has been reported that the dynein light chain (encoded by *dlc1*) and the Glued subunit of dynactin are indispensable for the accumulation of the dynein heavy chain at the cortex (Miki *et al.* 2002; Niccoli *et al.* 2004). However, it remains largely unknown how dynein associates the cell cortex.

We have shown previously that the Glued ortholog Ssm4p shares some function with the CLIP-170-like protein Tip1p in localizing Dhc1p (Niccoli *et al.* 2004). Tip1p, which regulates the stability of microtubules in interphase cells (Brunner and Nurse 2000), is not absolutely essential for the proper localization of Dhc1p. However, the *tip1*Δ *ssm4*Δ double mutant cannot localize Dhc1p along microtubules, although it can localize the protein to the SPB (Niccoli *et al.* 2004).

The dynein–dynactin complex is involved in nuclear migration in many organisms (Morris 2003). The nuclear positioning during mitosis in the budding yeast *Saccharomyces cerevisiae* may be one of the best-characterized examples. During mitosis in budding yeast, the nucleus migrates to the mother-bud neck and then into the neck (DeZwaan *et al.* 1997). Both movements require interaction of microtubules with the cortex, and the latter process is mediated by sliding of microtubules along the bud cortex, which requires dynein and dynactin

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TABLE 1

***S. pombe* strains used in this study**

Strain	Genotype
JV421	h^{90} *num1GFP<< kan*^{r} *tip1::ura4*$^{+}$ *ade6-M216 leu1*
JV422	h^{90} *num1:: kan*^{r} *tip1::ura4*$^{+}$ *ade6-M216 leu1*
JV471	h^{90} *tip1GFP<< kan*^{r} *ade6-M216 leu1*
JV545	h^{90} *num1:: kan*^{r} *tip1GFP<< kan*^{r} *ade6-M216 leu1*
JV626	h^{90} *num1GFP<< kan*^{r} *ade6-M216 leu1*
JV627	h^{90} *num1:: kan*^{r} *ade6-M216 leu1*
JV650	h^{90} *num1:: kan*^{r} *ssm4:: kan*^{r} *ade6-M216 leu1*
JV656	h^{90} *num1:: kan*^{r} *kan*^{r}*>> nmt1-GFP-dhc1 ade6-M210 leu1*
JV681	h^{90} *num1:: kan*^{r} *tip1::ura4*$^{+}$ *kan*^{r}*>> nmt1-GFP-dhc1 ade6-M216 leu1*
JV897	h^{90} *dhc1GFP<< LEU2 ade6-M216 leu1*
JV898	h^{90} *dhc1GFP<< LEU2 num1:: kan*^{r} *ade6-M216 leu1*
JW327	h^{90} *dhc1::ura4*$^{+}$ *ade6-M216 leu1 ura4-D18*
JW652	h^{90} *ssm4:: kan*^{r} *ade6-M216 leu1*
JW785	h^{90} *kan*^{r}*>> nmt1-GFP-dhc1 ade6-M216 leu1*
JX648	h^{90} *tip1::ura4*$^{+}$ *ade6-M216 leu1 ura4-D18*
JX650	h^{90} *ssm4::ura4*$^{+}$ *tip1::ura4*$^{+}$ *ade6-M216 leu1 ura4-D18*
JY450	h^{90} *ade6-M216 leu1*

(Stearns 1997; Adames and Cooper 2000; Yeh *et al.* 2000). Budding-yeast dynein heavy-chain Dyn1p shows discontinuous localization along microtubules with accumulation at the SPB and microtubule plus ends (Lee *et al.* 2003; Sheeman *et al.* 2003). It has been reported that the CLIP-170 ortholog Bik1p and the lissencephal protein LIS1 ortholog Pac1p are required to recruit Dyn1p onto microtubules (Lee *et al.* 2003; Sheeman *et al.* 2003). In addition, Num1p is known as a candidate for the cortical anchor of dynein (Bloom 2001). Num1p has a pleckstrin homology (PH) domain and localizes to the bud cortex (Farkasovsky and Kuntzel 1995; Heil-Chapdelaine *et al.* 2000; Farkasovsky and Kuntzel 2001). Loss of Num1p leads to a failure in dynein-dependent microtubule sliding and results in defective nuclear migration (Heil-Chapdelaine *et al.* 2000; Farkasovsky and Kuntzel 2001). In cells lacking Num1p, dynein localization at microtubule plus ends is enhanced, suggesting that Num1p may promote the transfer of dynein from microtubule plus ends to the cortical attachment site and anchor it there (Lee *et al.* 2003; Sheeman *et al.* 2003). Co-immunoprecipitation of Num1p with the dynein intermediate chain Pac11p (Farkasovsky and Kuntzel 2001) is consistent with this off-loading-anchoring model.

In the filamentous fungi *Aspergillus nidulans*, dynein is required for the distribution of nuclei within the syncytial hyphae (Xiang *et al.* 1994). The *apsA* mutants are defective in this distribution (Clutterbuck 1994). The *apsA* gene encodes a protein with structural similarities to *S. cerevisiae* Num1p (Fischer and Timberlake 1995). ApsA is a cortical protein and is involved in the regulation of dynein-dependent nuclear migration (Clutterbuck 1994; Suelmann *et al.* 1997; Efimov 2003).

We have identified a possible fission yeast homolog of Num1p and ApsA. The above results obtained in *S. cerevisiae* and *A. nidulans* led us to speculate that this protein might also participate in the regulation of dynein at the cell cortex. Thus, we set out to analyze this protein to elucidate the mechanism to anchor dynein on the cortex in fission yeast.

MATERIALS AND METHODS

Fission yeast strains, genetic procedures, and media: Table 1 summarizes *S. pombe* strains used in this study. General genetic procedures for *S. pombe* were according to Gutz *et al.* (1974). Complete medium YE, minimal medium SD, minimal medium MM (Moreno *et al.* 1991), synthetic sporulation medium SSA (Egel and Egel-Mitani 1974), and sporulation medium SPA (Gutz *et al.* 1974) were used. Transformation of *S. pombe* was done by a lithium acetate method (Okazaki *et al.* 1990). To monitor subcellular localization of Num1p, we replaced the chromosomal *num1* gene with the *num1-GFP* fusion gene according to Bahler *et al.* (1998). A strain carrying a fusion of the *tip1* and the GFP ORFs was constructed similarly. The tagged strains behaved in the same manner as parental strains with no tag during both vegetative growth and meiosis, indicating that the tagging did not interfere with the function of the relevant gene products.

Construction of truncated *num1* alleles: The *S. pombe* expression vectors pREP1 and pREP41 carried the strong and the medial thiamine-repressible promoters, respectively (Basi *et al.* 1993). The *num1* ORF was PCR amplified with a pair of primers, one carrying an *Nde*I site at the initiation codon and the other carrying a *Bam*HI site at the stop codon. PCR products were digested with *Nde*I and *Bam*HI and cloned in either pREP1 carrying the GFP ORF or pREP41 carrying three copies of the HA epitope, so that GFP or the HA epitope was fused to the C terminus of the *num1* ORF. To create a derivative carrying a truncated *num1* allele termed *num1-ΔRU*, two *Pst*I sites were introduced immediately upstream and downstream of the conserved repeating unit by PCR and a short *Pst*I

fragment was deleted, which resulted in the replacement of 56 amino acids of the repeating unit (244–299) with 2 amino acids encoded by the inserted *Pst*I site (L–Q). To create *num1-ΔPH*, which encoded truncated Num1p lacking the C-terminal PH domain (845–968), a *Bam*HI site was introduced immediately upstream of the PH domain by PCR. The two truncated genes were cloned in expression vectors as described above.

Fluorescence microscopy: To observe localization of Num1p-GFP and GFP-Dhc1p in living cells, strains JV626, JV656, JV681, JV897, JV898, and JW785 were cultured in MM medium at 30° up to the midlog phase, washed, and spotted onto SPA medium. After incubation for 6–8 hr at 30°, these cells were observed using a chilled Quantix CCD camera (Photometrics, Tucson, AZ) attached to a fluorescent microscope (Carl Zeiss, Axioplan 2) and the MetaMorph software (Universal Imaging, West Chester, PA). To visualize nuclei, cells were counterstained with Hoechst 33342. For live analysis of nuclear dynamics, cells were cultured in MM medium at 30° up to midlog phase, washed, shifted to MM–N medium, and incubated for 4–6 hr. These cells were then mounted on a thin layer of 1% agarose containing MM–N medium, which was attached to a glass slide. Live images were taken at room temperature (23°–25°).

Statistical analysis of the Num1p dot: For live analysis of Num1p-GFP, JV626 was monitored using a microscope (Nikon, TE2000) equipped with a spinning disk confocal unit (Yokogawa, CSU22) and a chilled CCD camera (Andor, iXon). Z-stacks were taken at 0.5-min intervals. Each measurement was done for 10 min. Only those dots that were absent in the first and the last frames were subjected to analysis. We assumed that the lifetime of a Num1p dot was 0.5 min multiplied by the number of frames in which it was detected.

Live analysis of microtubular dynamics: To visualize microtubules by GFP fluorescence, we constructed the plasmid pREP81-GFP-*atb2*, which expressed nonessential α-tubulin with GFP fused to its N terminus (Toda *et al.* 1984) from the weak thiamine-repressible promoter (Basi *et al.* 1993). Cells carrying this plasmid were processed as described above and imaged with confocal microscopes [Carl Zeiss (LSM5) and Nikon (TE2000) equipped with Yokogawa (CSU22)]. Z-stacks were taken at each time point with 10 focal planes spaced at 0.3- to 0.6-μm intervals. Tracking of microtubules was performed using ImageJ software (National Institutes of Health).

RESULTS

Fission yeast have a possible homolog of the budding yeast Num1p: To investigate the mechanism for cortical anchoring of dynein at meiotic prophase, we searched the *S. pombe* genome database and found a putative gene (SPBC216.02) encoding a weak homolog of *S. cerevisiae* Num1p. We call this gene *num1*. The homology between *S. pombe* Num1p and *S. cerevisiae* Num1p was not high (27% identity). However, they had a similar domain structure (Figure 1), with a coiled-coil domain at the N terminus and a PH domain at the C terminus. *S. cerevisiae* Num1p has repeats of nearly identical 64-amino-acid residues at its central region (Kormanec *et al.* 1991). The number of repeats varies from 1 to 24 among strains (Revardel and Aigle 1993). *S. pombe* Num1p had a single copy of the repeat unit just after the coiled-coil domain (Figure 1). The putative Ca^{2+} site in *S. cerevisiae* Num1p was not conserved in *S. pombe*.

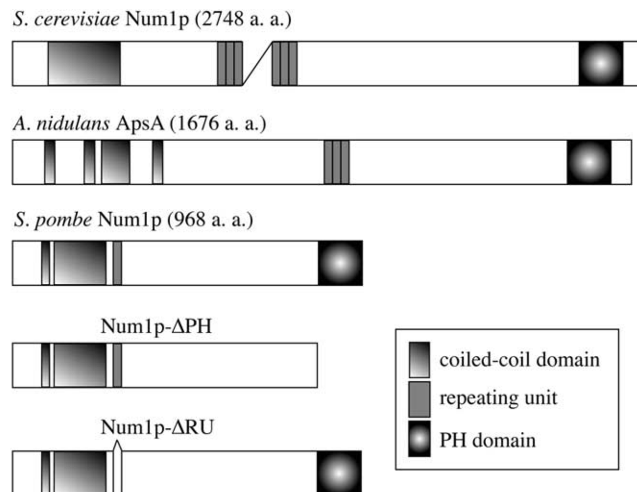

Figure 1.—Schematic of the structure of *S. pombe* Num1p and its *S. cerevisiae* and *A. nidulans* homologs. Num1p-ΔPH lacks the PH domain and Num1p-ΔRU lacks the single conserved repeating unit.

Num1p is expressed in a meiosis-specific manner and localizes at the cell cortex: To analyze expression and localization of *S. pombe* Num1p, we connected the ORF for GFP to the end of the chromosomal *num1* ORF in frame. This fusion gene did not express Num1p-GFP at a detectable level in mitotically growing cells (data not shown). In meiotic cells, however, green fluorescence of the protein could be observed (Figure 2). This was consistent with the expression profile of *num1* shown previously by DNA microarray assays (Mata *et al.* 2002). Num1p-GFP was localized to the cell cortex in a punctate pattern during karyogamy and meiotic prophase (Figure 2, A–C). Num1p-GFP remained there until anaphase of the first meiotic division (Figure 2D), disappeared during the course of anaphase, and was never detected through the rest of meiosis (data not shown). This expression pattern resembled that of *dhc1* (Yamamoto *et al.* 1999). Cortical spots of Num1p-GFP were not uniform in their behavior (Figure 2E), with some staying for only a short time as if blinking. We have measured the lifetime of the cortical Num1p dot as detailed in materials and methods. The results are shown in Figure 2F. A majority of the dots exited for an average lifetime of 2.5 min (91%; $n = 23$). However, there were some spots remaining for >10 min (9%). A correlation between this blink of Num1p-GFP fluorescence and the nuclear movement is currently uncertain.

Num1p is essential for the horse-tail nuclear movement: We next examined whether Num1p was involved in the same cellular function as the dynein–dynactin complex. We deleted the *num1* gene by replacing it with a kanamycin resistance gene. The *num1*Δ strain showed a phenotype highly similar to the *dhc1*Δ or the *ssm4*Δ mutant. Disruption of *num1* had no obvious effect on

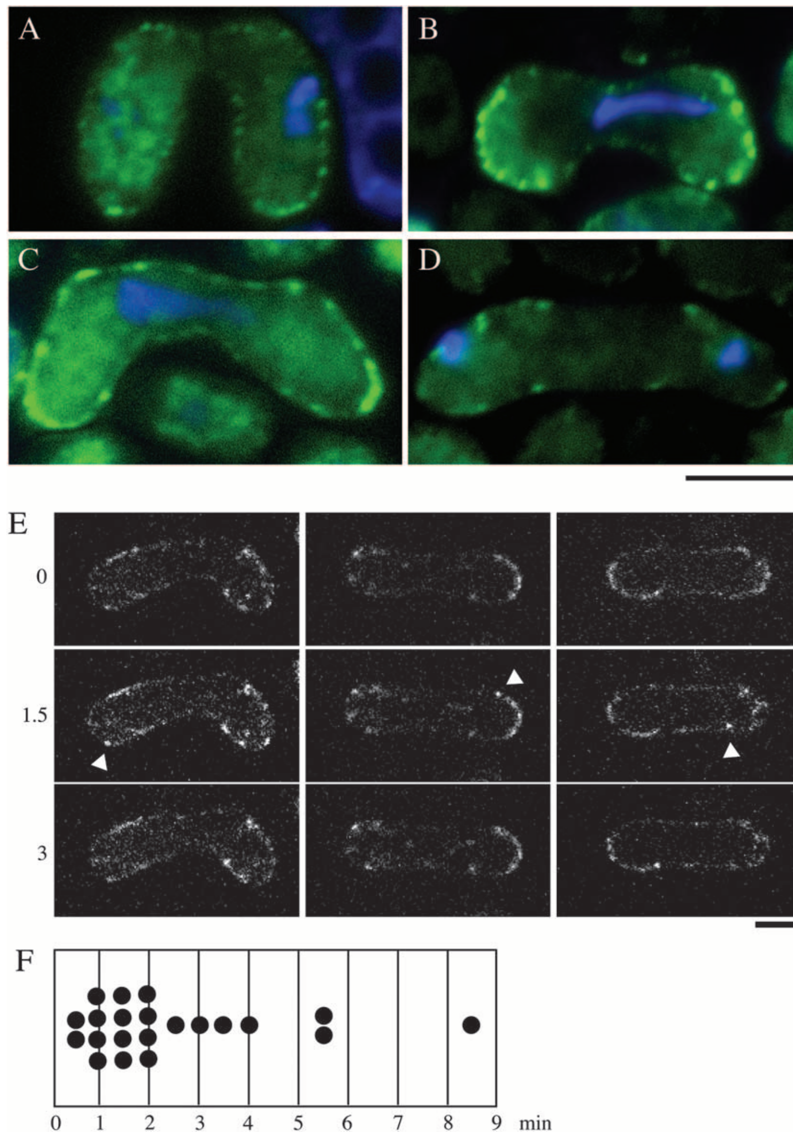

Figure 2.—Subcellular localization of Num1p in the course of meiosis. (A–D) Green fluorescence of Num1p-GFP in living cells. Homothallic haploid cells (JV626) carrying the *num1-GFP* fusion gene were starved for nitrogen to induce conjugation and subsequent meiosis. Nuclear DNA was stained with Hoechst 33342. GFP fluorescence is shown in green, and stained DNA in blue. Bar, 5 μm. Conjugating cells (A), prophase cells (B and C), and a cell at anaphase I (D) are shown. (E) Time-lapse recording of Num1p-GFP. Images were taken at 0.5-min intervals. Z-stacked images of three cells at 1.5-min intervals are shown. Arrowheads indicate GFP fluorescence, which went in and out during a filming period. Bar, 5 μm. (F) The lifetime of a blinking cortical Num1p dot.

cell growth and conjugation: The *num1*Δ strain showed a doubling time and a mating frequency that were indistinguishable from that of the wild type. However, the *num1*Δ strain frequently generated aberrant asci carrying fewer than four spores, as did the *dhc1*Δ or the *ssm4*Δ strain (Table 2) (Yamashita *et al.* 1997; Yamamoto *et al.* 1999; Niccoli *et al.* 2004). The frequency of aberrant asci formation was comparable among these strains. We constructed the *num1*Δ *ssm4*Δ double mutant and found that this strain produced aberrant asci at the same frequency as each single mutant (Table 2). This suggested strongly that Num1p might perform the same cellular function as the dynein–dynactin complex.

We then investigated if Num1p was essential for the horse-tail nuclear movement. In the *num1*Δ mutant, the nucleus did not show oscillatory movement and remained at the center of a cell (Figure 3), as was the case with the dynein–dynactin mutants (Yamamoto *et al.* 1999; Niccoli *et al.* 2004).

Microtubules fail to establish a lateral interaction with the cell cortex in the *num1* mutant: Microtubule dynamics at meiotic prophase is altered significantly in the dynein mutant (Yamamoto *et al.* 2001). We visualized microtubules in *num1*Δ cells using GFP-tagged α-tubulin. Figure 3 shows images of a cell in a single focal plane. In *num1*Δ cells, microtubules did not show a lateral interaction with the cell cortex, which was evident in wild-type cells (see also Figure 4). We then compared microtubule dynamics in meiotic prophase in wild-type and *num1*Δ cells, using an optical sectioning microscope (Figure 4). The growth and shrinkage rates of microtubules in *num1*Δ cells were comparable to those in wild type during the horse-tail period (Table 3). These observations appears to be distinct from those obtained with *dhc1*Δ cells, which have shown a faster shrinkage rate and a slower elongation rate than wild-type cells (Yamamoto *et al.* 2001), if we take into account that the rates for wild-type cells are similar in their

TABLE 2

Number of spores per ascus in the *num1* and other mutants

	% of asci carrying the following no. of spores:			
Strain	One	Two	Three	Four
Wild type	<0.1	<0.1	1.1	98.9
*dhc1*Δ	0.7	8.6	8.6	82.1
*ssm4*Δ	0.5	6.8	8.1	84.6
*num1*Δ	0.7	8.0	8.0	83.3
*num1*Δ *ssm4*Δ	0.3	7.9	8.4	83.4
*tip1*Δ	<0.1	1.7	6.9	91.4
*ssm4*Δ *tip1*Δ	1.4	10.9	24.6	63.1
*num1*Δ *tip1*Δ	1.1	11.7	25.1	62.1

Sporulation was induced in each strain on SSA medium at 30°. Spores were scored after 3 days. More than 400 zygotes were examined for each strain.

measurements (3.4 ± 1.4 μm/min for elongation and 6.4 ± 3.4 μm/min for shrinkage) and in ours (3.4 ± 0.8 μm/min for elongation and 5.1 ± 2.0 μm/min for shrinkage). In *num1*Δ cells, microtubules grew until they reached the cell ends, but they stopped elongation without curving around and then shrank (Figure 4B), unlike in wild-type cells (Figure 4A). Ninety-two percent of microtubules in *num1*Δ cells underwent catastrophe within 2 min of contacting the cell cortex ($n = 59$). In contrast, 80% of microtubules that interacted with the cell cortex laterally in wild-type cells remained at the cell ends for >2 min ($n = 15$). Thus, Num1p appeared to be essential for the interaction between microtubules and the cell cortex during the horse-tail period.

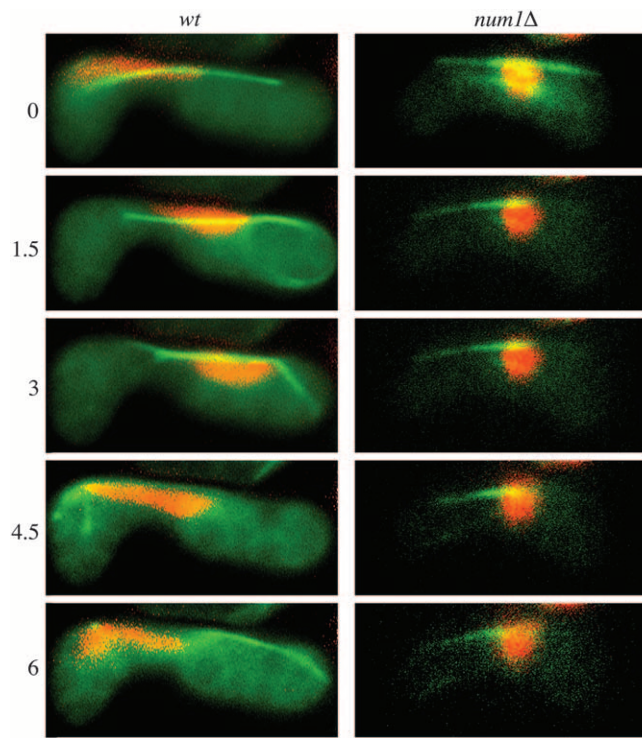

FIGURE 3.—Nuclear behavior in wild-type and *num1*Δ zygotes. Chromosomal DNA in zygotes (JY450 or JV627) was stained with Hoechst 33342 and monitored. The numbers on the left indicate time in minutes. Microtubules were visualized simultaneously by GFP-tagged α-tubulin. Stained DNA is shown in red, and GFP fluorescence in green. Bar, 5 μm.

Num1p is required to anchor dynein on the cortex: We examined localization of Dhc1p in *num1*Δ cells. Dhc1p, tagged with GFP and expressed from the authentic *dhc1* promoter on the chromosome, was seen on the SPB and microtubules during meiotic prophase in *num1*Δ cells, as in wild-type cells (YAMAMOTO *et al.* 1999) (Figure 5). The same localization pattern was observed when expression of GFP-tagged Dhc1p was driven from the strong *nmt1* promoter (data not shown). However, we never detected Dhc1p-GFP on the cell cortex of *num1*Δ. Dhc1p on the cortex has been supposed to generate a pulling force to drive nuclear oscillation (YAMAMOTO *et al.* 2001). Our observation suggested that Num1p was essential to anchor Dhc1p on the cortex.

The C-terminal PH domain and the central repeating unit are essential for the function of Num1p: To dissect regions that are important for the function of Num1p, we created two truncated versions of this protein, namely Num1p-ΔPH, which lacked the PH domain at the C terminus, and Num1p-ΔRU, which lacked the conserved repeating unit (Figure 1). We transformed *num1*Δ cells with plasmids expressing these Num1p variants tagged with GFP. Num1p-ΔRU could localize to the cell cortex, whereas cortical localization of Num1p was abolished by the deletion of the PH domain (Figure 6, A and B). To see if the function of Num1p was affected by these deletions, we observed behavior of the nucleus at meiotic prophase in *num1*Δ cells expressing either Num1p-ΔPH or Num1p-ΔRU (Figure 6B). Unlike cells expressing wild-type Num1p, cells expressing Num1p-ΔPH showed no nuclear movement, which appeared to be consistent with the delocalization of Num1p-ΔPH from the cell cortex. Num1p-ΔRU localized to the cell cortex properly, but this variant also could not drive the

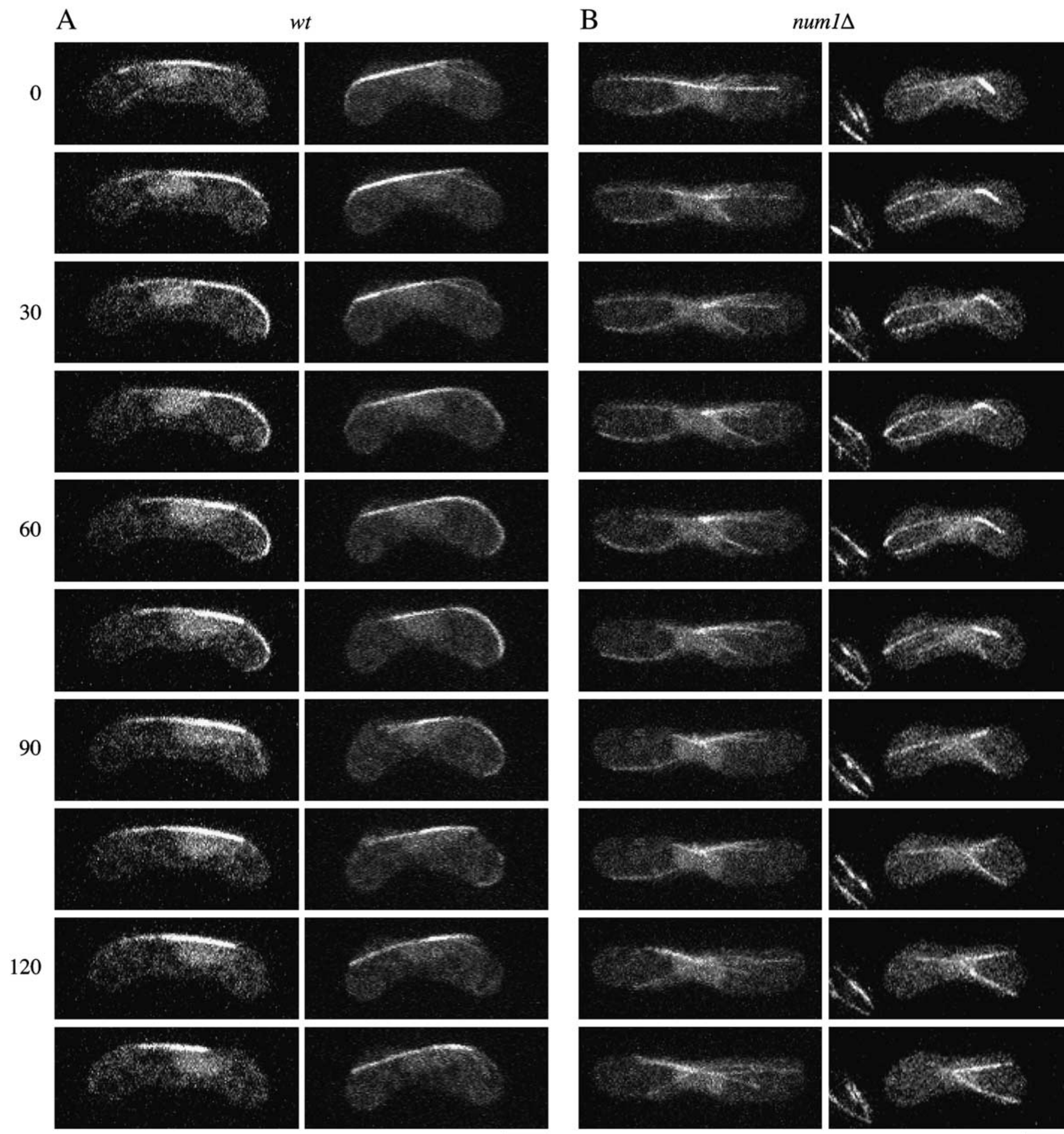

FIGURE 4.—Microtubule behavior in wild-type and *num1*Δ zygotes. (A) Wild type (JY450) and (B) *num1*Δ (JV627) homothallic haploid cells expressing GFP-tagged α-tubulin were starved for nitrogen to induce conjugation and meiosis. Confocal z-step series were recorded at 15-sec intervals and the projection images constructed are shown. The numbers indicate time in seconds. Bar, 5 μm.

horse-tail movement. Aberrant spore formation of *num1*Δ cells was rescued by neither Num1p-ΔPH nor Num1p-ΔRU (Table 4), suggesting that these variants were not functional.

Although Num1p-ΔRU could localize to the cell cortex, it was not functional. We tested the possibility that Num1p-ΔRU could not anchor dynein on the cortex. In *num1*Δ cells expressing Num1p-ΔRU, GFP-Dhc1p, expressed from the inducible *nmt1* promoter, was found to localize to the SPB and microtubules. However, we could not detect accumulation of Dhc1p on the cortex among cells carrying microtubules reaching the cell surface (0/7), even though Dhc1p was expressed from the strong *nmt1* promoter (Figure 6C). We also failed to detect a cortical GFP-Dhc1p signal in *num1*Δ cells expressing either Num1p-ΔPH (0/10) or no Num1p derivative (0/12), as expected, whereas cells expressing intact Num1p showed a cortical Dhc1 dot at a considerable frequency (4/10) (Figure 6C).

Num1p functions independently of the CLIP-170-like protein Tip1p: In addition to the Glued ortholog Ssm4p, the CLIP-170-like protein Tip1p is involved in localization of Dhc1p in fission yeast (NICCOLI *et al.* 2004). In the *tip1*Δ *ssm4*Δ double mutant, Dhc1p totally

TABLE 3

Growth and shrinkage rates of microtubules during meiotic prophase

Strain	Growth rate (μm/min)	Shrinkage rate (μm/min)
Wild type	3.4 ± 0.8 (n = 12)[a]	5.1 ± 2.0 (n = 12)[a]
*num1*Δ	3.2 ± 0.8 (n = 18)[a]	5.3 ± 1.7 (n = 13)[a]

Rates are shown in mean ± SD. The number of microtubules observed is indicated by n.

[a] t-Test showed no significant difference between wild-type and mutant values ($P > 0.05$).

loses its microtubular localization, although it can localize to the SPB (Niccoli *et al.* 2004).

Tip1p has been shown to localize to microtubule tips and cell ends in interphase cells (Brunner and Nurse 2000). During shmooing growth, Tip1p localizes to the nongrowing cell end and in the cytoplasm as dots in a row (Niccoli and Nurse 2002; Niccoli *et al.* 2004). We thus investigated how Tip1p was involved in meiosis and whether it might cooperate with Num1p during the horse-tail movement. Tip1p was observed evidently at the cortex of cell ends during meiotic prophase (Figure 7A), although fluorescence of Tip1p-GFP in meiotic cells was weaker than that in mitotic interphase cells (data not shown). Cytoplasmic dots in a row were also observed in some zygotes. However, the localization of Tip1p in *num1*Δ cells appeared to be the same as that in wild-type cells (Figure 7A). Conversely, Num1p appeared to localize properly to the cell cortex in the absence of Tip1p (Figure 7B).

To further examine the relationship between Tip1p and Num1p, we constructed the *num1*Δ *tip1*Δ double mutant. During mitotic growth, the *num1*Δ *tip1*Δ mutant showed a phenotype similar to that of the *tip1*Δ mutant. The *tip1*Δ mutant produced aberrant asci having fewer than four spores, as did the *dhc1*Δ or *ssm4*Δ mutants, but at a lower frequency. The *ssm4*Δ *tip1*Δ double mutant showed an exaggerated phenotype compared to each single mutant (Niccoli *et al.* 2004) (Table 2). The *num1*Δ *tip1*Δ mutant produced aberrant asci at nearly the same frequency as the *ssm4*Δ *tip1*Δ mutant (Table 2). We then observed localization of Dhc1p in the *num1*Δ *tip1*Δ mutant. It was similar to that in the *num1*Δ single mutant; *i.e.*, Dhc1p localized to microtubules and the SPB but not to the cell cortex (Figure 7C). This contrasts with the results in the *ssm4*Δ *tip1*Δ mutant, in which Dhc1p lost its microtubular localization (Niccoli *et al.* 2004). These observations altogether suggest that Num1p and Ssm4p are likely to contribute differently to the localization of Dhc1p. Ssm4p, in collaboration with Tip1p, may load Dhc1p onto microtubules independently of Num1p. At the cell cortex, Num1p anchors Dhc1p, and Ssm4p is also required for this anchoring (Niccoli *et al.* 2004).

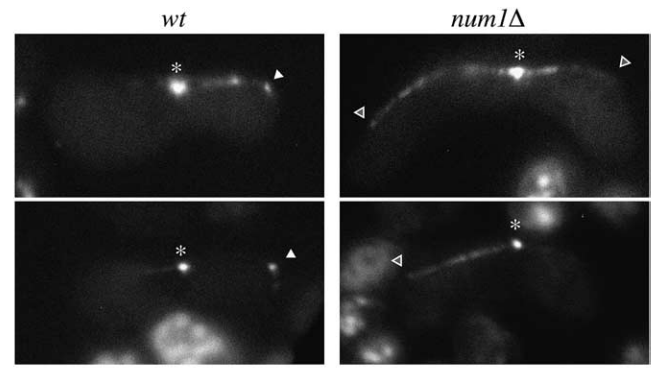

Figure 5.—Localization of Dhc1p in *num1*Δ cells. Wild-type (JV897) and *num1*Δ (JV898) homothallic haploid cells carrying *dhc1-GFP* were starved for nitrogen and observed under the fluorescence microscope. Asterisks and solid arrowheads indicate GFP fluorescence at SPB and on the cell cortex, respectively. Open arrowheads indicate possible microtubule contact sites on the cell cortex of *num1*Δ cells, where no accumulation of Dhc1p-GFP was detected. Bar, 5 μm.

DISCUSSION

In this study we have characterized a novel cortical protein, Num1p, in fission yeast. Num1p is required for the horse-tail nuclear movement. The driving force of this movement is thought to be generated by the dynein–dynactin complex localized to the site where microtubule plus ends contact the cell cortex (Yamamoto *et al.* 2001; Niccoli *et al.* 2004). As shown in this study, the dynein heavy chain Dhc1p cannot accumulate at this site in the absence of Num1p. This suggests strongly that Num1p may be a cortical dynein anchor. *S. cerevisiae* Num1p has been suggested to be a cortical factor anchoring dynein (Heil-Chapdelaine *et al.* 2000; Farkasovsky and Kuntzel 2001). In *S. cerevisiae num1*Δ cells, dynein heavy-chain Dyn1p concentrates at microtubule plus ends, suggesting that *S. cerevisiae* Num1p may unload Dyn1p from the end of microtubules when they reach the cortex (Lee *et al.* 2003; Sheeman *et al.* 2003). However, in our observations we have never seen concentration of dynein at the microtubule ends in *S. pombe* cells lacking Num1p. Thus, although the function of Num1p as the cortical anchor for dynein seems to be conserved in the two yeast species, the mechanisms to recruit dynein may be different. Recently, it has been reported in *S. cerevisiae* that dynein may be targeted to the microtubule plus end by kinesin Kip2p (Carvalho *et al.* 2004). It remains to be seen whether a similar mechanism operates in localizing dynein in *S. pombe.*

Both the central conserved repeating unit and the C-terminal PH domain are found to be essential for the function of *S. pombe* Num1p. This is in clear contrast to *S. cerevisiae* Num1p and *A. nidulans* ApsA. *S. cerevisiae* Num1p lacking the repeating unit and *A. nidulans* ApsA lacking the PH domain are reported to retain some residual activity (Farkasovsky and Kuntzel 1995; Suelmann *et al.* 1997). However, *S. pombe* Num1p lacking

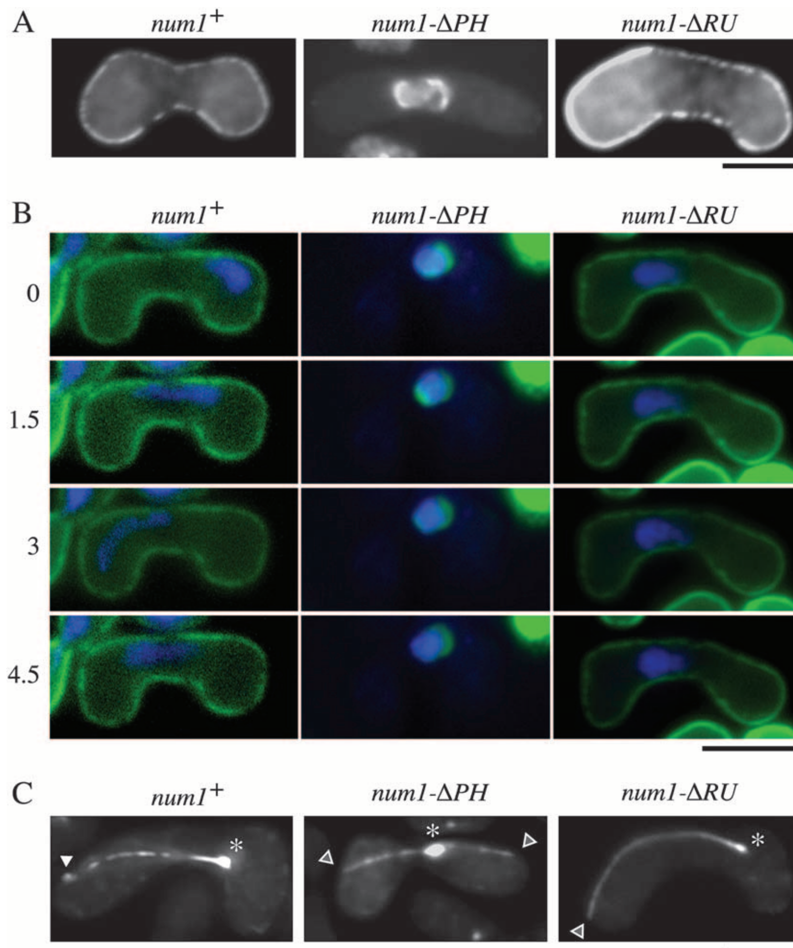

Figure 6.—Domain analysis of Num1p. (A) *num1Δ* cells (JV627) were transformed with a plasmid expressing Num1p-GFP, Num1p-ΔPH-GFP, or Num1p-ΔRU-GFP from the *nmt1* promoter. Transformants were subjected to nitrogen starvation and observed under the fluorescence microscope. Bar, 5 μm. (B) Nuclear movement during meiotic prophase in the same set of transformants as in A. Chromosomal DNA of each transformant stained with Hoechst 33342 is shown in blue. Fluorescence of GFP-tagged Num1p and its variants is shown in green. The numbers on the left indicate time in minutes. Bar, 5 μm. (C) Localization of Dhc1p in cells expressing Num1p-ΔPH or Num1p-ΔRU. *num1Δ* homothallic haploid cells (JV656) carrying *GFP-dhc1* were transformed with a plasmid expressing Num1p, Num1p-ΔPH, or Num1p-ΔRU from the *nmt1* promoter and analyzed as described in Figure 5. Transformants were starved for nitrogen and observed under the fluorescence microscope. Asterisks and solid arrowheads indicate GFP fluorescence at SPB and on the cell cortex, respectively. Open arrowheads indicate possible microtubule contact sites on the cell cortex of *num1Δ* cells. Bar, 5 μm.

either of these two domains appears to have no activity, even if expressed from a multi-copy plasmid. As demonstrated in this study, the central conserved repeating unit of *S. pombe* Num1p is not necessary for the cortical localization of the protein and hence is likely to be involved in the interaction with dynein. In *S. cerevisiae*, Num1p interacts with the dynein intermediate-chain Pac11p, depending on the presence of the dynein heavy chain (Farkasovsky and Kuntzel 2001). It is intriguing and remains to be clarified critically whether *S. pombe* Num1p associates with the dynein complex in a similar manner, although to date we have not been successful in detecting physical interaction of Num1p with the dynein intermediate-chain Dic1p by either two-hybrid assay or immunoprecipitation.

We have shown in this study that Num1p and a CLIP-170-like protein Tip1p have distinct roles in localizing dynein. We have shown previously that the Glued subunit of dynactin Ssm4p regulates dynein localization in cooperation with Tip1p (Niccoli *et al.* 2004). In the absence of Ssm4p, Dhc1p cannot accumulate at the cell cortex, as in *num1Δ* cells. Tip1p itself is not required for

TABLE 4

Number of spores per ascus in the *num1Δ* strain carrying a mutant *num1* allele

	% of asci carrying the following no. of spores:			
Plasmid	One	Two	Three	Four
num1^{+}	<0.1	0.7	2.1	97.2
num1-ΔPH	0.9	7.1	8.7	83.3
num1-ΔRU	0.6	6.6	7.2	85.6
None	0.9	6.4	7.0	85.7

A *num1Δ* homothallic haploid strain was transformed with a plasmid carrying each gene indicated. Transformants were examined for their ability to produce spores, as described in Table 2.

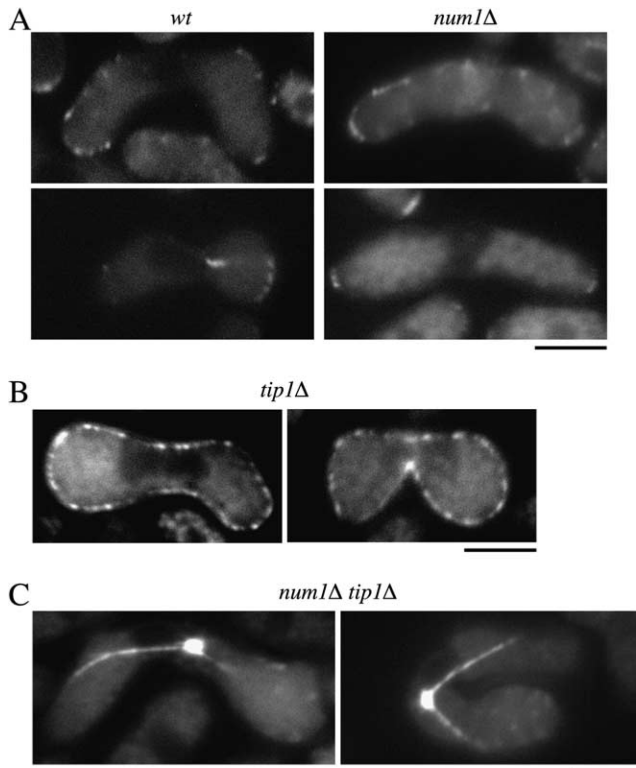

Figure 7.—Functional independency of Num1p and Tip1p. (A) Localization of Tip1p during meiotic prophase in wild-type and *num1*Δ cells. Homothallic haploid wild-type (JV471) and *num1*Δ cells (JV545) carrying the *tip1-GFP* fusion gene were starved for nitrogen to induce conjugation and subsequent meiosis and observed under the fluorescence microscope. Bar, 5 μm. (B) Localization of Num1p in *tip1*Δ cells. Homothallic haploid *tip1*Δ cells (JV421) carrying the *num1-GFP* fusion gene were starved for nitrogen to induce conjugation and meiosis and observed under the fluorescence microscope. Bar, 5 μm. (C) Localization of Dhc1p in *num1*Δ *tip1*Δ cells. Homothallic haploid *num1*Δ *tip1*Δ cells (JV681) carrying *GFP-dhc1* were starved for nitrogen and observed under the fluorescence microscope. Bar, 5 μm.

the localization of Dhc1p, but Dhc1p fails to localize on microtubules in the *ssm4*Δ *tip1*Δ mutant. In the *num1*Δ *tip1*Δ mutant, however, Dhc1p localizes on microtubules, only failing to accumulate at the cell cortex. These observations suggest that Ssm4p and Tip1p collaborate to recruit Dhc1p on microtubules, whereas at the cell cortex both Num1p and Ssm4p participate in anchoring Dhc1p.

Regulation of dynein localization, especially anchoring of dynein on cellular organelles, is crucial for many migratory processes, and a variety of regulators have been identified. Many regulators are common among different systems, although the role of each factor may vary from organism to organism. To date, however, we have been unable to find an ortholog of Num1p in organisms other than fungi. In metazoans, spectrin, which associates to membranous organelles through its PH domain, plays an essential role for dynein-dependent vesicle transport by linking the dynein–dynactin complex to the membrane (Holleran *et al.* 2001; Muresan *et al.* 2001). In Drosophila oocytes, an ortholog of the Lissencephaly protein DLis-1 localizes along the cortex and is required for the recruitment of dynein to the cortex (Swan *et al.* 1999). It remains to be examined whether these factors function in the same manner as Num1p.

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