Current Biology 20, 1029–1034, June 8, 2010 ©2010 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2010.04.017

Report

The Cortical ER Network Limits the Permissive Zone for Actomyosin Ring Assembly

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Summary

Precise positioning of the cellular division plane is important for accurate segregation of genetic material and determination of daughter cell fates. Here we report a surprising connection between division site positioning and the organization of the cortical endoplasmic reticulum (ER). The cortical ER is an interconnected network of flat cisternae and highly curved tubules sharing a continuous lumen [1, 2]. Stabilization of high curvature by reticulon and DP1 family proteins contributes to formation of tubules [3–5]. We show that in the fission yeast *Schizosaccharomyces pombe*, the ER network is maintained by a set of three membrane proteins: reticulon/Rtn1p, DP1/Yop1p, and a newly identified evolutionarily conserved protein, Tts1p. Cells lacking the ER domain sustained by these proteins exhibit severe defects in division plane positioning as a result of abnormal dispersion of a key regulator of division site selection, Mid1p, along the cell cortex. This triggers delocalized assembly of actomyosin cables and compromises their compaction into a single medially positioned ring. We propose that the cortical ER network restricts the lateral motion of Mid1p and hence generates a permissive zone for actomyosin ring assembly precisely at the cell equator.

Results and Discussion

The *Schizosaccharomyces pombe* genome encodes one reticulon and one DP1/Yop1 protein, which we term Rtn1p (SPBC31A8.01C) and Yop1p (SPCC830.08C). Rtn1p-GFP predominantly localized to the peripheral (cortical) endoplasmic reticulum (ER) and was largely excluded from the nuclear envelope (NE), consistent with localization in budding yeast [6]. On the other hand, Yop1p-GFP localized to both the peripheral ER and NE (Figure 1A). Interestingly, Rtn1p and Yop1p accumulated at the cell equator during mitosis, following assembly of the actomyosin ring marked by the myosin light chain, Rlc1p (Figure 1A). From a genetic screen for modulators of Cut11p function in spindle pole body anchorage at the NE [7] (unpublished data), we identified an evolutionarily conserved transmembrane protein (SPBC1539.04) with a strikingly similar subcellular distribution. This protein, Tts1p (*tet*ra-spanning protein 1), colocalized with Rtn1p and Yop1p in the peripheral ER and was also found at the NE (Figure 1B). The compartment marked by Tts1p, Rtn1p, and Yop1p was distinct from membranes containing the ER resident protein oligosaccharide-transferase Ost1p (see Figure S1A available online). Tts1p was enriched at the cell equator during mitosis together with Rtn1p and Yop1p, unlike Ost1p and the translocon subunit Sec63p [8] (Figure 1A; data not shown). Thus, the peripheral ER in fission yeast is organized as an intricately compartmentalized network of high-curvature (tubular) and low-curvature (cisternal) elements.

Mass spectrometry analysis of proteins copurified with the TAP-tagged Tts1p identified Rtn1p as a Tts1p-interacting partner (Figure S1B). We confirmed their interaction by dual coimmunoprecipitation (Figure 1C). Furthermore, Rtn1p interacted with Yop1p (Figure S1C), consistent with reports from budding yeast [3]. We also detected an association between Tts1p and Yop1p by coimmunoprecipitation (Figure S1C). Thus, our data suggest that Rtn1p, Yop1p, and Tts1p colocalize in a subcompartment of the ER and physically associate.

We wondered whether Rtn1p, Yop1p, and Tts1p function together in shaping ER membranes. We examined ER morphology with fluorescent markers for different ER compartments in wild-type and mutant genetic backgrounds. In *rtn1*Δ cells, both Yop1p-GFP- and Tts1p-GFP-marked membranes were significantly depleted from the lateral cortex (Figures 2A and 2B), suggesting diminishment of peripheral tubules. Tts1p and Yop1p in *rtn1*Δ cells were still clearly excluded from the cisternal compartment marked by Ost1p (Figure S2A). Consistent with a conversion to more cisternal ER, Ost1p in *rtn1*Δ cells localized extensively along the lateral cortex, unlike its typical intermittent pattern in wild-type cells (Figure S1A).

Similarly, Rtn1p-GFP showed decreased occupancy at the cell cortex in both *yop1*Δ and *tts1*Δ cells, and its occupancy was further diminished in the double *yop1*Δ*tts1*Δ mutant (Figure 2C). We performed similar analyses for other single and double deletions, with Yop1p-GFP (Figure 2A) or Tts1p-GFP (Figure 2B) as markers for the tubular ER. The reduction in occupancy of tubular ER markers was statistically significant in all cases, with double-deletion strains exhibiting augmented phenotypes. This was not due to compromised marker protein expression (Figure S2B). Of the three proteins, Rtn1p had the largest influence on the distribution of putative tubular ER markers.

As compared to the wild-type, the cortical ER domain visualized by Ost1p-mCherry in the triple *tts1*Δ*rtn1*Δ*yop1*Δ mutant cells (subsequently called *try*Δ) was more prominent and continuous, with occasional extended breaks (Figure S2C). To address how the deficiency of Rtn1p, Yop1p, and Tts1p affected general ER structure, we constructed artificial luminal ER markers, GFP-AHDL and mCherry-AHDL. Because the ER possesses a continuous intraluminal space, these markers localized to both cisternal and tubular compartments (Figure S2D). In wild-type cells, the cortical ER appeared as an elaborate and regularly distributed membranous network underlying the plasma membrane (Figure 2D, top; n = 50 cells). We observed that this regular network structure was disrupted in *try*Δ cells: the cortical ER now mostly appeared as continuous sheet-like membranes (Figure 2D, bottom; n = 50 cells). Additionally, we noticed irregular membrane accumulations and occasional breaks at the cortex. Because the geometry

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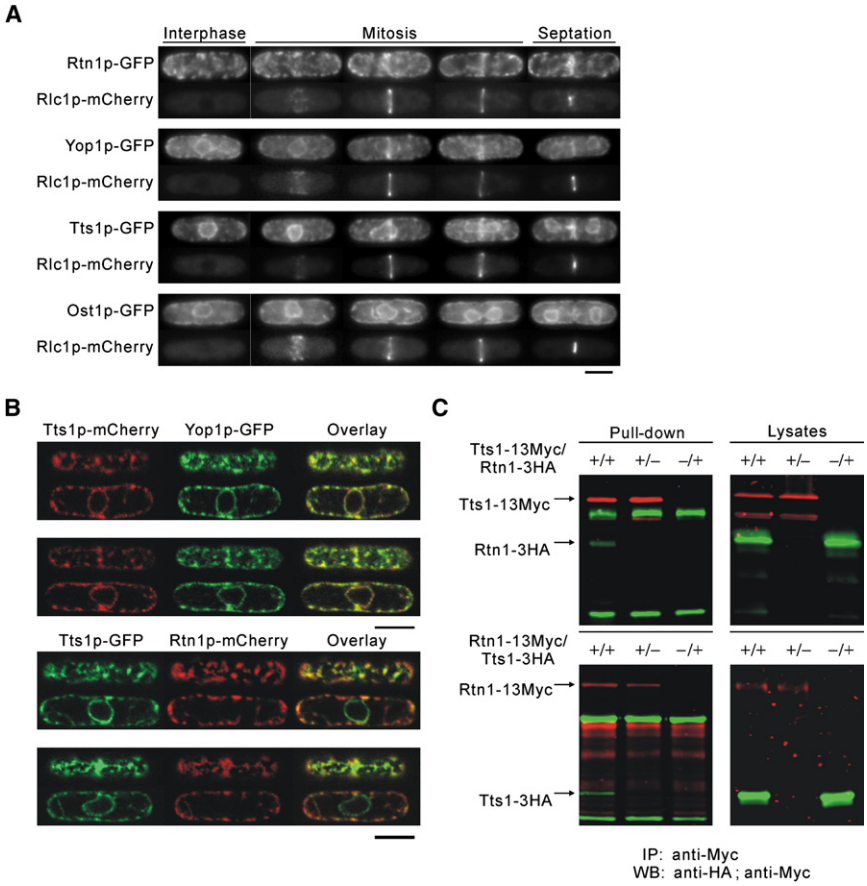

Figure 1. Rtn1p, Yop1p, and Tts1p Colocalize in the Cortical Endoplasmic Reticulum and Accumulate at the Mitotic Cell Equator

(A) Localization of GFP-fused proteins at cell-cycle stages indicated by coexpressed myosin light chain Rlc1p-mCherry as the actomyosin ring marker. Shown are maximum Z projections of epifluorescence micrographs.

(B) Scanning confocal micrographs of cells expressing indicated proteins. Shown are top and middle planes from Z stacks. Scale bars represent 5 μm.

(C) Coimmunoprecipitation of the indicated proteins from native cell extracts. Samples were probed with the anti-Myc (red) and anti-HA (green) antibodies.

of fission yeast cells impedes the resolution of fine details of cortical ER organization, we visualized GFP-AHDL in spheroplasts where the cell wall was removed and cells assumed a spherical shape. This allowed us to clearly document the transition between the intricate ER network in wild-type cells and continuous sheet-like membranes in a *try*Δ genetic background (Figure 2E). Taken together with work in other systems [3], our results indicate that Rtn1p, Yop1p, and Tts1p collaborate to maintain the tubular ER at the cell periphery.

Surprisingly, we observed a progressive failure in division site positioning as cells lost Tts1p, Yop1p, and Rtn1p. *S. pombe* cells position actomyosin rings in the cell center and divide perpendicular to the long axis of the cell. Single mutants for each *try* gene produced a low incidence of tilted and off-center septa. The proportion increased in double and triple mutant combinations. Interestingly, the deficiency of Rtn1p that individually had the strongest effect on ER structure consistently caused a more prominent cytokinesis defect. The triple mutant *try*Δ cells showed the highest incidence of multiple septa and long-axis septa (Figure 3A). The vast majority of *try*Δ cells (92.3% ± 1.8%; n = 1500 cells) failed to normally position septa and resembled cells lacking the anillin-like protein Mid1p that determines actomyosin ring positioning [9–12] (Figures 3A and 3B). Deletion of *mid1* in a *try*Δ genetic background did not exacerbate division site mispositioning, suggesting that Mid1p and the TRY proteins function in the same epistasis group (Figures 3A and 3B). The kinase Pom1p functions as a negative regulator of division site selection [13, 14], and cells lacking both Mid1p and Pom1p were reported as inviable [11]. We obtained mutant cells lacking the *try* genes together with *pom1* at a frequency much lower than expected from tetrad analysis (17% of the expected yield, 88 progeny). The quadruple mutant cells were severely retarded for growth and exhibited extreme septum positioning defects, including frequent tip septa (Figure S3A). This further suggests that the TRY proteins function together with Mid1p in a pathway that is distinct from the Pom1p pathway.

Mid1p shuttles between the nucleus and cell cortex during interphase, but upon entry into mitosis it redistributes to the medial cortex, where it is detectable as distinct nodes [15]. We found that Mid1p-GFP exited normally from the nucleus in triple mutant cells and was recruited to the cortex (Figure 3C, 20 of 23 wild-type and 8 of 10 *try*Δ cells completed Mid1p export within 4 min following spindle pole body [SPB] separation). The total levels of Mid1p-GFP were comparable in wild-type and *try*Δ cells (Figure S3B). Intriguingly, Mid1p spread along a larger area of the cortex and failed to compact into a tight ring (Figure 3C, see kymographs for Mid1p-GFP time evolution). As a result of this abnormal dispersal, the cortical domain occupied by Mid1p was much broader in early mitotic *try*Δ cells as compared to the wild-type (Figure 3D). Notably, we observed that Mid1p-GFP was now largely present in cortical nodes of lower mean fluorescence intensity (Figure S3C; n = 140, p = 0.0013, Kolmogorov-Smirnov test). Unlike wild-type cells that compacted rings during early mitosis, 10 of 15 *try*Δ cells failed to compact Mid1p into rings, and ring formation was strongly delayed in 5 of 15 *try*Δ cells. We did not observe mislocalization of Pom1p, the negative regulator of Mid1p localization, during interphase (Figure S3D).

Given that cortical Mid1p failed to compact into rings in *try*Δ cells, we examined whether it recruited essential ring components such as myosin II and F-actin by using Rlc1p-mCherry and the GFP-tagged Rng2p calponin homology domain as markers. In *try*Δ cells, Rlc1p-mCherry promptly associated with all cortical Mid1p-GFP nodes, as in the wild-type (Figures 3E and 3F; n = 15 cells). Furthermore, actin filaments appeared around the cortical nodes marked by Rlc1p-mCherry in both wild-type and *try*Δ cells (Figures 3G and 3H; n = 20 cells). The timing of actin recruitment to the cortex was not affected (2.5 ± 0.9 min after SPB separation in wild-type, n = 9 cells; 2.9 ± 1.0 min in *try*Δ cells, n = 13 cells; Figure S3E). Thus,