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The *Saccharomyces cerevisiae* Fin1 protein forms cell cycle-specific filaments between spindle pole bodies

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The *FIN1* gene from the yeast *Saccharomyces cerevisiae* encodes a basic protein with putative coiled-coil regions. Here we show that in large-budded cells a green fluorescent protein-Fin1 fusion protein is visible as a filament between the two spindle pole bodies. In resting cells the protein is undetectable, and in small-budded cells it is localized in the nucleus. During late mitosis it localizes on the spindle pole bodies. Filaments of cyano fluorescent protein-tagged Fin1 colocalize with filaments of green fluorescent protein-tagged Tub1 only in large-budded cells. By electron and atomic force microscopy we showed that purified recombinant Fin1p self-assembles into filaments with a diameter of ≈ 10 nm. Our results indicate that the Fin1 protein forms a cell cycle-specific filament, additional to the microtubules, between the spindle pole bodies of dividing yeast cells.

The 14-3-3 proteins comprise a family of highly conserved regulatory proteins that interact with more than 100, mostly phosphorylated, proteins (1–5). In this way they are involved in many cellular processes such as signaling, cell cycle control, apoptosis, and cytoskeletal dynamics. In a two-hybrid screen to identify interaction partners of the *Saccharomyces cerevisiae* 14-3-3 protein Bmh2p (6, 7) we found the *YDR130c* ORF (M.J.v.H. and G.P.H.v.H., unpublished data). For reasons detailed below we named this ORF *FIN1* (filaments in between nuclei 1). *FIN1* encodes a protein of 291 aa with a predicted pI of 10 containing two putative coiled-coil regions at its C terminus. A *fin1* null mutant is viable on both minimal and rich growth media. Genome-wide analysis of gene expression during the cell cycle showed that *FIN1* is expressed significantly higher during the G₂-to-M transition (8). Overexpression of a gene encoding 6 \times His-tagged Fin1p using the *GALI* promoter results in lethality in haploid cells and very poor growth of diploid cells, which became strongly deformed (M.J.v.H. and G.P.H.v.H., unpublished data). Because this overexpression resulted in the accumulation of filamentous structures resembling the neurofibrillary tangles found in cells of patients with Alzheimer's disease (9, 10), we investigated the cellular behavior of the Fin1 protein in more detail. In this study we showed that the Fin1 protein can form a cell cycle-specific filament, additional to the microtubules, between the spindle pole bodies of dividing yeast cells.

Materials and Methods

Strains and Media. Yeast strain GG3036 (*MATa ura3-52 trp1-289 pUG36[FIN1]*) was obtained by transformation of CENPK113-9D (P. Kötter, Goethe University, Frankfurt) with pUG36[*FIN1*]. pUG36[*FIN1*] was made by cloning a PCR fragment with the *FIN1* ORF flanked by restriction sites for *Bam*HI and *Sal*I into pUG36 (U. Güldener and J. H. Hegemann, unpublished data). In pUG36[*FIN1*], *FIN1* is tagged with yeGFP at its N terminus and expressed under control of the *MET25* promoter. MAS101, containing an integrated *TUB1*-green fluorescent protein (*GFP*) fusion was obtained from A. Murray (11). Plasmid pRUL1001 was made by replacing the GFP-

containing *Xba*I-*Bam*HI fragment of pUG34 (U. Güldener and J. H. Hegemann, unpublished data) by a cyano fluorescent protein (CFP)-containing *Xba*I-*Bam*HI fragment. The latter fragment was made by PCR on plasmid pDH3 (D. Haley, Yeast Resource Center, University of Washington, Seattle) using the primers 5'-AAATCTAGAATGAACAGTAAAGGAGAA-GAAGTT-3' and 5'-AAAGATCCACTAGTTTTGTAT-AGTTCATCCATGCC-3'. *FIN1* was cloned into pRUL1001 as described above yielding pRUL1005. MAS101 was transformed with pRUL1005 yielding strain GG3060. Yeast was grown in MY medium supplemented as required with adenine, leucine, histidine, tryptophan, and uracil (12).

Microscopy. 4',6-Diamidino-2-phenylindole (DAPI) staining and indirect immunofluorescence microscopy were performed on glutaraldehyde-fixed cells as described (13). During confocal fluorescence microscopy with a Leica DMIRBE, GFP was excited at 488 nm, and emission was detected at 514–564 nm; CFP was excited at 457 nm, and emission was detected at 464–500 nm.

In Vitro Filament Formation and Atomic Force Microscopy. The construction of yeast strains allowing the isolation of 6 \times His-tagged Fin1 protein will be described elsewhere. Cells were disrupted by vortexing in the presence of glass beads in 8 M urea/0.5 M NaCl/20 mM Tris-HCl, pH 8.0/1 mM sodium vanadate and protease inhibitors (Roche, Mannheim, Germany), and the 6 \times His-Fin1p was isolated by Ni²⁺-nitrilotriacetic acid agarose affinity chromatography in the presence of 8 M Urea/0.5 M NaCl/20 mM Tris-HCl, pH 8.0. The protein was dialyzed against 25 mM NaCl/5 mM sodium phosphate, pH 7.5 and 10 μ l containing 200 ng of 6 \times His-Fin1p was applied on a freshly cleaved mica surface for 30 sec. Atomic force microscopy was done in air with a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA). For electron microscopy, protein samples were applied to Formvar carbon-coated grids, and negative staining was done with 2% phosphotungstic acid in water for 2 min.

Two-Hybrid Screening. pRUL558 was made by cloning a PCR fragment with the *FIN1* ORF with restriction sites for *Bam*HI and *Sal*I at the ends into pGBDK-C2. In this plasmid the *FIN1* ORF is fused to the GAL4 binding domain. pGBDK-C2 was made from pGBD-C2 by replacement of the ampicillin resistance marker by a kanamycin resistance marker (M.J.v.H. and

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Abbreviations: GFP, green fluorescent protein; CFP, cyano fluorescent protein; DAPI, 4',6-diamidino-2-phenylindole.

See commentary on page 4757.

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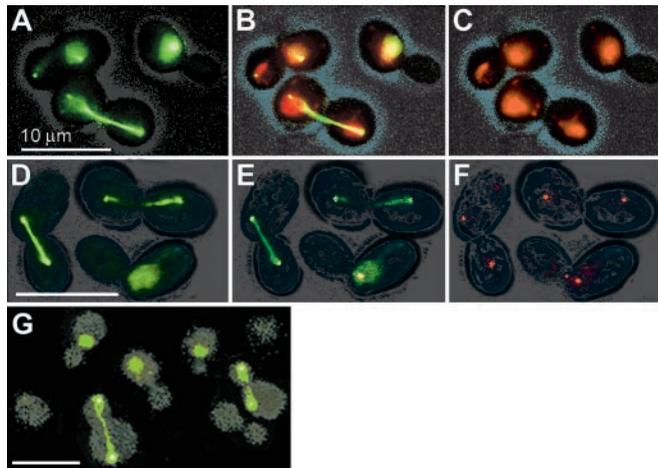


Fig. 1. Subcellular localization of GFP-Fin1p. The nuclei of GG3036 cells were stained with DAPI, and images were captured by using filters for GFP (A) and DAPI. The blue color obtained with the DAPI filter was converted into red by computer manipulation (C), and the images were superimposed (B). Spindle pole bodies were visualized in GG3036 cells by indirect immunofluorescence microscopy using the anti-Spc98p antiserum (15) and tetramethylrhodamine B isothiocyanate (TRITC)-labeled secondary antibodies. Images were captured by using the TRITC (D) and GFP filter (F) and were superimposed (E). *GFP-FIN1* was integrated at the *FIN1* locus, and the subcellular localization of GFP-Fin1p was determined (G). In G, extreme offline image enhancement was required to visualize GFP.

G.P.H.v.H., unpublished data). The two-hybrid screen was done as described by James *et al.* (14). Positive interactions were confirmed by retransformation of strain HF7c with the isolated library plasmid in combination with pRUL558.

Results

Subcellular Localization of Fin1p. We studied the intracellular localization of the Fin1 protein using an N-terminal GFP fusion. To improve the signal we used the *MET25* promoter, allowing mild overexpression. Cells with this construct only exhibited a slight reduction in growth rate (μ_{\max} , 0.20 h^{-1} versus 0.26 h^{-1} for the control strain), suggesting that this level of overproduction gave no major adverse effects. The localization of Fin1p varied depending on the stage of a cell in the cell cycle (Fig. 1A). Interestingly, in large-budded cells fluorescent filaments were seen between the mother and daughter cell. Superimposing the localization of nuclei (Fig. 1C) and GFP-Fin1p in DAPI-stained cells clearly showed that the Fin1p filaments extend between the two nuclei of a dividing cell (Fig. 1B). In other cells, the Fin1 protein is present in the nucleus in a nonfilamentous form. Spindle pole bodies were visualized by indirect immunofluorescence microscopy using anti-Spc98p antibodies (14). Superimposing the localization of Spc98p (Fig. 1F) on that of the GFP-Fin1 protein (Fig. 1D) clearly shows that the Fin1p filaments extend between the spindle pole bodies, and the bright centers at the ends of the filaments colocalize with these organelles (Fig. 1E). A similar GFP-Fin1p localization was observed when the *GFP-FIN* gene was integrated into the genome and expressed under control of the *FIN1* promoter, although fluorescence was very weak and offline image enhancement was necessary to observe the filaments (Fig. 1G).

Cell Cycle-Dependent Localization of Fin1p. To investigate the cell cycle-dependent localization of Fin1p further, we followed single cells using time-lapse confocal fluorescence microscopy. In cells early in the cell cycle, hardly any fluorescence was detectable. Later, a nuclear localization of Fin1p became apparent (Fig. 2,

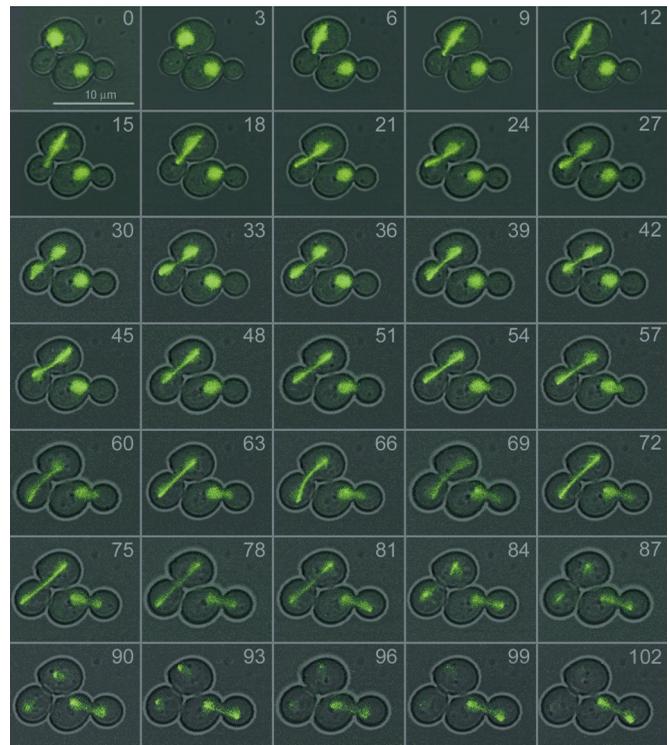


Fig. 2. Time-lapse confocal laser fluorescence microscopy of GG3036 cells. Cells were incubated at 22°C , and images were captured at the indicated times (in minutes). The total cell cycle was estimated to be ≈ 280 min. The later part of the cell cycle is shown.

time 0). During the next stages the Fin1 protein rapidly became localized in a filament between the mother and daughter cells. This localization remained for a relatively long time. Quantification of the fluorescence showed that the amount of GFP-Fin1p varied less than 20% between 0 and 78 min. Subsequently, the filament rapidly broke down, and the protein was seen as single dots in each cell (Fig. 2, time points 75–93 min). Finally, it disappeared completely, thus returning to the situation at the beginning of the cell cycle (Fig. 2, time 102). Quantification of the fluorescence indicated that at 102 min the amount of GFP-Fin1p had decreased to less than 10% of the amount before 78 min (Fig. 2). The cell cycle-dependent expression of the Fin1 protein is not an artifact caused by the *MET25* promoter, because the expression of other genes controlled by this promoter is not cell cycle-dependent (data not shown). Cells synchronized by α -factor arrest behaved similarly. In arrested cells (G_1 phase of the cell cycle), hardly any fluorescent protein was visible. Western blotting with an anti-GFP antibody confirmed the low level of the fusion protein in these cells ($\approx 10\%$ of the amount present in large-budded cells; data not shown). After removal of α -factor, the GFP-Fin1 fusion protein first accumulated in the nucleus of small-budded cells followed by the formation of filaments in large-budded cells. The amount of fusion protein detected by Western blotting increased considerably after release from the α -factor block (data not shown).

Localization of Fin1p Relative to Microtubules. Because the filaments formed by the Fin1 protein resemble microtubules, we visualized Fin1p as a fusion with CFP and Tub1p as a fusion with GFP in the same cell. In small-budded cells GFP-Tub1p is present in small filaments (Fig. 3C, bottom cells), whereas CFP-Fin1p is nonfilamentous (Fig. 3A). Superimposing both figures clearly shows a different localization (Fig. 3B). Filaments

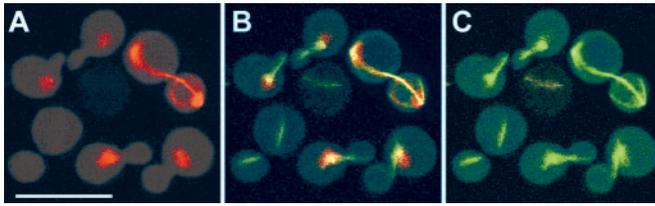


Fig. 3. Confocal laser fluorescence microscopy of GG3060 cells expressing *TUB1-GFP* and *CFP-FIN1*. (A) CFP fluorescence (red). (C) GFP fluorescence. (B) Superimposing A and C. (Scale bar, 10 μ m.)

of both CFP-Fin1p (Fig. 3A, upper cells) and GFP-Tub1p (Fig. 3C) are present in large-budded cells with apparent colocalization (Fig. 3B).

In Vitro Filament Formation. To investigate whether the Fin1 protein can form filamentous structures independently of tubulin, we isolated 6 \times His-tagged Fin1 protein from yeast in the presence of 0.5 M NaCl and 8 M Urea and analyzed the structures formed after dialysis against low-salt buffers. Electron (Fig. 4A) and atomic force (Fig. 4B) microscopy clearly show the presence of filaments of proteins with a diameter of \approx 10 nm. These results indicate that *in vitro* the Fin1 protein is able to self-assemble into 10-nm filamentous structures similar to other intermediate filament-forming proteins.

Interaction Partners of Fin1p. To obtain insight into the function of the Fin1p protein we searched for interacting proteins using the yeast two-hybrid system. A total number of 1.2×10^8 transformants were obtained, of which 340 were His-positive, and 44 also had β -galactosidase activity. The library plasmids were isolated from these 44 positives, and we identified the Fin1 protein itself, Reb1p, Fir1p, and Wss1p. Both the Reb1 and Fir1 proteins are involved in RNA metabolism. The Reb1 protein is a DNA-binding protein required for termination of RNA polymerase I transcription (16, 17), and the Fir1 protein (18) has been identified as a factor interacting with the Ref2 protein, which is involved in 3' mRNA processing (19). A *wss1* mutation can weakly suppress a *SMT3* mutation, which is involved in ubiquitin-like protein degradation in relation to the function and/or structure of the kinetochore. In a large-scale search for protein-protein interactions the Fin1 protein was found to interact with the Glc7 protein phosphatase (20).

Discussion

In this study we have shown that the Fin1 protein is present in a filamentous structure extending between the spindle pole

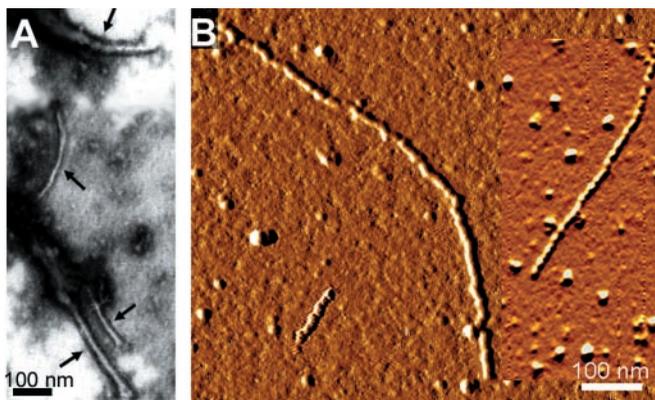


Fig. 4. Electron (A) and atomic force (B) microscopic analysis of structures formed by 6 \times His-Fin1p.

bodies in dividing cells. In large-budded cells these structures appear to colocalize with nuclear microtubules. However, there is no evidence for a direct physical interaction between the Fin1 protein and the tubulin proteins Tub1p, Tub2p, and Tub3p. In our two-hybrid screen for interaction partners of the Fin1 protein we did not find any of the *TUB* genes. Furthermore, others did not find Fin1p after using Tub1p as bait, whereas they did find the microtubule-binding protein Bim1p (21). In addition, no interaction between the Fin1 protein and the Tub proteins was found in large scale analyses of yeast protein-protein interactions (20, 22). It is highly likely that Fin1p can form filaments by itself *in vivo*. We have found an interaction between Fin1p fused to the Gal4 binding domain and Fin1p fused to the Gal4 activating domain in the yeast two-hybrid system. In addition, purified 6 \times His-tagged Fin1 protein binds to immobilized 6 \times His-tagged Fin1 protein during surface plasmon resonance measurements (data not shown). Analysis of purified 6 \times His-tagged Fin1p preparations exposed to low salt concentrations by electron and atomic force microscopy (Fig. 4) revealed that Fin1p is able to self-assemble into 10-nm filamentous structures. These filaments resemble filaments of other intermediate filament-forming proteins such as Mdm1p (23). However, the role of other proteins such as tubulins in the filament formation in the intact cell remains to be elucidated.

To deepen the insight into the function of the Fin1 filaments we performed a two-hybrid screen for interaction partners of the Fin1 protein, and two interaction partners were identified that were involved in RNA metabolism, i.e., Reb1p and Fir1p. The Fir1 protein interacts not only with proteins involved in RNA processing but also with the spindle pole/kinetochore component Spc24p (24–26) and with Spo12p (27), a protein probably involved in meiosis and exit from mitosis. As the Fin1 filaments extend between the two spindle pole bodies of a dividing cell, the Fin1 protein may link chromosome segregation with RNA metabolism. Evidence for a link between the chromosome segregation apparatus and RNA metabolism has been obtained also by others. In screens for proteins interacting with enzymes involved in RNA processing, two spindle pole body/kinetochore components involved in microtubule nucleation, Spc24p and Tid3p, were identified (24, 28). In addition, the centrioles of higher eukaryotes and yeast contain RNA, which is required for the formation of the mitotic spindle in dividing cells (29, 30). However, because a *FIN1* disruption is not lethal, the Fin1 protein either is not essential or other proteins can take over the function of the Fin1 protein. Alternatively, the Fin1 filaments may be part of the postulated “spindle matrix,” which may help organize and stabilize the spindle microtubules (31, 32).

We noticed a striking resemblance between the behavior of the Fin1 protein and that of the tau protein in cells of patients with Alzheimer’s disease. Despite the difference in their primary structure and the absence of binding of Fin1p to tubulin, both proteins form filaments, high intracellular levels of both proteins lead to abnormal cells with an accumulation of filamentous structures, and both proteins are binding partners for the 14-3-3 proteins (33). Further research may show whether filament formation by the Fin1 protein in yeast cells indeed can serve as a model for filament formation by the tau protein.

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