Supplemental material

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Figure S1. The nMT array emanates from an unduplicated SPB and eventually deforms the nucleus. (A) Nuclear membrane deformation induced by nMT arrays in quiescent cells observed by EM. Yellow arrows, MT; red arrowheads, nuclear membrane. Bar, 500 nm. (B) Nuclear membrane deformation induced by nMT array in quiescent cells expressing GFP-Tub1 and Nup2-RFP or Tub1-GFP and Htb1-RFP. Corresponding proliferating cells in G1 are shown for comparison. Bar, 2 μ m. (C) Fluorescence quantification of various SPB-GFP components in proliferating G1 cells and in 3-d-old quiescent cells (63 > n > 102; two experiments). "p" indicates that no significant differences were detected by Student's *t* test. Bar, 2 μ m. (D) nMT array visualized in WT cells by EM. Yellow arrows, MTs; red arrowheads, nuclear membrane; pink arrow, SPB. Bar, 100 nm.



Figure S2. The nMT array contains highly stable MTs. (A) Variation of nuclear MT length in function of time (second) in proliferating cells. Cells express GFP-Tub1 (green) and Nup2-RFP (red). Bars, 2 μ m. N = 1, *n* is indicated. (B) Variation of the nMT array length in function of time measured for 4-dold Bim1-GFP-expressing cells (*n* = 16), for 6-dold Dad2-GFP-expressing cells (*n* = 17), for 4-dold Stu2-GFP-expressing cells (*n* = 17). Examples of corresponding time lapse are shown. Time is in minutes (two experiments). Bar, 2 μ m. (C) Nuf2-GFP dots detected along nMT array (arrow) are immobile. Several time lapse images of 4-dold cells are shown. Time is in minutes. Bar, 2 μ m. (D) MT organizations in proliferating G1 cells and 4-dold quiescent cells before and after a 90-min treatment with indicated drugs. Bar, 2 μ m. (E) MT organizations in 5-dold quiescent cells before and after a 90-min treatment with indicated.

Figure S3. The nMT array reorganizes the nucleus and is required for cell viability in quiescence. (A) Relocalization of the nucleolus in quiescent cells observed by immuno-EM. The electron-dense zone is the nucleolus (blue arrow); yellow arrows, MTs; red arrowheads, nuclear membrane. Bar, 100 nm. (B) Localization of various kinetochore proteins in 4-d-old quiescent cells. (C) Representative quiescent cells (4 d) for which TetO repeated sequences were integrated next to the chromosome IV centromere and expressing TetR-GFP (green) and CFP-Tub1 (red). (D) Distances between DNA proximal to chromosome IV centromere and the SBP (Spc42-RFP). Distances were measured in proliferating G1 cells and in 3-d-old quiescent cells (n > 200 from a single experiment). (E) Images of MAP deletion mutants expressing Bim1-3GFP grown for 4 d in YPDA before (top) or after 1 h nocodazole treatment (22.5 µg/µL; bottom). (F) Images of MAP deletion mutants expressing GFP-Tub1 or Tub1-RFP grown for 4 d in YPDA. Wild-type cell expressing GFP-Tub1 and Nup2-RFP is shown as a control. (G) In the absence of nMT array, kinetochores localize at SPB proximity. The distribution of the distances measured between the SPB and Spc24-GFP is shown (two experiments; for WT, n = 301; $arp 1\Delta$, n = 218; and $dyn1\Delta$, n = 202). Corresponding images are shown. (H) Dynein and dynactin complexes localize on the SPB cytoplasmic side. Quiescent cells coexpressing Arp1-3GFP or Dyn1-3YFP and Tub1-RFP or Nup2-RFP are shown. Left panels are average line scan evaluating the position of Arp1-3GFP and Dyn1-3YFP (green line) toward the nuclear membrane (Nup2-RFP, red line) in quiescent cells (4 d) obtained using the indicated number of cells (n) from a single experiment. The orange portion schematizes the nucleus. (I) Percentage of dead cells (gray bar) and bi-nucleated cells (blue bars) scored using methylene blue and Hoechst staining, respectively, after 7 d of culture. Dashed bars indicate budded cells. (J) Percentage of cells with actin bodies scored using phalloidin staining after 7 d of culture. (K) Mutants without nMT array have a reduced viability in guiescence. Colony-forming unit ability measured for cells grown 2 (yellow), 7 (light blue), or 14 d (dark blue) at 30 or 37°C. Strains that assemble an nMT array in quiescence (WT and kip2A) and a sac6 Δ deletion strain that is known to have a drastically reduced viability in quiescence (Sagot et al., 2006) were used as controls. (L) Sorbitol has no effect on cell survivability in quiescence. Cells were grown 7 or 14 d in YPDA without (white bars) or with 1 M sorbitol (gray bars) at 30°C, and their capacity to form a colony was scored. In K and L, percentages are given using wild-type cells as reference. Bars: (B-H) 2 µm.



Table S1 is provided as a Microsoft Excel file.

Reference

Sagot, I., B. Pinson, B. Salin, and B. Daignan-Fornier. 2006. Actin bodies in yeast quiescent cells: an immediately available actin reserve? *Mol. Biol. Cell*. 17:4645–4655. http://dx.doi.org/10.1091/mbc.E06-04-0282