Dispatch
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Meiosis: Organizing Microtubule Organizers

During meiosis in fission yeast, the zygote nucleus undergoes microtubule-driven oscillatory movements that ultimately serve to promote genetic recombination. An essential component of this is a meiosis-specific consolidation of microtubule-organizing activity to the the spindle pole body, driven by the novel coiled-coil protein mcp6/hrs1p.

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In certain types of eukaryotic cells, the microtubule cytoskeleton is co-opted to create specialized structures, such as axons in neurons, or flagella in spermatozoa. Even in non-specialized cells, however, the microtubule cytoskeleton can be transiently altered in interesting ways. The archetypical example of this is the mitotic spindle, which rapidly assembles during mitosis to effect chromosome segregation and then disassembles equally quickly. Although spindle formation involves a wholesale reorganization of cellular components and is, appropriately, incredibly complex, smaller organizational changes in the cytoskeleton can similarly illuminate our understanding of cytoskeletal regulation. Recent studies in fission yeast [1,2] have now revealed that a single protein can be responsible for a major change in microtubule organization during the early stages of meiosis.

During fission yeast meiosis, after fusion of the cell nuclei of the two mating-partners, the resulting zygote nucleus undergoes sweeping oscillatory movements in the cell [3–5]. These 'horsetail' movements — so called because the nucleus, led by the spindle pole body, the yeast centrosome equivalent, resembles the shape of a horsetail — are driven by microtubules and the force-generating enzyme cytoplasmic dynein. Strikingly, mutants in which the movements do not occur, including mutants in dynein itself [5], show much reduced recombination between homologous chromosomes, indicating that these movements are more than a cytological curiosity.

The horsetail movements are accompanied by changes in nuclear organization of chromosomes: in vegetative (mitotically growing) cells, the interphase centromeres are associated with the spindle pole body, but in meiotic cells it is the telomeres that associate with the spindle pole body [3,6]. Collectively, these modifications and movements within the nucleus and the cytoplasm are thought to allow for a more efficient homology search among the *Schizosaccharomyces pombe* chromosomes, and thus to promote proper recombination.

A key feature of horsetail movement is that it involves a rearrangement of the microtubule cytoskeleton. To appreciate this properly requires a brief explanation of interphase microtubule organizing centers in fission yeast. In higher eukaryotic cells, microtubules are centrally organized in a radial array, nucleated from the centrosome, where microtubule 'minus' ends are associated with the conserved microtubule-nucleating γ-tubulin complex [7], while microtubule 'plus' ends are more likely found towards the cell periphery. By contrast, interphase microtubules in vegetative fission yeast run along the long axis of the cylindrically shaped cells [8], and are nucleated from sites in the cell middle that may include the spindle pole body, additional sites on the surface of the nucleus, and satellite sites on microtubules themselves [9–11] (Figure 1).

Nucleation from all of these sites involves the coiled-coil protein mto1p (previously known as mbo1/mod20p) [9,11,12], which acts to recruit the γ-tubulin complex [13,14] to the varied microtubule organizing centers. Thus, although the basic mechanism of microtubule nucleation via the γ-tubulin complex is thought to be conserved between fission yeast and higher eukaryotes, the specific modes of organizing the microtubule-organizing centers may be different.

During the horsetail stage of meiosis, however, fission yeast microtubules become centrally organized by the spindle pole body, such that, at least schematically, microtubule organization more closely resembles that of higher eukaryotic cells [4] (Figure 1). In conjunction with microtubule dynamic instability and dynein-mediated force production on microtubules, this centralized organization is an essential component of the system that drives the spindle-pole-body-led horsetail movements.

In this context, a major outstanding question is what is responsible for the microtubule reorganization. Specifically, how are the microtubule organizing centers themselves reorganized to create the conditions favorable for horsetail movements? Recent work, published independently in the *Journal of Cell Science* by Saito *et al*. [1] and in this issue of *Current Biology* by Tanaka *et al*. [2], has identified a novel protein, mcp6/hrs1p, as playing a major role in this process.

Although the two groups initiated their work from somewhat different angles, both were searching the *S. pombe* genome database for coiled-coil proteins specifically upregulated during meiosis, and both ended up focusing on a 327 residue protein with no apparent orthologs, which they alternatively named meiosis-specific coiled-coil protein 6 (mcp6p) [1] or horsetail protein 1 (hrs1p) [2].

Mcp6/hrs1p is specifically expressed upon induction of meiosis, but both RNA and protein levels decline

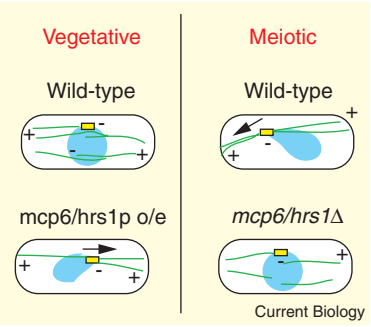

Figure 1. Distributed versus centralized microtubule organization in vegetative and meiotic cells in relation to mcp6/hrs1 expression.

Cells are either wild-type, overexpress mcp6/hrs1 (*mcp6/hrs1 o/e*) or lack mcp6/hrs1 (*mcp6/hrs1Δ*), as shown. The nucleus is in blue, the spindle pole body in yellow, and microtubules in green. Arrows indicate direction of nuclear movements, and plus and minus signs indicate microtubule plus and minus ends. Cell shape is schematic (zygotes from cell mating do not have this regular shape).

considerably by the beginning of the first meiotic division [1], suggesting that the main function of the protein may be performed before this time. Further hints to possible function came from fusing green fluorescent protein (GFP) to mcp6/hrs1p, as the fusion protein localized to the spindle pole body early in the meiotic program but then disappeared prior to the first meiotic division.

Because both the localization and timing of mcp6/hrs1p expression suggested a role in some aspect of horsetail movements, the two groups [1,2] deleted the gene in order to test the possibility directly. As might be expected for a gene expressed in meiosis, mcp6/hrs1p was found not to be essential for vegetative growth, but meiotic defects were immediately apparent in the deletion mutants, the main defects being an absence of horsetail movements and a reduction in recombination.

Interestingly, when the two groups [1,2] examined microtubule distribution in the *mcp6/hrs1Δ* cells during the period corresponding to the horsetail stage, they found that microtubules were not centrally organized at the spindle pole body, but rather resembled those of vegetative interphase cells (Figure 1). Tanaka *et al*. [2] further found that, at this time, alp4p, a conserved component of the γ-tubulin complex [14], was dispersed around the nucleus in *mcp6/hrs1Δ* mutants instead of concentrated at the spindle pole body as in wild-type cells. These experiments indicate that mcp6/hrs1p is likely to play an important role directly at the spindle pole body to promote the centralized microtubule organization required for horsetail movements.

Although mcp6/hrs1p is normally expressed only in meiosis, Tanaka *et al*. [2] went on to express it ectopically in vegetative cells to see what, if any, effects this might produce. Amazingly, nuclei in many of these cells now exhibited unusual oscillatory movements, as well as an altered interphase microtubule distribution, now centralized from the spindle pole body (Figure 1). The nuclear movements were not as pronounced as true meiotic horsetail movements, but they would not be expected to be, as some of the other proteins required for meiotic horsetail movements, including cytoplasmic dynein, are specifically expressed only in meiosis. So although this 'pseudo-horsetail' movement might perhaps best be considered primarily as an indicator of the new microtubule organization, the ectopic expression experiment confirms that mcp6/hrs1p is likely to play a major role in consolidating microtubule minus ends to the spindle pole body, and suggests that mcp6/hrs1p can do this largely without any other meiosis-specific proteins.

But how is this achieved mechanistically? Is mcp6/hrs1p a new type of microtubule nucleator? Does it bind or capture microtubule minus ends? Does it interact with other proteins at the spindle pole body? Localization studies of ectopically expressed hrs1p-GFP showed it to be not only at the spindle pole body but also at another fission yeast microtubule-organizing center, the cell division site [15,16], suggesting that mcp6/hrs1p might specifically interact either with microtubule minus ends or with proteins directly involved in microtubule nucleation [2].

Tanaka *et al*. [2] tried to identify mcp6/hrs1p-interacting proteins by testing likely candidates. Interestingly, in co-immunoprecipitation experiments in vegetative cells overexpressing hrs1p, they were able to show association of mcp6/hrs1p with the γ-tubulin complex component alp4p and also with mcp6/hrs1p itself, and these interactions also occurred in (budding) yeast two-hybrid experiments. In further experiments they also showed that mcp6/hrs1p interacts with mto1p, the γ-tubulin complex-recruiting protein. This is particularly interesting because the spindle pole body localization of ectopically expressed mcp6/hrs1p in vegetative cells, as well as its ability to centralize microtubule organization, appears to be dependent on mto1p [2].

Is it now possible to formulate a specific model of how mcp6/hrs1p functions to organize the microtubule organizing centers of fission yeast? A provisional model, proposed by Tanaka *et al*. [2], roughly follows these lines. First, mto1p at the spindle pole body nucleates microtubules at the horsetail stage, by recruiting γ-tubulin complexes to the spindle pole body. After nucleation, a given microtubule, with its associated γ-tubulin complex, might then be prone to release from the spindle-pole-body-associated mto1p, but this would normally be prevented by a tethering function provided by hrs1p, which could bind simultaneously to mto1p on one hand and the γ-tubulin complex protein alp4p on the other. As mcp6/hrs1p also associates with itself, it is not difficult to imagine that this could generate a larger multimeric structure that further 'glues' the microtubule minus ends in place at the spindle pole body.

Even at this level of specificity, however, additional data are probably needed to firm up the

model, in particular with regard to the role of mto1p. Tanaka *et al*. [2] suggest that, like mcp6/hrs1p, mto1p is probably important for horsetail microtubule organization in meiosis, but loss of mto1p can lead, either directly or indirectly, to a wide range of defects in microtubule behavior in vegetative cells, affecting not only microtubule distribution but also microtuble dynamics and possibly microtubule-based transport [9,11,12].

In this regard, the ectopic vegetative cell expression system, in which fragments and/or mutant forms of mcp6/hrs1p can be assayed simultaneously for microtubule-organizing function and for protein–protein interactions, will undoubtedly serve as a very useful 'test tube' for probing the functional importance of the mcp6/hrs1p–mto1p interaction, as well as how interactions of mcp6/hrs1p with itself and the γ-tubulin complex can build up a new type of microtubule organizing center in fission yeast.

References

1. Saito, T.T., Tougan, T., Okuzaki, D., Kasama, T., and Nojima, H. (2005). Mcp6, a meiosis-specific coiled-coil protein of Schizosaccharomyces pombe, localizes to the spindle pole body and is required for horsetail movement and recombination. J. Cell Sci. *118*, 447–459.
2. Tanaka, K., Kohda, T., Yamashita, A., Nonaka, N., and Yamamoto, A. (2005). Hrs1p on the meiotic SPB organises astral microtubule arrays for oscillatory nuclear movement. Curr. Biol. *15*, this issue.
3. Chikashige, Y., Ding, D.Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M., and Hiraoka, Y. (1994). Telomere-led premeiotic chromosome movement in fission yeast. Science *264*, 270–273.
4. Ding, D.Q., Chikashige, Y., Haraguchi, T., and Hiraoka, Y. (1998). Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells. J. Cell Sci. *111*, 701–712.
5. Yamamoto, A., West, R.R., McIntosh, J.R., and Hiraoka, Y. (1999). A cytoplasmic dynein heavy chain is required for oscillatory nuclear movement of meiotic prophase and efficient meiotic recombination in fission yeast. J. Cell Biol. *145*, 1233–1249.
6. Chikashige, Y., Ding, D.Q., Imai, Y., Yamamoto, M., Haraguchi, T., and Hiraoka, Y. (1997). Meiotic nuclear reorganization: switching the position of centromeres and telomeres in the fission yeast Schizosaccharomyces pombe. EMBO J. *16*, 193–202.
7. Job, D., Valiron, O., and Oakley, B. (2003). Microtubule nucleation. Curr. Opin. Cell Biol. *15*, 111–117.
8. Hagan, I.M. (1998). The fission yeast microtubule cytoskeleton. J. Cell Sci. *111*, 1603–1612.
9. Sawin, K.E., Lourenco, P.C., and Snaith, H.A. (2004). Microtubule nucleation at non-spindle pole body microtubule-organizing centers requires fission yeast centrosomin-related protein mod20p. Curr. Biol. *14*, 763–775.
10. Janson, M.E., Setty, T.G., Paoletti, A., and Tran, P.T. (2005). Efficient formation of bipolar microtubule bundles requires microtubule-bound gamma-tubulin complexes. J. Cell Biol. *169*, 297–308.
11. Zimmerman, S., and Chang, F. (2005). Effects of {gamma}-tubulin complex proteins on microtubule nucleation and catastrophe in fission yeast. Mol. Biol. Cell *16*, 2719–2733.
12. Venkatram, S., Tasto, J.J., Feoktistova, A., Jennings, J.L., Link, A.J., and Gould, K.L. (2004). Identification and characterization of two novel proteins affecting fission yeast gamma-tubulin complex function. Mol. Biol. Cell *15*, 2287–2301.
13. Horio, T., Uzawa, S., Jung, M.K., Oakley, B.R., Tanaka, K., and Yanagida, M. (1991). The fission yeast gamma-tubulin is essential for mitosis and is localized at microtubule organizing centers. J. Cell Sci. *99*, 693–700.
14. Vardy, L., and Toda, T. (2000). The fission yeast gamma-tubulin complex is required in G(1) phase and is a component of the spindle assembly checkpoint. EMBO J. *19*, 6098–6111.
15. Heitz, M.J., Petersen, J., Valovin, S., and Hagan, I.M. (2001). MTOC formation during mitotic exit in fission yeast. J. Cell Sci. *114*, 4521–4532.
16. Zimmerman, S., Tran, P.T., Daga, R.R., Niwa, O., and Chang, F. (2004). Rsp1p, a J domain protein required for disassembly and assembly of microtubule organizing centers during the fission yeast cell cycle. Dev. Cell *6*, 497–509.

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RNA Turnover: Unexpected Consequences of Being Tailed

In eukaryotic cells, the 3′ poly(A) tails found on mRNA influence their stability and translation. The discovery of a second nuclear poly(A) polymerase complex has fueled a series of reports defining a new and unexpected role for 3′ end poly(A) tails in the nuclear surveillance and turnover of noncoding RNAs and intergenic mRNAs of unknown function.

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The life cycle of RNA begins with transcription and ends upon degradation; in between are several processing steps that are required for maturation and function. One aspect of RNA maturation is 3′ end processing, which for most RNA occurs through endonucleolytic cleavage, and/or exonucleolytic digestion, and subsequent addition of polyadenylate (poly(A)) tails to mRNA and CCA to tRNA, or no addition in the case of rRNA, snRNA and snoRNAs. The synthesis of mRNA 3′ poly(A) tails is carried out by a conventional nuclear localized poly(A) polymerase, and the functions of poly(A) tails are to stabilize, facilitate localization and enhance translation of mRNAs.

Recent studies have confirmed the identity of a second nuclear poly(A) polymerase in yeast that is evolutionarily conserved throughout eukaryotes. Genetic and biochemical characterization of this poly(A) polymerase, Trf4p, and its associated factors [1,2] establishes a new function for poly(A) tails in targeting the degradation of aberrant non-coding RNAs — tRNA, rRNA and snRNA — and intergenic mRNAs of unknown function [3].

The first poly(A) polymerase enzymatic activity, Pap1, was identified in *Escherichia coli*, but the identification of poly(A)$^+$ RNAs in bacteria lagged behind this discovery. As it turns out, a small percentage of bacterial mRNA is polyadenylated by bacterial Pap1, and in some cases polyadenylation is associated with increased mRNA turnover. Noncoding RNAs can also be polyadenylated in *E. coli*, and the presence of the poly(A) tail on noncoding RNAs has been shown to control the expression of a