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Kinetochore asymmetry defines a single yeast lineage

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Asymmetric cell division is of fundamental importance in biology as it allows for the establishment of separate cell lineages during the development of multicellular organisms. Although microbial systems, including the yeast Saccharomyces cerevisiae, are excellent models of asymmetric cell division, this phenotype occurs in all cell divisions; consequently, models of lineage-specific segregation patterns in these systems do not exist. Here, we report the first example of lineage-specific asymmetric division in yeast. We used fluorescent tags to show that components of the yeast kinetochore, the protein complex that anchors chromosomes to the mitotic spindle, divide asymmetrically in a single postmitotic lineage. This phenotype is not seen in vegetatively dividing haploid or diploid cells. This kinetochore asymmetry suggests a mechanism for the selective segregation of sister centromeres to daughter cells to establish different cell lineages or fates. These results provide a mechanistic link between lineage-defining asymmetry of metazoan cells to unicellular eukaryotes.

Results

Method to Track Nonencoded Protein in Yeast. We reasoned that if a unique lineage does exist in yeast, it would be established during the emergence of a new strain. Under normal circumstances there are only 2 moments in the yeast life cycle when a new strain is created: when 2 haploid yeast cells mate to create a new diploid and during meiosis when a diploid cell produces 4 new haploid spores. We decided to study meiosis, because this is a likely instance in which molecular programs would be reset within the nucleus. In addition, the transition from diploid to haploid allowed us to develop a simple method to follow the segregation of protein without having to consider the confounding effects of transcription. In a diploid strain, we tagged the protein encoded by 1 allele of a gene with yellow fluorescent protein (YFP) and the other allele with cyan fluorescent protein (CFP). We next induced this diploid strain to enter meiosis and produce 4 haploid spores, each of which inherits only one of the tagged alleles, but both of the tagged proteins. In this way, we were able to observe the nonencoded protein inherited from the original diploid cell as it segregated from the spore to its daughter cells (Fig. 1). Differential levels of transcription in progeny cells could confound our results, but our method of quantitating nonencoded protein avoids this problem.

Asymmetry of Mtw1. We first examined MTW1, a homolog of the mammalian MIS12 gene, whose protein forms part of the conserved Mtw1/MIND kinetochore complex that promotes biorientation of the mitotic spindle (15, 16). Elegant studies have shown that Mtw1 protein levels can be assessed using fluorescent intensity of tagged protein (17). Using time-lapse fluorescence microscopy, we followed individual spores as they divided and were able to track the segregation of both nonencoded and total Mtw1 protein that localized to the kinetochore.

For each spore, we first quantitated the total amount of both yellow (YFP) and cyan (CFP)-tagged Mtw1 protein in the kinetochore, as the spore went through 3 rounds of cell division (Figs. 2 and 3 and Fig. S2). At the first division, a spore divides to give a mother (m) and bud (b) (Figs. 1 and 2). In the second round of division, each cell divides again into mother and bud: mm, mb (descended from the mother) and bm, bb (descended from the bud). In the third round of division, each of the 4 cells divides again into mother and bud: mmm, mbmb, etc. Because all of the yeast kinetochores colocalize to a single locus in the nucleus, the tagged Mtw1 protein is visualized as a single fluorescent focus (actually 2 foci before mitosis). It was this fluorescence that was used to quantify the levels of the tagged

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Fig. 1. A diagram of the double-fluorescent tag method. Two haploid cells containing a given gene fused to either one of CFP (blue) or YFP (yellow) fluorescent tags were created. These 2 haploids were mated together to form a diploid that contained the gene in question, tagged with heterozygous fluorescent markers (CFP/YFP, shown as green). After sporulation, the individual haploid spores only contained either the CFP or YFP tagged gene, but inherited both CFP and YFP fluorescent protein from the parental diploid. Because the spores divided to produce successive generations of progeny, the protein that is no longer genetically encoded was diluted away. In this case, we illustrate a CFP spore that loses its inherited YFP protein (i.e., becomes more blue) in successive generations.

kinetochore protein immediately after cytokinesis. The ratio of both nonencoded and total protein in the mother versus the bud (m/b) was calculated for 3 rounds of division to assess potential asymmetry.

Through this analysis we found that the kinetochore of the mother cells contains more Mtw1 protein than the bud, but only in the mother cell lineage directly descended from the spore. In the first division, the kinetochore of the m cell contains over twice as much nonencoded Mtw1 as that of the b cell, on average (m/b ratio of 2.1) (Fig. 2). In the second round of division, Mtw1 segregates asymmetrically from the m cell to the new mother (mm, with a m/b ratio of 2.3) but segregates symmetrically from the b cell to its daughters (m/b ratio of 0.9). In the third division, Mtw1 segregates preferentially from the mm cell to the third generation mother cell (mmm, with a m/b ratio of 2.5) while segregating evenly in all other divisions (m/b ratios of 1.1, 1.2 and 1.5). The mean m/b ratio of nonencoded Mtw1 segregation in the mother lineage is 2.2 (SEM = 0.22, n = 85), which is significantly different (P = 8.8 × 10^-6 using a 2-tailed t test with unequal variance) from that of the nonmother lineage (mean m/b ratio of 1.1, SEM = 0.07, n = 46; see Table S1).

This asymmetry is not restricted to the nonencoded protein; when examining total Mtw1 protein (which includes both encoded and nonencoded) at the kinetochore in both the spores and their descendants, the same asymmetric segregation pattern is observed (ratios shown in parentheses in Fig. 2). By distinguishing nonencoded from total protein at the kinetochore, we show that this asymmetric phenotype is not due to mother-specific overexpression of the MTW1 gene in this lineage, but instead can be attributed to differential protein segregation. Although not all mother lineage divisions show asymmetric Mtw1 segregation, the frequency of asymmetric division in the mother lineage is much higher than the other lineages, summarized in Fig. S3. We note that the mother-lineage divisions that do not show Mtw1 asymmetry, instead segregate symmetrically (mean m/b ratio = 1.1). Because it has been found that there is an intrinsic asymmetry in the 4 spores of a tetrad (18), it is possible that not all spores in a tetrad show lineage-specific asymmetry. However, we cannot easily test this hypothesis because we are unable to analyze all 4 products of a single tetrad.

Finally, we examined the segregation of total Mtw1 protein at the kinetochore in vegetatively growing haploid and diploid cells and found that it is symmetrical (m/b ratios of 1.2) irrespective of whether the protein was tagged with either YFP or CFP (Fig. 2). Thus, the asymmetric Mtw1 segregation phenotype is specific to the postmeiotic mother lineage.

Symmetric Division of Histones. Next, we tested whether the asymmetric segregation pattern of Mtw1 that was observed could be explained by nonspecific asymmetric segregation of all nuclear proteins in this pedigree of cells. It is known that, in yeast, damaged protein preferentially localizes to mother cells (19) and it is possible that this phenotype is more pronounced in postmeiotic cells than in haploid or diploid cells. It is also possible that the asymmetric Mtw1 phenotype is caused by some unforeseen postmeiotic effector caused by the fluorescent protein tags. To test these hypotheses, we repeated our experiments by differentially tagging the alleles that encode the H2A histone protein, Hta1. A diploid strain containing both HTA1-YFP and HTA1-CFP (W7912) was sporulated and the amounts of both nonencoded and total Hta1 in the nucleus of individual spores and their progeny were quantitated (Fig. 4). Hta1 protein (either nonencoded or total) segregated equally to the mother and bud at the first, second and third postmeiotic divisions (ratio of 0.96, 0.89 and 0.84 respectively, Table 1). The average m/b ratio for all divisions of the mother lineage (mean m/b ratio = 0.90) is not significantly different from those of the other lineages (mean m/b ratio = 0.88). These data indicate that the asymmetric segregation of Mtw1 is not a general feature of postmeiotic nuclear proteins in yeast. Additionally, two other proteins, TetR (the tetracycline repressor) and Rad52 (a central DNA repair protein), segregate equivalently in both mother and non-mother lineages (SI Methods).

Asymmetry of 3 Other Kinetochore Proteins. To explore whether the postmeiotic asymmetric segregation was unique to Mtw1, we examined 3 other kinetochore proteins, Ask1, Ndc10 and Ctf19. These proteins are each part of separate kinetochore complexes associated with either the centromeric DNA (Ndc10 and Ctf19) or the spindle microtubules (Ask1). We chose Ndc10 in partic-
ular because it is required to localize Mtw1 to the kinetochore (20). We sporulated 3 diploid strains containing differentially
tagged Ask1, Ndc10 or Ctf19 (W7908, W7910 and W7909
respectively) and observed the spores through subsequent divi-
sions. Like Mtw1, the nonencoded protein at the kinetochore
segregates asymmetrically only in the pedigree of mother cells
descended directly from the spore and not in the other lineages
(Table 1 and Fig. S2). As before, total protein in the kinetochore
segregates asymmetrically in the same asymmetric pattern as nonencoded
protein. We saw no asymmetric segregation of these 3 proteins
in either vegetatively growing haploid or diploid cells (Table 1).
Consequently, the lineage-specific asymmetry phenotype ap-
pears to be a feature of the whole kinetochore and is not a unique
feature of the Mtw1 protein.

Discussion
We have identified a unique pattern of asymmetric protein
segregation in a single postmeiotic pedigree of S. cerevisiae,
deﬁned by the haploid spore and the mother lineage descended
from it. Mother cells derived from a bud do not show this
phenotype, nor do buds themselves. To the best of our knowl-
dge, this is the ﬁrst report of a phenotype in microorganisms
that is passed down within, and deﬁnes, 1 single lineage of cells
in a population (Fig. S1).

We ﬁnd that 4 distinct kinetochore proteins—Ndc10, Ctf19,
Mtw1, and Ask1—show the same asymmetric phenotype after
meiosis. Ndc10, a member of the CBF3 complex, is part of the
centromere-bound inner kinetochore (21). Ctf19 and Mtw1 are
part of the COMA and MIND complexes respectively, which
appear to bridge the inner and outer kinetochore (22); Ask1, a
member of the DAM/DASH complex, is part of the outer
kinetochore and interacts with the spindle microtubules (23).
Because these 4 complexes span the kinetochore, it seems likely
that the postmeiotic asymmetry is a general feature of the whole
kinetochore. It is important to note that our results do not
address the issue of whether or not the kinetochore complex
to itself is disassembled during S phase (17, 24, 25).

The function of the kinetochore asymmetry described here is
not yet known. It is possible that this asymmetry is linked to that
of the yeast spindle pole body, which is the yeast microtubule-
organizing center (centrosome) that separates the kinetochores
during cell division. The spindle pole body (SPB) proteins in

Fig. 3. A spore derived from the diploid strain W7247, MTW1-YFP/MTW1-CFP, is shown dividing 3 times. The images are arranged as in Fig. 2. Each image shows a differential interference contrast (DIC) image (Upper) and below a fluorescent YFP image (YFP, the white scale bar is 2.5 μm). Right images in each example are contrast enhanced to illustrate the (a)symmetry of each division – the kinetochore foci are not located inside the dashed circle. For clarity, only the encoded protein is shown, in this case YFP. The quantitative analysis of the fluorescence levels in this lineage is summarized in Dataset S1 (Mtw1 spore AM). More examples or Mtw1 asymmetry are provided in the Fig. S2.

Fig. 4. A spore derived from diploid strain W7912, HTA1-YFP/HTA1-CFP, is shown dividing 3 times; the images are arranged as in Fig. 3. The encoded CFP protein is shown, artificially colored to 480 nm. (Scale bar: 2.5 μm.) The dashed circles indicate the position of the nucleus in the contrast-enhanced images. The quantitative analysis of this lineage can be found in Dataset S1 (Hta1 spore 1-2).
yeast divide asymmetrically (26), the old spindle pole segregates to the bud, although this phenotype is not confined to a single lineage. This SPB asymmetric division is controlled by the cyclin dependent kinase Cln4-Cdc28 (27). In addition, a unique pattern of sister chromatid segregation to daughter cells immediately after mitosis, a tempting hypothesis is that nonrandom chromatid segregation, thereby preventing stem cells from inheriting and accumulating DNA replication errors specifically by the asymmetrically dividing kinetochore, allowing cosegregation of centromere strands on the basis of their orientation. Our prediction is that, in this case, an unusually low rate of dicentric chromosome breakage would be observed in the first division after meiosis and subsequently in the postmeiotic mother lineage.

Immortal chromosome strands in yeast seem unlikely, however, because of the occurrence of sister chromatid exchange (SCE). After DNA replication, homologous recombination between the 2 sister chromatids before mitosis would negate strand asymmetry because the recombined chromosome arms would be a mix of old and new DNA strands. Because SCE is estimated to be a frequent event in mitotic yeast (36, 37), it is unlikely that a whole chromosome strand would persist intact in yeast for multiple generations. One possibility is that SCE is inhibited in the postmeiotic yeast lineage to allow a chromatic DNA strand to remain distinct from its sister. To explore whether recombination is specifically inhibited in the postmeiotic mother lineage, we tested if a central homologous recombination protein, Rad52, forms foci in spores and their immediate descendents. Rad52 foci are indicative of double strand break repair by homologous recombination (38). An absence of these foci in the postmeiotic lineage suggests but does not prove an absence of SCE. However, we find that Rad52 foci are seen in both dividing spores and in the mother cells at the next division (“m” in Figs. 1 and 2; SI Methods), suggesting that recombination is active in this lineage of cells. Nevertheless, Rad52 foci may not be indicative of SCE and, indeed, SCE may be inhibited in the dividing spores.

An alternative view to “immortal strands” is that the kinetochore asymmetry found in the postmeiotic cells preferentially segregates only centromeric DNA to a single lineage. Because budding yeast centromere sequences are unidirectional, they have the potential to direct strand asymmetry. This strand distinction could be provided either by the centromere sequences themselves or by the direction of DNA synthesis through the centromere region. This latter idea has been proposed as a means to drive nonrandom chromatid segregation (11) and recent experiments demonstrate that such a mechanism exists in Escherichia coli (39). If a centromeric DNA or protein “mark” is established during meiosis, it could be bound or retained specifically by the asymmetrically dividing kinetochore, allowing cosegregation of centromere strands on the basis of their orientation (Fig. S4). For example, if kinetochores bind preferentially to a single strand of the centromere, this could facilitate strand discrimination. It should be possible to test this hypothesis using a dicentric chromosome in which both centromeres are aligned in the same orientation. Our prediction is that, in this case, an unusually low rate of dicentric chromosome breakage would be observed in the first division after meiosis and subsequently in the postmeiotic mother lineage.

Table 1. Segregation of non-encoded (and total) protein during post-meiotic cell divisions

<table>
<thead>
<tr>
<th></th>
<th>Hta1</th>
<th>Ask1</th>
<th>Ndc10</th>
<th>Ctf19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haploid</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.92</td>
<td>1.2</td>
<td>1.15</td>
<td>1.2</td>
</tr>
<tr>
<td>Diploid</td>
<td>0.88</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>(0.91)</td>
<td>(1.0)</td>
<td>(1.1)</td>
<td>(1.1)</td>
</tr>
</tbody>
</table>

The mean m/b ratios of histone H2A (Hta1) and three kinetochore proteins (Ask1, Ndc10, Ctf19) are shown for post-meiotic cell divisions originating with the spore (0), as shown in Figure 2. The mean m/b ratios are shown both for non-encoded and, in parentheses, total protein. The asymmetric m/b ratios for the mother lineage are shaded and in bold. T-tests of the m/b ratios for nonencoded protein comparing the mother lineage against the other lineages are significantly different (P values of 4 x 10^-4, 2 x 10^-4 and 1 x 10^-3 for Ask1, Ndc10 and Ctf19 respectively, two tailed, unequal variance). However, the segregation of histone Hta1 is equivalent in the mother and non-mother lineages (Hta1 gives a p values of 0.98, two tailed, unequal variance). Additionally, the mean m/b ratio of both haploid and diploid cells for Ask1, Ndc10 and Ctf19 are ~1 (all cell divisions are combined). These values are in parentheses since they are measurements of total protein; n/t indicates “not tested”. The raw quantitation data are in Dataset S1 together with a table of summary statistics, Table S1.
It is possible that kinetochore asymmetry is important for the evolution of centromeres. Although the kinetochore is highly conserved, centromere sequences and their DNA binding proteins appear to be diverging rapidly in metazoans (13, 40). It is suggested that the meiotic drive, which selects for specific products of meiosis, is responsible for this process during metazoan female meiosis (14, 40). Because female meiosis results in a single oocyte, as the other polar bodies degenerate, there is the potential to preferentially segregate specific alleles into the egg. Such meiotic drive is suggested to underlie the rapid divergence of centromeres and, by extension, drive speciation (41). Interestingly, recent sequencing studies from Saccharomyces para-
doxus, a close relative of S. cerevisiae, show that the CDE II centromere sequences are undergoing unusually rapid divergence (42). This observation suggests that, like the centromeres of metazoan yeast, centromeres are also subject to drive. However, unlike female meioses, all 4 of the yeast meiotic products are viable. Hence, drive would need to be accomplished in another way. For example, if parental centromere strands are confined to a single lineage in postmeiotic yeast, then perhaps the kinetochore asymmetry described here enables a “postmeiotic drive” to select for retention of evolving centromere sequences in the mother lineage. Centromeres whose sequences bind optimally to the coevolving kinetochore proteins would be preferentially retained in a single lineage derived from the spore, whereas evolutionarily less fit “old” centromeres would be distributed equally throughout the rest of the population.

Yeast has proven to be a useful model for a wide range of fundamental processes of multicellular organisms. Our results show that an additional unexpected phenotype—a stem cell-like, lineage-specific asymmetric cell division—is present and can be studied in this genetically tractable microorganism. Indeed, it should be possible to screen the yeast gene disruption library to identify the genes responsible for this lineage-specific asymmetry.

Materials and Methods

Strains and Media. The yeast strains used in this study are listed in Table S2. The sporulation medium consisted of 2.5 g/L yeast extract, 1 g/L glucose, 20 g/L potassium acetate, 1.5% w/v vol. bacto-agar, supplemented with 75 mg/L adenine, L-histidine, L-lysine, L-methionine, L-tryptophan and uracil adjusted to pH 7.0. Synthetic complete medium plus adenine (SC + Ad) consists of 1.72 g/L yeast nitrogen base, 5 g/L ammonium sulfate, 20 g/L glucose and potassium acetate, 1.5% wt/vol bacto-agar, supplemented with 75 mg/L ad-

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