light resetting pathway becomes functional or, alternatively, that an oscillating clock cannot be entirely initiated de novo. In mammals, the circadian clock located in the suprachiasmatic nuclei (SCN) starts oscillating during late fetal life, and expression of the bimomo et al. gene clock Per2 was observed in the SCN just before or at birth (4, 9, 25). However, the discovery of circadian oscillators in peripheral organs and in tissue culture cells indicates that circadian clock function does not necessarily require the completion of long and complex developmental processes such as vertebrate brain development (10, 17, 26). In Drosophila, Per is expressed throughout development, and the onset of circadian behavioral rhythm is light independent; however, its synchronization requires a light-entraining signal (27). Our data show that developing zebrafish embryos inherit maternal circadian clock gene products and perhaps also the phase of their clock.

References

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20. F. Delaunay, C. This, O. Marchand, V. Lauder, B. This, data not shown.
28. C. This and B. Thisse, unpublished data.
30. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
31. The AB zebrafish strain was kept at 28°C in a LD 14:10 cycle with lights on at 0900 hours (ZT 0). For most experiments, adults were crossed overnight, resulting in spawning and fertilization at about ZT 0, the next morning. Embryos were raised at 28°C in petri dishes containing 0.003% phenolthiourea to prevent pigmentation. Lighting conditions were either LD 14:10, LD 14:10 that was shifted 8 hours forward, LD 8:8, LL, or DD. Delayed development was obtained by keeping embryos at 23°C in LL. For desynchronizing development from the light/dark cycle, adults in LD 14:10 were crossed at ZT 3, and embryos fertilized at ZT 12 the same day were kept in LL. Embryos were fixed for 12 to 16 hours in 4% paraformaldehyde/phosphate–buffered saline at 4-hour intervals and stored in methanol. Unfertilized oocytes were obtained by squeezing mature females under anesthesia at 4-hour intervals.

Requirement of the Spindle Checkpoint for Proper Chromosome Segregation in Budding Yeast Meiosis

Marion A. Shonn,1* Robert McCarroll,2 Andrew W. Murray1,3*

The spindle checkpoint was characterized in meiosis of budding yeast. In the absence of the checkpoint, the frequency of meiosis I missegregation increased with increasing chromosome length, reaching 19% for the longest chromosome. Meiosis I nondisjunction in spindle checkpoint mutants could be prevented by delaying the onset of anaphase. In a recombination-defective mutant (spa1Δ), the checkpoint delays the biochemical events of anaphase I, suggesting that chromosomes that are attached to microtubules but are not under tension can activate the spindle checkpoint. Spindle checkpoint mutants reduce the accuracy of chromosome segregation in meiosis I much more than that in meiosis II, suggesting that checkpoint defects may contribute to Down syndrome.

Meiosis I differs from mitosis and meiosis II. In meiosis I, the two sister chromatids remain attached to each other and move to one spindle pole, segregating away from the paired centromeres of the homologous chromosome (Fig. 1A) (1). We investigated the meiotic role of the spindle checkpoint, which keeps cells with misaligned chromosomes from starting anaphase by preventing the activation of the anaphase-promoting complex (APC, also known as the cyclosome) (2, 3). The spindle checkpoint detects kinetochore microtubules that are not attached to microtubules (4, 5) and the absence of tension at kinetochores that are attached to microtubules (6). Mutations in the budding yeast MAD1, MAD2, MAD3, BUB1, BUB3, and MPS1 genes eliminate the spindle checkpoint (7, 8).

To follow meiotic chromosome segregation, we targeted green fluorescent protein (GFP) to bind a specific chromosome. Tan-
dem repeats of the bacterial lactose operator (LacO) sequence were integrated close to the centromere and seen by binding of a protein fusion between the lactose repressor and GFP (GFP-LacI) (9, 10). By marking both homologs of a chromosome, we can determine the pattern of chromosome segregation in meiosis I. If chromosome segregation was normal in both meiotic divisions, all four spores inherit a single copy of the marked locus (Fig. 1A). Meiosis I nondisjunction, the segregation of both homologs to the same spindle pole, produces two spores that lack the marked locus and two spores that have two copies (Fig. 1A).

We analyzed the meiotic segregation of GFP-marked chromosome IV in wild-type and mad2Δ cells (Fig. 1B). Chromosome IV nondisjoined in 19% of mad2Δ tetrads (Fig. 1C), showing that the spindle checkpoint is required for proper chromosome segregation in meiosis I. The frequency of nondisjunction in meiosis II was statistically indistinguishable between wild-type and mad2Δ cells (11). In mad2Δ diploids, the frequency of nondisjunction in meiosis I increased with increasing chromosome length (Fig. 1C) (12). The pattern of spore inviability confirmed that spindle checkpoint mutants suffer from nondisjunction in meiosis I. The mad1Δ and mad2Δ strains produced an excess of tetrads with two viable and two dead spores. Statistical analysis suggests that nondisjunction events are clustered rather than randomly distributed among all cells (Table 1).

How does the spindle checkpoint improve the fidelity of chromosome segregation in meiosis I? One possibility is that both members of some pairs of homologous chromosomes attach to the same spindle pole and that a checkpoint-dependent delay allows them to reorient and attach to opposite poles (Fig. 1A). This hypothesis predicts that anaphase would occur earlier in mad2Δ cells. Using the SK1 background, which gives the best synchrony of sporulation (13, 14), we could not detect a change in timing of meiosis I chromosome segregation or spindle breakdown in mad2Δ diploids (15). However, anaphase begins only 10 min earlier in checkpoint-defective mitotic cells (16), a change that we could not detect in the much less synchronous meiotic cell cycle. A second prediction is that imposing a metaphase delay should eliminate meiosis I nondisjunction in spindle checkpoint mutants. Weak expression of a nondegradable form of the anaphase inhibitor Pds1 (17) delayed the start of anaphase I and produced two-spored asci (18). After the metaphase I delay, the GFP-marked chromosome IV segregated correctly in both wild-type and mad2Δ cells (Fig. 1D).

Thus, artificially delaying anaphase of meiosis I can prevent nondisjunction in spindle checkpoint mutants. We believe that the formation of two-spored asci in cells expressing nondegradable Pds1 reflects a fixed interval between the formation of a

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**Table 1.** Spore viability in spindle checkpoint mutants. Distribution of spore viability in wild-type (WT), mad1Δ, and mad2Δ tetrads. Viability was assessed by dissecting tetrads. If nondisjunction events are not clustered, we can use the Poisson distribution and the frequency of tetrads with four viable spores to calculate the mean number of nondisjunction events per cell as 0.844. Another value of the same parameter, 1.35, was derived using the cytologically measured nondisjunction frequencies for several chromosomes (Fig. 1C). The fraction of four-spored tetrads predicted using this average nondisjunction frequency is significantly different from that observed ($\chi^2$ test, $P < 0.01$). Bold values differ markedly from those for the wild type.

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**Fig. 1.** The spindle checkpoint is required for proper chromosome segregation in meiosis I. (A) Chromosome segregation in wild-type cells (left) results in one GFP-marked LacO array (GFP/LacO) in each of four spores that can be detected by fluorescence microscopy. Nondisjunction in meiosis I (right) results in GFP/LacI in two of four spores. (B) Chromosome segregation was assessed in tetrads by fluorescence microscopy in wild-type and mad2Δ strains that have LacO arrays near the centromere of chromosome IV (9). (C) Chromosomes III, IV, VII, and VIII were marked with a LacO array integrated near the centromere (12). Chromosome segregation in wild-type and mad2Δ cells was assessed in tetrads by fluorescence microscopy. MI, meiosis I. (D) A metaphase delay rescues the chromosome segregation defect in mad2Δ. A nondegradable form of Pds1 (PDS1-ΔDB) was expressed from the copper-inducible CUP1 promoter (18). Cells sporulated in 100 μM CuSO4 arrest with a single short spindle. Cells sporulated in 5 μM CuSO4 accumulated short spindles, but completed a single meiotic division in which the segregation efficiency of chromosome IV was similar in wild type (WT) and mad2Δ. In the absence of copper, cells continued through a second division to form tetrads. Nondisjunction frequency in the absence of copper was determined in tetrads.
meiosis I spindle and spore formation. If anaphase of meiosis I is sufficiently delayed, spores will form before meiosis II occurs.

In mitosis, the spindle checkpoint arrests cells that lack a mitotic spindle. Does this checkpoint have a similar function in meiosis? We incubated wild-type cells in sporulation medium containing the microtubule poison benomyl (19). The cells arrested with a single mass of DNA and two closely spaced spindle pole bodies (Fig. 2, A and C). In contrast, benomyl-treated, checkpoint-deficient mad2Δ cells completed two meiotic divisions (Fig. 2B), although they suffered massive missegregation in both meiosis I and meiosis II (15).

We investigated how the linkage between homologous chromosomes regulates the spindle checkpoint. This linkage is created by reciprocal recombination and causes tension on the kinetochore-microtubule connections as they pull against each other. Tension is thought to signal to the checkpoint that the homologs are attached to opposite poles (6). The spo11Δ mutant completely abolishes meiotic recombination, preventing linkage of homologous chromosomes (20) and causing random segregation of GFP-marked homologs at meiosis I (15, 21). In insect spermatocytes, the absence of tension at the kinetochore can activate the spindle checkpoint in meiosis (6). However, because animal kinetochores have many microtubule binding sites, the effect of tension could be indirect, causing some sites to lose their microtubules and thus activate the checkpoint. In contrast, yeast kinetochores are captured by a single microtubule in mitotic cells (22), and data from meiotic spindles suggests that single microtubules attach to kinetochores in meiotic divisions as well (23). The absence of a mechanical link between homologs (spo11Δ) does not appear to affect microtubule attachment of a GFP-marked homolog in meiosis I (24), suggesting that experiments in yeast directly measure the effects of tension, as opposed to effects of destabilizing the link between kinetochores and microtubules.

The kinetochores of linked homologs pull against each other, thus preventing spindle elongation until the linkage dissolves (25, 26). To follow the state of the cell cycle machinery, we examined the destruction of Pds1 as a biochemical marker for the start of anaphase. In wild-type meiosis I, Pds1 behaved exactly as it does in mitosis: it was present in cells with a single short spindle (metaphase I) and absent from cells with a long spindle and two separated DNA masses (anaphase I), showing that the APC had been activated (Fig. 3A). Although DNA staining suggested that spo11Δ cells had completed anaphase of meiosis I, spindle elongation had occurred before the biochemical events that trigger chromosome separation: Pds1 was present in 77% of spo11Δ cells with a single short spindle (metaphase I) and absent from cells with a long spindle and two separated DNA masses (anaphase I), showing that the APC had been activated (Fig. 3A). Although DNA staining suggested that spo11Δ cells had completed anaphase of meiosis I, spindle elongation had occurred before the biochemical events that trigger chromosome separation: Pds1 was present in 77% of spo11Δ cells with a single short spindle (metaphase I) and absent from cells with a long spindle and two separated DNA masses (anaphase I), showing that the APC had been activated (Fig. 3A). Although DNA staining suggested that spo11Δ cells had completed anaphase of meiosis I, spindle elongation had occurred before the biochemical events that trigger chromosome separation: Pds1 was present in 77% of spo11Δ cells with a single short spindle (metaphase I) and absent from cells with a long spindle and two separated DNA masses (anaphase I), showing that the APC had been activated (Fig. 3A).
demonstrating that without a mechanical link between homologous chromosomes, the meiosis I spindle elongates even though the APC has not been activated, as do spindles in mitotic cells with unlinked sister chromatids (27, 28).

If the spindle checkpoint senses kinetochore tension, checkpoint components should be required to stabilize Pds1 in spo11Δ mutants. Pds1 had been destroyed in every spo11Δ mad2Δ cell that produced two DNA masses on a long meiosis I spindle, indicating that APC activation had occurred at the same time as spindle elongation (Fig. 3A). Furthermore, in spo11Δ mutants with an intact checkpoint, there were more cells with two DNA masses (suggesting a delay in completing meiosis I) (29), and the completion of meiosis II was delayed by about 1 hour compared to spo11Δ mad2Δ cells (Fig. 3B). Because chromosomes attached to and segregated to the spindle in elongating (31), the threshold required with increased maternal age and chromosomes whose recombination events are far from the centromere (35). These chromosomes apparently nondisjoin more often in older mothers because of age-dependent loss of a factor required for accurate segregation (36, 37). Our findings suggest that the spindle checkpoint is a candidate for this factor.

Does spindle elongation in spo11Δ cells reflect the absence of a connection between homologs? We restored centromere linkage to spo11Δ mutants by removing Spo13, a protein required to prevent sister kinetochores from separating at anaphase of meiosis I. In the spo11Δ spo13Δ double mutant, a single mitosis-like division occurs: the sister chromatids of the unrecombined chromosomes attach to and segregate to opposite poles (31). In the double mutant, 50% of the meiosis I spindles were short, indicating that sister kinetochores prevent the spindle from elongating (15), and long spindles were only seen in cells that lacked Pds1 (Fig. 3A). Thus, restoring the bipolar attachment of kinetochores made spindle elongation and chromosome segregation depend on APC activation.

We have shown that the budding yeast spindle checkpoint, which is largely dispensable in wild-type mitosis (7), plays a critical role in meiotic chromosome segregation. This difference may reflect the different chromosome linkages in mitosis and meiosis I. In meiosis, sister chromatid cohesion forces sister kinetochores to face opposite spindle poles. In meiosis I, homologs are linked at sites of recombination that can be far from the kinetochores, creating a floppy linkage (32–34). If the nearest recombination event is further from the centromere on long chromosomes, this idea may explain why long chromosomes preferentially nondisjoin in checkpoint-defective cells.

Our findings may be relevant to Down syndrome. This birth defect is mostly caused by nondisjunction of chromosome 21 in meiosis I and is correlated with increased maternal age and chromosomes whose recombination events are far from the centromere (35). These chromosomes apparently nondisjoin more often in older mothers because of age-dependent loss of a factor required for accurate segregation (36, 37). Our findings suggest that the spindle checkpoint is a candidate for this factor.