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35. Histology and immunocytochemistry were performed as described (*5*) using antibodies to glial fibrillary acidic protein (DAKO, Carpinteria, CA) or SOD1 (*5*, *25*). For restaining of sections after treatment with hemotoxylin and eosin (H&E), coverslips were removed and decolorized with 1% HCl in 70% ethanol before immunocytochemistry.
36. We are grateful to L. B. Corson for critical reading of the manuscript and to H. Hwang for her technical assistance. This work was supported by grants to D.W.C. from NIH (NS 27036) and the ALS Association. L.I.B. was supported by a postdoctoral fellowship from the Muscular Dystrophy Association. Salary support for D.W.C. is provided by the Ludwig Institute for Cancer Research. S.K. is supported by a Grant-in-Aid for Scientific Research (09680744) from the Ministry of Education, Science, Sports and Culture of Japan.

19 May 1998; accepted 28 July 1998

Regulation of Meiotic S Phase by Ime2 and a Clb5,6-Associated Kinase in *Saccharomyces cerevisiae*

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Cyclin-dependent kinase (Cdk) mutations that prevent entry into the mitotic cell cycle of budding yeast fail to block meiotic DNA replication, suggesting there may be fundamental differences between these pathways. However, S phase in meiosis was found to depend on the same B-type cyclins (Clb5 and Clb6) as it does in mitosis. Meiosis differs instead in the mechanism that controls removal of the Cdk inhibitor Sic1. Destruction of Sic1 and activation of a Clb5-dependent kinase in meiotic cells required the action of the meiosis-specific protein kinase Ime2, thereby coupling early meiotic gene expression to control of DNA replication for meiosis.

In eukaryotes, both mitotic chromosomal DNA replication (S phase) and chromosome segregation (M phase) are triggered by cyclin-dependent kinases (Cdks). In budding yeast, specific B-type cyclins activate the principal Cdk, Cdc28, to control entry into S phase. Late in G_1, a series of events leads to activation of the S-phase promoting factor (SPF), a complex of Cdc28 with either of two similar B-type cyclins, Clb5 and Clb6 (*1–3*). Before S phase, the Clb5,-6/Cdc28 kinase is maintained in an inactive state by its association with the CDK inhibitor (CDI) Sic1. Targeting of Sic1 for degradation requires activity of the Cdc28 kinase in conjunction with the G_1-specific cyclins Cln1 and Cln2 (*4–7*). In mutants lacking Clb5 and Clb6, S phase occurs but is delayed, because other B-type cyclins (*CLB1–4*) that arise later in the cell cycle can also fulfill this function (*8*).

In meiosis, the G_1- to S-phase transition is controlled differently. The temperature-sensitive CDK mutation that blocks mitotic S phase, *cdc28-4*, has no effect on meiotic S phase (*9*). Thus, Cdc28 might render mitotic DNA synthesis dependent on growth conditions, while being dispensable for meiotic S phase (*10*). One possibility is that the meiosis-specific kinase encoded by *IME2*, which has sequence similarity to Cdc28 and is required for meiotic S phase (*11–13*), replaces Cdc28 in an SPF-like function that is required in meiosis. To find out, we have reinvestigated genetic interactions that influence DNA replication in meiosis.

Yeast strains mutant for the G_1-specific cyclins *CLN1,-2,-3* are blocked in mitosis at G_1 (*14*, *15*) but form viable spores to the same extent as do wild-type (WT) cells (*16*, *17*). Likewise, mutations eliminating components of transcription factors for the *CLN1* and *CLN2* genes (*SBF* and *MBF*) cause mitotic G_1 arrest (*18*) but fail to block meiosis (*16*, *17*). These findings are consistent with a differential control of S phase between mitosis and meiosis, but the results of introducing mutations in *CLB5* and *CLB6* (*19*) are not. Although a strain deleted for *CLB6* is fully proficient in sporulation, a *clb5*Δ homozygote fails to sporulate (*16*) (Fig. 1C). Because different genetic backgrounds vary in sporulation efficiency, we also tested *clb5*Δ in two other strain backgrounds (W303 and YK) and obtained similar results (*16*, *19*). Meiotic S phase (*20*) in a *clb5*Δ homozygote was delayed about 3 hours relative to the WT (Fig. 1B) and meiotic progression was blocked either in G_2 (60% of cells) or in meiosis I (40% of cells) (Fig. 1, A and C). The delay of S phase did not result from delayed initiation of meiosis, for the increase in transcription of both *IME2* (an early meiotic gene) and *CLB6* occurred on schedule and to even higher levels than that in WT cells (Fig. 1D) (*20*). Assessment of intragenic recombination within the *HIS4* locus revealed no His^+ progeny (*21*), indicating either that *CLB5* is required for recombination or that recombinants are inviable. We conclude that *CLB5* function is required for scheduled entry into S phase and that *clb5*Δ homozygotes, despite their capacity for extensive DNA replication, are unable to progress normally through subsequent stages of meiosis. These latter anomalies might reflect either qualitative defects in the newly replicated DNA or an additional requirement for *CLB5* function in the transition from G_2 to meiosis I. The finding that *CLB5* is also activated by the G_2-specific meiotic transcription factor Ndt80 (*22*) favors the latter explanation.

Because *CLB6* is functionally redundant with *CLB5* in mitosis (*1–3*), we also tested strains deleted for both genes (*16*). In both genetic backgrounds tested, sporulation failed to occur and DNA replication was impaired to a greater extent than in cells lacking *CLB5* alone (Fig. 1, B and C), which indicates that either *CLB5* or *CLB6* can act to promote meiotic DNA synthesis. Flow cytometric profiles showed late and incomplete DNA replication, and further progression through the meiotic pathway did not occur (Fig. 1, A and B) (*21*).

Because both the Clb5 and Clb6 cyclins and the Ime2 kinase are required for meiotic DNA replication (Fig. 1) (*11–13*), they might constitute a single complex that promotes the G_1-S transition in meiosis (Fig. 4B2). Alternatively, Ime2 might mediate entry into S phase indirectly by controlling removal of an inhibitor, such as Sic1 (Fig. 4B3). The target of this inhibitor would be an unspecified meiotic Cdk (designated X) that is activated by association with Clb5 and Clb6, and the role of Ime2 in meiosis would parallel that of Cln1,-2/Cdc28 in mitosis. In this case, deletion of *SIC1* might render *IME2* dispensable for DNA replication. Indeed, deletion of *SIC1* in *ime2*Δ cells restored DNA replication to the level and kinetics observed for the control *sic1*Δ strain (Fig. 2). Thus, Sic1 acts by blocking entry into meiotic S phase, and Ime2, instead of promoting entry directly, may con-

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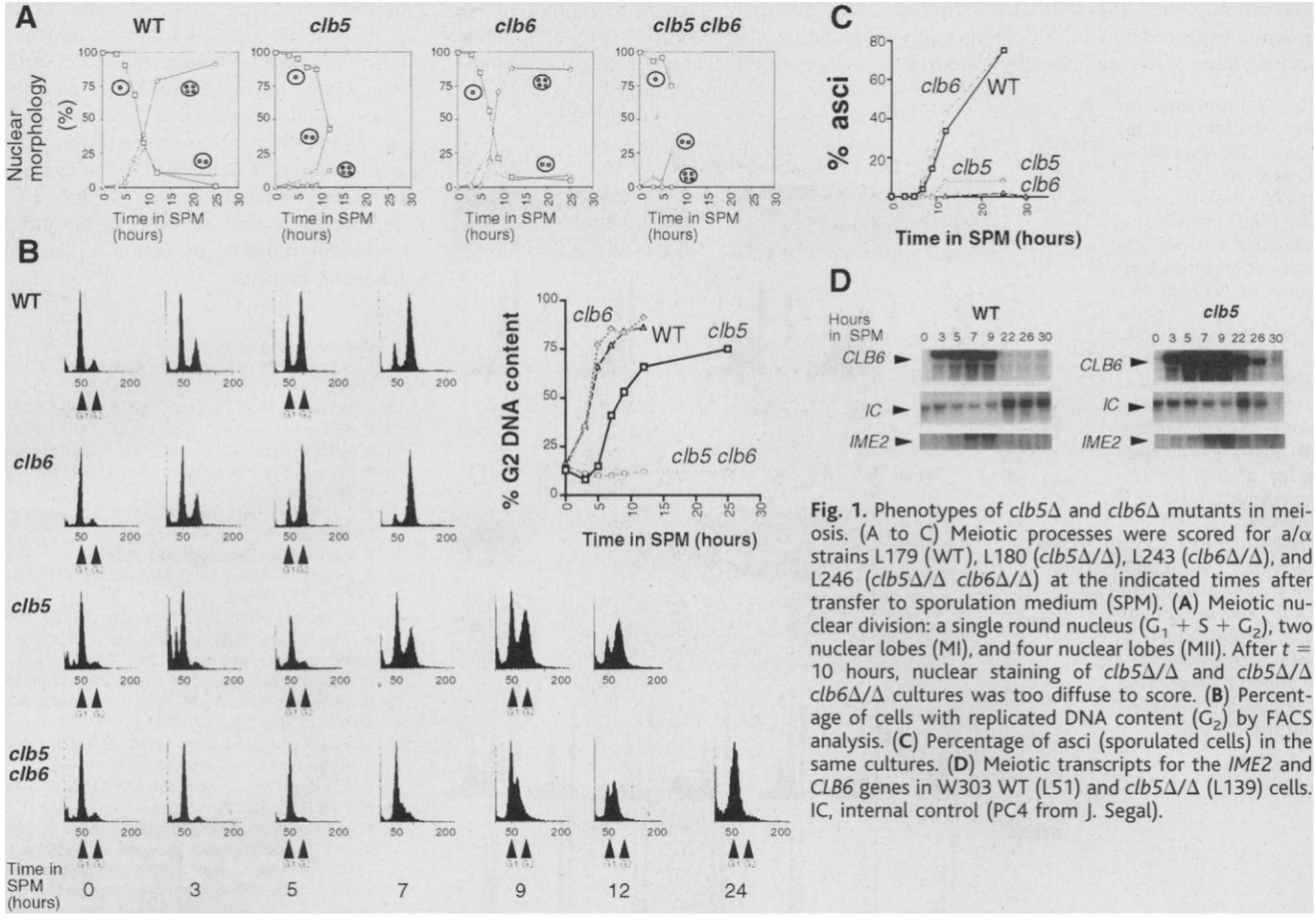

Fig. 1. Phenotypes of *clb5*Δ and *clb6*Δ mutants in meiosis. (A to C) Meiotic processes were scored for a/α strains L179 (WT), L180 (*clb5*Δ/Δ), L243 (*clb6*Δ/Δ), and L246 (*clb5*Δ/Δ *clb6*Δ/Δ) at the indicated times after transfer to sporulation medium (SPM). (**A**) Meiotic nuclear division: a single round nucleus (G_1 + S + G_2), two nuclear lobes (MI), and four nuclear lobes (MII). After t = 10 hours, nuclear staining of *clb5*Δ/Δ and *clb5*Δ/Δ *clb6*Δ/Δ cultures was too diffuse to score. (**B**) Percentage of cells with replicated DNA content (G_2) by FACS analysis. (**C**) Percentage of asci (sporulated cells) in the same cultures. (**D**) Meiotic transcripts for the *IME2* and *CLB6* genes in W303 WT (L51) and *clb5*Δ/Δ (L139) cells. IC, internal control (PC4 from J. Segal).

trol it indirectly by mediating destruction of the SPF inhibitor Sic1.

If Ime2 regulates the amount of Sic1, *ime2*Δ cells should accumulate Sic1 protein and Clb5-associated kinase should be absent from *ime2*Δ cells undergoing meiosis. Protein immunoblot analysis of the meiotic cells (*23*) demonstrated that Sic1 was lost near the time of S phase in WT cells but accumulated in *ime2*Δ cells under identical conditions (Fig. 3A). Furthermore, histone H1 kinase assays (*23*) on extracts of meiotic cells showed a transient increase in Clb5-associated kinase in WT cells, but not in *ime2*Δ cells, around the time of DNA replication (t = 6 to 10 hours; Fig. 3B). In *ime2*Δ cells, Sic1 persisted and Clb5 kinase activity was absent (Fig. 3, A and B). The presence of increased Clb5-associated kinase activity in the WT at late time points (up to 10 hours) might result from persistent *CLB5* transcription in G_2 (*22*), consistent with a possible role for this kinase in meiosis I.

We have shown here that meiotic SPF has two predominant characteristics expected for a cyclin B/Cdc28 complex—dependence on Clb5,-6 (Figs. 1 and 3B) and inhibition by Sic1 (Figs. 2 and 3A). In addition, we have ruled out Ime2 as an essential component of meiotic SPF (Fig. 2). Lacking a *cdc28* allele that is specifically defective in Clb5,-6 activation in vivo, we cannot exclude the possibility that enzyme X is Cdc28 itself. It has been shown that cdc2, the enzyme in *Schizosaccharomyces pombe* that is analogous to Cdc28, is required for meiotic S phase, but a relevant cyclin is unidentified (*24*). In vertebrates, Cdk functions needed for the G_1/S transition are performed by several Cdks (*25*) and meiosis-specific activities remain unresolved.

How might meiosis have evolved from a mitotic cycle? Because the precondition in yeast for mitosis (growth conditions) differs so markedly from that for meiosis

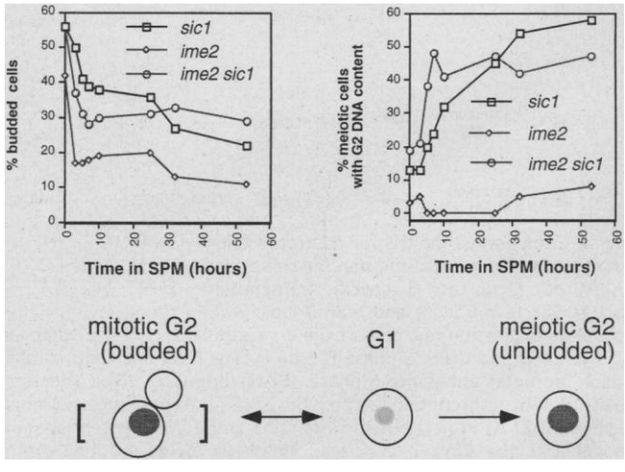

Fig. 2. Effect of *SIC1* deletion on meiotic DNA replication in an *ime2*Δ strain. FACS analysis of SK1 a/α strains bearing *sic1*Δ/Δ (L182), *ime2*Δ/Δ (L213), and *ime2*Δ/Δ *sic1*Δ/Δ (L234). Because many *sic1*Δ cells remain blocked in G_2 of mitosis after transfer to sporulation medium (*27*), the percentage of budded G_2 cells was subtracted from the total percentage of cells with replicated DNA to yield the percentage of meiotic cells with G_2 DNA content. Electron microscopy confirmed the presence of condensed chromosomes and other meiotic features in the unbudded cells of the *ime2*Δ/Δ *sic1*Δ/Δ culture (*21*).

(starvation), the *SBF/MBF* transcription program triggered by Cln3/Cdc28 for mitosis may have been supplanted by a meiosis-specific early transcription program triggered by *IME1* (*16, 26*) (Fig. 4, A and B). However, the demonstrated conservation in meiosis of roles for the same cyclins (Clb5,-6) and their inhibitor (Sic1) as in mitosis strongly implicates Clb5,-6/Cdk complexes in the control of meiotic S phase. This suggests that functions of the cyclin B/Cdk complex in controlling DNA replication were conserved in the evolution of meiosis, whereas the role of Cln1,-2/Cdk complexes in Sic1 removal in the mitotic cycle was replaced by action of the Ime2 kinase in meiosis.

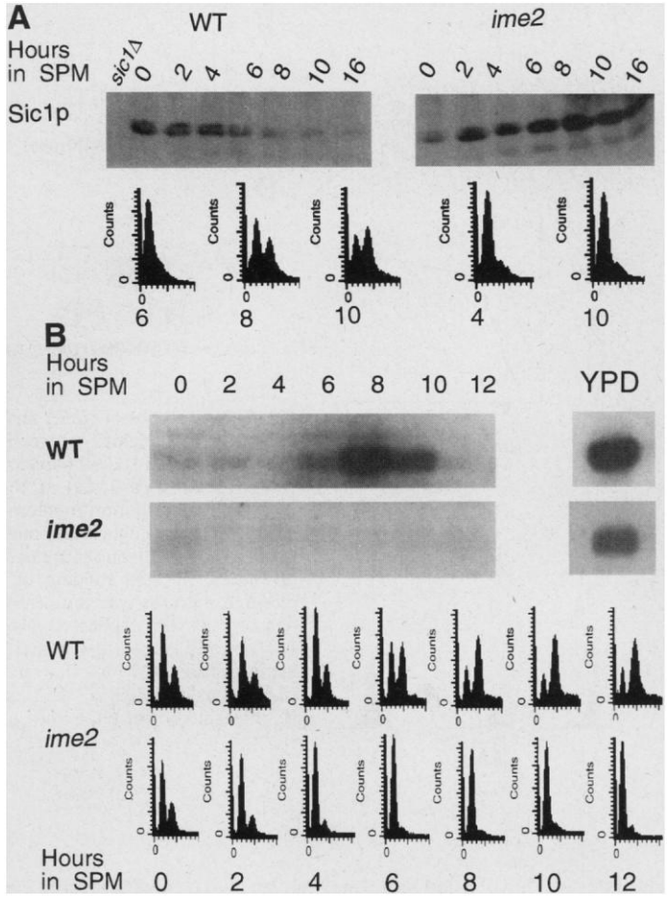

Fig. 3. Amounts of Sic1 protein (Sic1p) and Clb5-associated kinase in WT and *ime2*Δ meiotic cells. (**A**) (Top) Protein immunoblot analysis (*23*) with polyclonal antibody to Sic1 in WT (L179) and *ime2*Δ (L213) during a meiotic time course. (Bottom) FACS analyses of DNA replication. The *sic1*Δ control is L182. (**B**) Histone H1 kinase assay of anti-HA immunoprecipitates (*23*) from WT (L535) and *ime2*Δ (L545), both carrying two *CLB5-HA3* alleles. Note the faint kinase activity due to remaining mitotic S-phase cells at *t* = 0 and 2 hours. YPD, samples from cycling mitotic cultures.

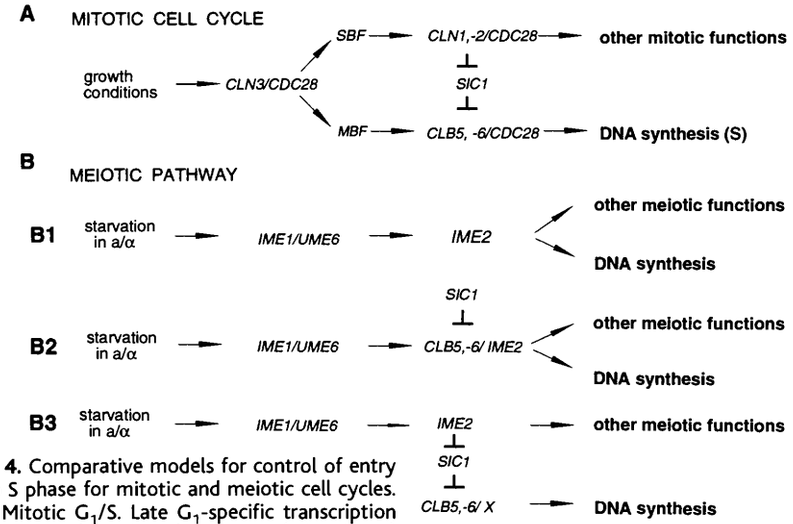

Fig. 4. Comparative models for control of entry into S phase for mitotic and meiotic cell cycles. (**A**) Mitotic G_1/S. Late G_1-specific transcription factors SBF (Swi4/Swi6) and MBF (Mbp1/Swi6) stimulate expression not only of the G_1 cyclins (*CLN1*, *CLN2*) that target Sic1 for proteolysis but also of *CLB5* and *CLB6*. S-phase B-type cyclins Clb5 and Clb6 combine with Cdc28 to form SPF, which promotes entry into S phase. (**B**) Meiotic G_1/S. (**B1**) After transcription from Ime1/Ume6 (early meiotic transcription factors), the Ime2 (inducer of meiosis) kinase directly triggers entry into S phase. (**B2**) As in (B1) but showing Clb5 and Clb6 as potential activators of Ime2. (**B3**) Revised model. Like the Cln1,-2-associated kinase in model A, Ime2 promotes S phase indirectly by triggering destruction of Sic1, thus allowing increased activity of an unidentified kinase (X) associated with Clb5 and Clb6.

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17. The *SBF* and *MBF* deletion mutants were created by integration of a *pGAL1-10::RME1* construct in K1107 (L43) and multiple crosses to *swi4*Δ, *swi6*Δ and *mbp1*Δ strains. *swi4*Δ/Δ *swi6*Δ/Δ (L44) and *swi4*Δ/Δ *mbp1*Δ/Δ (L453) cultures were enabled to grow mitotically by overexpression of the *RME1* gene [W. M. Toone *et al.*, *EMBO J.* **14**, 5824 (1995); L. Dirick, unpublished data]. The triple *CLN*-deleted strain (L49), kept alive by *CLN3* under *pGAL1-10* promoter, comes from a cross between K2759 and K3130.
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scribed in (*1*). Transformation of all three *clb5Δ* strains with the WT *CLB5* gene on a CEN plasmid [Ycplac111::CLB5 (LEU2); D74] restored sporulation.

20. For fluorescence-activated cell sorter (FACS) analysis, 1-ml aliquots of cultures were fixed in 70% ethanol, treated overnight with ribonuclease (RNase) A (300 μg/ml) at 37°C, rinsed in phosphate-buffered saline (PBS), and stained with propidium iodide (40 μg/ml in PBS); 10,000 cells per sample were analyzed on a Becton-Dickinson FACSscan. The same propidium iodide–stained cells were used to record chromosome segregation by fluorescence microscopy. RNA extraction and RNA blotting conditions were as described in (*2*).
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23. Protein immunoblot analysis of Sic1 was done as described [U. Surana *et al.*, *EMBO J.* **12**, 1969 (1993); A. Amon *et al.*, *Cell* **77**, 1037 (1994)] with a 1:2000 dilution of polyclonal antibody to Sic1 (M. Tyers). Proteins were detected with the enhanced chemiluminescence detection system (ECL; Amersham). Kinase assays were done as described in [U. Surana *et al.*, *EMBO J.* **12**, 1969 (1993)] starting from 140 μg of protein, except for *ime2Δ* in YPD where 70 μg was used. The monoclonal antibody to hemagglutinin (HA), 12CA5, was used at a 1:100 dilution to immunoprecipitate the HA3-tagged Clb5 proteins (*8*). Specificity was confirmed on an SK1 WT strain carrying untagged *CLB5* (L111).
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16 October 1997; accepted 12 August 1998

Fertilization Defects in Sperm from Mice Lacking Fertilin β

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Fertilin, a member of the ADAM family, is found on the plasma membrane of mammalian sperm. Sperm from mice lacking fertilin β were shown to be deficient in sperm-egg membrane adhesion, sperm-egg fusion, migration from the uterus into the oviduct, and binding to the egg zona pellucida. Egg activation was unaffected. The results are consistent with a direct role of fertilin in sperm-egg plasma membrane interaction. Fertilin could also have a direct role in sperm-zona binding or oviduct migration; alternatively, the effects on these functions could result from the absence of fertilin activity during spermatogenesis.

The sperm surface protein fertilin, a member of the ADAM family, is a heterodimer composed of α and β subunits (*1*–*4*). The ADAM family is comprised of structurally related cell surface proteins proposed to have cell adhesion activity, protease activity, or both (*5*). Fertilin α and β are made as precursors in spermatogenic cells and are processed before sperm maturation is complete (*2*, *6*). Both precursors are composed of the multiple domains found in all ADAM family membrane proteins: pro-, metalloprotease, disintegrin, cysteine-rich, transmembrane, and cytoplasmic domains (*4*, *7*). Proteolytic processing removes the pro- and metalloprotease domains, leaving an NH_2-terminal disintegrin domain on mature forms of the α and β subunits (*3*, *8*). Processed fertilin may promote sperm-egg binding, fusion, and egg activation (*9*–*11*).

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We disrupted the fertilin β gene, which shows testis-specific expression, by deleting exon 14. This exon encodes a region of the disintegrin domain that has been predicted to include the active binding site of fertilin β (*9*, *12*) (Fig. 1A). Fertilin β, using this active site, apparently binds to an egg integrin $\alpha_6\beta_1$ (*13*). Homozygous (fertilin $\beta^{-/-}$) mutant mice were identified by polymerase chain reaction (PCR) and Southern (DNA) blot analyses of genomic DNA (Fig. 1B). Fertilin $\beta^{-/-}$ mice (both male and female) are viable and develop normally. Both precursor and processed fertilin β proteins were absent from spermatogenic cells and mature sperm, respectively, from fertilin $\beta^{-/-}$ male mice (Fig. 1C). In mice lacking fertilin β, the level of fertilin α precursor was reduced (Fig. 1C). Fertilin α may be degraded when unable to form a heterodimer with β. Similar loss of one subunit of a plasma membrane heterodimer has been observed in other cases when the gene

Table 1. Phenotypic analysis in fertilin $\beta^{+/+}$ and fertilin $\beta^{-/-}$ mice. Data in sperm analysis (*20*) represent the mean ± SEM of *n* individual measurements indicated in parentheses. Cauda epididymal sperm were used for motility analysis. Motility parameters were measured by computer-assisted sperm analysis (*21*). Additional motility parameters measured, including amplitude of lateral head displacement, beat cross frequency, mean angle of deviation, average path velocity, and straight line velocity, were not different between +/+ and −/−. Also, there was no time-dependent difference during capacitation in all the motility parameters between +/+ and −/−. The values shown for acrosome reaction are from sperm incubated in medium with 3% BSA. At a lower concentration of BSA (0.5%), the values for acrosome reaction were also not different between +/+ and −/−. The numbers of eggs observed in egg activation (*20*) are shown in parentheses.

Parameter	Genotype	
	+/+	−/−*
Sperm analysis		
Number of sperm		
Epididymal (×10^7)	3.8 ± 1.0 (4)	4.4 ± 0.7 (4)
Ejaculated (×10^6)	3.2 ± 0.9 (7)	3.7 ± 0.3 (8)
Motility		
Motile sperm (%)	67 ± 8 (16)	62 ± 5 (17)
Curvilinear velocity (μm/s)	266.0 ± 7.1 (4)	245.8 ± 20.4 (3)
Linearity (%)	21.5 ± 2.9 (4)	20.6 ± 4.4 (3)
Straightness (%)	62.9 ± 5.4 (4)	62.9 ± 7.5 (3)
Acrosome reaction (%)		
Incubation time (min)		
0	14 ± 4 (2)	15 ± 4 (2)
120	39 ± 7 (2)	38 ± 2 (2)
270	57 ± 6 (4)	60 ± 3 (4)
Egg activation		
Fused eggs with Ca^{2+} oscillations (%)	100 (219)	98.2 (56)
Fused eggs with polar body formation (%)	100 (29)	82.4 (17)

* Values for all parameters are not significantly different from +/+ ($P > 0.3$, Student's *t* test).