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Speedy A–Cdk2 binding mediates initial telomere–nuclear envelope attachment during meiotic prophase I independent of Cdk2 activation

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Telomere attachment to the nuclear envelope (NE) is a prerequisite for chromosome movement during meiotic prophase I that is required for pairing of homologous chromosomes, synapsis, and homologous recombination. Here we show that Speedy A, a noncanonical activator of cyclin-dependent kinases (Cdks), is specifically localized to telomeres in prophase I male and female germ cells in mice, and plays an essential role in the telomere–NE attachment. Deletion of *Spdya* in mice disrupts telomere–NE attachment, and this impairs homologous pairing and synapsis and leads to zygotene arrest in male and female germ cells. In addition, we have identified a telomere localization domain on Speedy A covering the distal N terminus and the Cdk2-binding Ringo domain, and this domain is essential for the localization of Speedy A to telomeres. Furthermore, we found that the binding of Cdk2 to Speedy A is indispensable for Cdk2's localization on telomeres, suggesting that Speedy A and Cdk2 might be the initial components that are recruited to the NE for forming the meiotic telomere complex. However, Speedy A-Cdk2–mediated telomere–NE attachment is independent of Cdk2 activation. Our results thus indicate that Speedy A and Cdk2 might mediate the initial telomere–NE attachment for the efficient assembly of the telomere complex that is essential for meiotic prophase I progression.

meiosis | telomere | Speedy A | Cdk2 | germ cells

In mammals, the progression of meiotic prophase I is largely dependent on the dynamic movement of chromosomes along the nuclear envelope (NE) (1, 2). A prerequisite for faithful chromosome movement is the anchoring of telomeres to the transmembrane LINC (linker of nucleoskeleton and cytoskeleton) complex that bridges chromatin to the cytoskeleton (3).

In recent years, several meiosis-specific structural molecules that mediate telomere–NE attachment have been identified in mice, such as TERB1 (telomere repeat binding bouquet formation protein 1), SUN1 (Sad1 and UNC84 domain containing 1), KASH5 (Klarsicht/ANC-1/Syne/homology 5), TERB2 (telomere repeat binding bouquet formation protein 2), and MAJIN (membrane-anchored junction protein), and mice lacking any one of SUN1, KASH5, TERB1, TERB2, or MAJIN display impaired telomere attachment and are sterile (4–8). Moreover, cyclin-dependent kinase 2 (Cdk2) is localized to telomeres in mouse spermatocytes and prophase I oocytes (9), and loss of *Cdk2* leads to sterility in both male and female mice (10, 11).

Speedy/RINGO (Rapid inducer of G2/M progression in oocytes) proteins are atypical noncyclin Cdk activators that were first discovered in *Xenopus laevis* as proteins that induce G2/M transition during oocyte maturation (12, 13). Multiple members of the Speedy family have since been identified in mammals (14–16). Speedy proteins activate Cdks independently of cyclins, and they are characterized by their highly conserved, ∼140-aa central Cdk-binding core, called the Ringo domain (14, 17). In mice, four homologs of Speedy have been identified: Speedy A, Speedy B1a, Speedy B1b, and Speedy B3 (14, 16, 17). Both mouse Speedy A and the human homolog Spy1 are able to induce meiotic resumption when injected into *Xenopus* oocytes (14, 17). However, the in vivo physiological role of mammalian Speedy A is unknown.

In the present study, we generated *Spdya* knockout mice and studied the functional roles of Speedy A in mammals. We found that distinct from *Xenopus*, Speedy A in mice is not involved in oocyte maturation. Instead, Speedy A is only specifically expressed in male and female germ cells at meiotic prophase I, and it is localized to the telomeres. Loss of Speedy A in mice impairs telomere–NE attachment in early meiosis, perturbs homologous recombination, and leads to infertility in both sexes. Moreover,

Significance

In meiotic prophase I, telomere attachment to the nuclear envelope is a prerequisite for subsequent prophase events, such as homologous pairing and recombination. In this study, we show that Speedy A, a noncanonical activator of cyclin-dependent kinases (Cdks), is essential for telomere attachment to the nuclear envelope in mice. We have identified a telomere localization domain in Speedy A, which covers the protein's distal N-terminus and Cdk2-binding Ringo domain but excludes its Cdk-activation domain. Furthermore, we found that the binding of Cdk2 to Speedy A is essential for Cdk2's localization to telomeres. Our results suggest that Speedy A–Cdk2 binding might mediate the initial assembly of the meiotic telomere complex, a process that is independent of Cdk2 activation.

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The authors declare no conflict of interest.

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we found that the proper attachment of telomeres to the NE is dependent on a telomere localization domain (TLD) on Speedy A, as defined by this study, whereas the C terminus of Speedy A, which is required for Cdk2 activation, is dispensible for the attachment of telomeres to the NE. Furthermore, we showed that the binding of Cdk2 to Speedy A is required for Cdk2 to localize onto telomeres. These results suggest that the function of Speedy A goes beyond that of a noncanonical activator of Cdk as previously suggested (14, 15, 18, 19). Rather, the binding between Speedy A and Cdk2 might mediate the initial assembly of the telomere–NE complex that is essential for meiotic prophase I progression.

Results

Speedy A Is Specifically Expressed in Meiotic Germ Cells and Is Localized to Telomeres. To study the functional roles of Speedy A in development, we first investigated the expression of Speedy A protein in various tissues by Western blotting. We found that Speedy A was specifically expressed in the adult testis and embryonic ovary when meiotic prophase I occurs, but not in the adult ovary or in the adult liver, lung, spleen, kidney, or intestine (Fig. 1*A*).

To further reveal the expression pattern of Speedy A in germ cells, we isolated different types of male germ cells by BSA gradient sedimentation (20). In the subsequent Western blotting, we found that Speedy A expression was undetectable in spermatogonia, but its expression began to be observed in preleptotene germ cells (Fig. 1*B*). The expression of Speedy A then decreased in pachytene cells and became almost undetectable in round spermatids and elongated spermatids (Fig. 1*B*). In contrast, the protein level of the 33-kDa isoform of Cdk2 (p33) remained constant between spermatogonia and spermatocytes, but the level of the 39-kDa isoform of Cdk2 (p39) (21) increased from preleptotene to pachytene cells (Fig. 1*B*). Moreover, Speedy A was first observed in testes at postnatal day (PD) 12 (Fig. 1*C*), which was concurrent with up-regulation of p39 Cdk2 (Fig. 1*C*). At PD12, Speedy A exhibits as a doublet and it is likely that this is caused by phosphorylation (Fig. 1*C*).

We also examined *Spdya* mRNA expression in the female germ cells at various developmental time points by RT-PCR. *Spdya* mRNA was not expressed in mitotic female primordial germ cells at 11.5 days postcoitum (dpc), but its expression was up-regulated in female germ cells once they entered meiosis at 14.5 and 17.5 dpc (Fig. 1*D*). The *Spdya* mRNA levels then decreased in prenatal oocytes at 18.5 and 19.5 dpc, when they became arrested at dictyate, and the *Spdya* mRNA levels were almost undetectable in the adult ovary (Fig. 1*D*). These results show that in females, *Spdya* expression is specific to germ cells undergoing meiotic prophase I.

To determine the subcellular localization of Speedy A, we performed immunofluorescent staining of PD18 spermatocytes and found that in preleptotene male germ cells where telomeres are partially internal within the cell nucleus (Fig. 2 *A*–*C*, arrowheads), Speedy A was only detectable on telomeres that had already localized to the NE, as shown by the colocalization with TRF1 (telomeric repeat-binding factor 1) (Fig. 2 *A*–*C*, arrows). At the leptotene stage—when almost all telomeres have attached to the NE—the Speedy A and TRF1 signals completely overlapped, indicating localization on telomeres that had attached to the NE (Fig. 2 *D*–*F*, arrows). The colocalization of Speedy A and TRF1 on the NE persisted throughout the zygotene and pachytene stages (Fig. 2 *G*–*L*, arrows).

Using a spreading method that breaks the NE (22), we found that Speedy A also formed a linear element along the XY body in pachytene-spermatocytes (Fig. S1 *A*–*C*, arrowheads) except for its expression on telomeres (Fig. S1 *A*–*C*, arrows). Speedy A began to disappear from telomeres at the diplotene stage (Fig. S1 *D*–*F*, arrows), and it was undetectable in metaphase spermatocytes (Fig. S1 *G*–*I*, arrows). The localization of Speedy A on telomeres at the pachytene stage and its disappearance at the

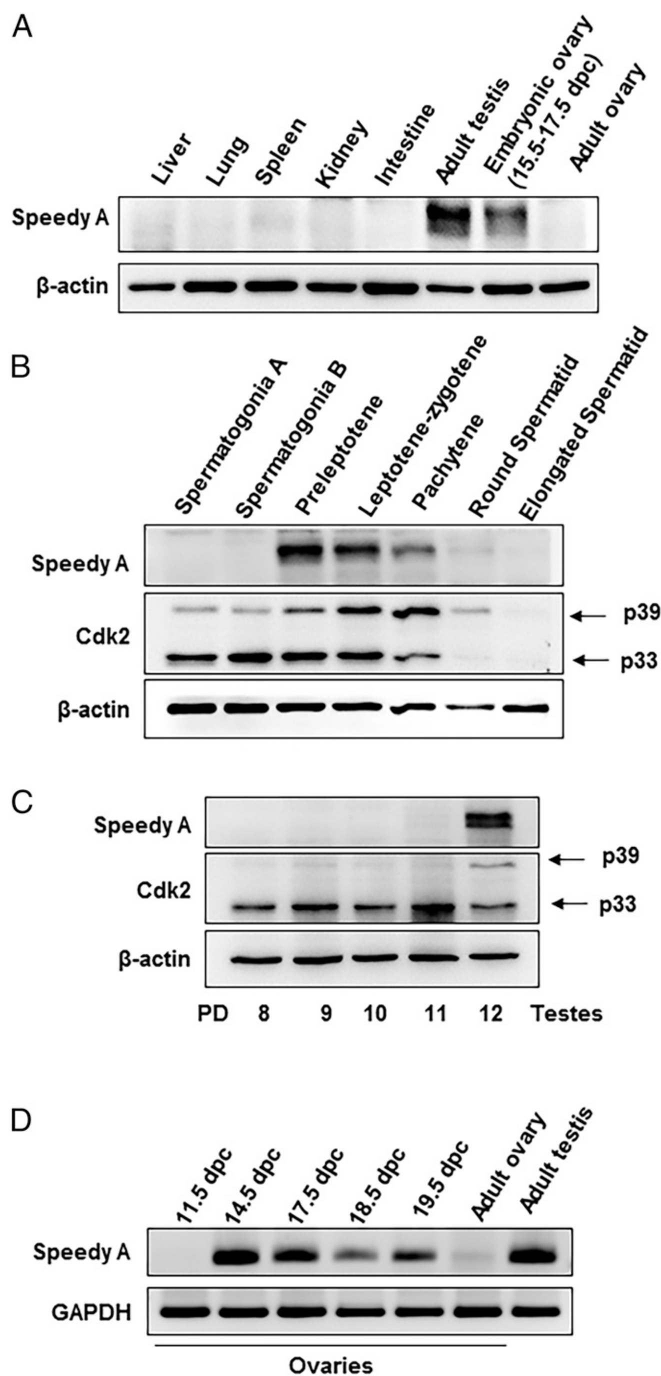

Fig. 1. Speedy A is specifically expressed in meiotic germ cells at prophase I. (*A*) Western blot of Speedy A in different tissues indicating that Speedy A was specifically expressed in testes and embryonic ovaries. (*B*) Western blot of Speedy A and Cdk2 in isolated male germ cells, demonstrating that Speedy A was expressed at high levels in the preleptotene stage and decreased afterward, whereas the expression of the 39-kDa isoform of Cdk2 increased from the preleptotene to the pachytene stage. (*C*) Western blots for Speedy A and Cdk2 in testes of different ages showing that Speedy A started to be highly expressed at PD12, which was concurrent with p39 Cdk2 up-regulation. For *A*–*C*, β-actin was used as the loading control, and 40-μg lysate was loaded in each lane. The experiments were repeated more than three times each. (*D*) RT-PCR detection of *Spdya* in female primordial germ cells and germ cells isolated from different stages of the embryonic ovary by FACS, showing that Speedy A was absent at 11.5 dpc, up-regulated at 14.5 and 17.5 dpc, and then down-regulated at 18.5 and 19.5 dpc. *Gapdh* was used as the loading control. The experiments were repeated more than three times.

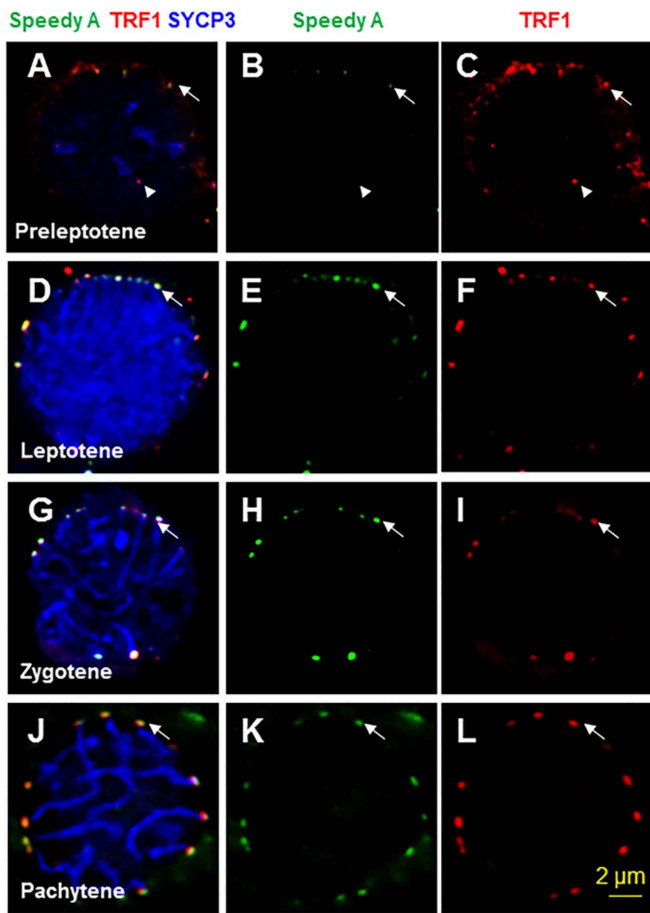

Fig. 2. Speedy A is localized to telomeres in structurally preserved spermatocytes during meiotic prophase I. Immunofluorescent staining of structurally preserved PD18 wild-type spermatocytes. Telomeres were stained with TRF1. (*A–C*) In preleptotene cells, Speedy A was only detected on telomeres that were on the NE (arrows). Telomeres inside the nucleus lacked the Speedy A signal (arrowheads). (*D–L*) In leptotene, zygotene, and pachytene cells, Speedy A and TRF1 signals overlapped on telomeres (arrows).

diplotene stage were also seen during prophase I in female germ cells (Fig. S1 *J–O*, arrows).

Knockout of Speedy A Leads to Loss of Male and Female Germ Cells. To study the functional roles of Speedy A in vivo in the female germ line, we generated *Spdya*$^{flox/flox}$ mice (Fig. S2). In contrast to findings in *Xenopus* (12, 13), tissue-specific deletion of *Spdya* in postnatal mouse oocytes using *Zp3-Cre* (23, 24) did not lead to any changes in oocyte maturation (Fig. S3 *A–D*) or female fertility (Fig. S3*E*), indicating that Speedy A is dispensable for oocyte maturation in mice.

However, in conventional *Spdya*$^{-/-}$ mice (as validated in Fig. 3*A*), we observed infertility in both males and females. These mice exhibited atrophic gonads (Fig. 3 *B* and *C*), and further histological analysis revealed that—in sharp contrast to normal spermatogenesis in *Spdya*$^{+/+}$ males at PD7, PD18, and PD75 (Fig. 3 *D*, *F*, and *H*, arrowheads)—*Spdya*$^{-/-}$ males had normal testes at PD7 (Fig. 3*E*) but displayed a loss of differentiated spermatocytes at PD18 (Fig. 3*G*, arrowhead) and lacked round and elongated spermatids at PD75 (Fig. 3*I*, arrowhead).

In females, no apparent difference in oocyte number was observed between *Spdya*$^{+/+}$ (Fig. 3*J*, arrow) and *Spdya*$^{-/-}$ (Fig. 3*K*, arrow) ovaries at 17.5 dpc. However, compared with the *Spdya*$^{+/+}$ ovary (Fig. 3 *L* and *N*, arrows), a reduction in oocytes was observed in the *Spdya*$^{-/-}$ ovary at PD1 (Fig. 3*M*, arrow), which led to a complete loss of oocytes by PD5 (Fig. 3*O*, arrow).

Telomere–NE Attachment Was Impaired in *Spdya*$^{-/-}$ Spermatocytes Leading to Meiotic Arrest Before Telomere Cap Exchange. To investigate the reasons for germ cell loss during prophase I in *Spdya*$^{-/-}$ mice, we analyzed telomere distribution patterns in structurally preserved spermatocytes by staining for TRF1 and synaptonemal complex protein 3 (SYCP3). In leptotene and zygotene cells, most of the TRF1 signal was localized to NE in *Spdya*$^{+/+}$ spermatocytes (Fig. 4 *A* and *C*, arrows). In contrast, in *Spdya*$^{-/-}$ spermatocytes a considerable proportion of the TRF1 signal was found to be within the nuclei (Fig. 4 *B* and *D*, arrowheads). The telomere attachment defect was similar in *Spdya*$^{-/-}$ female germ cells; although all telomeres were on the NE in *Spdya*$^{+/+}$ female germ cells (Fig. 4*E*, arrows), in *Spdya*$^{-/-}$ cells several telomeres were observed inside the nucleus (Fig. 4*F*, arrowheads), and the chromosome morphology showed that these cells did not progress beyond the zygotene stage (Fig. 4*F*).

In *Spdya*$^{+/+}$ spermatocytes at the pachytene stage, the central element SYCP1 (synaptonemal complex protein 1) was found along autosomal chromosomes (Fig. S4*A*, arrow), the DNA double-strand

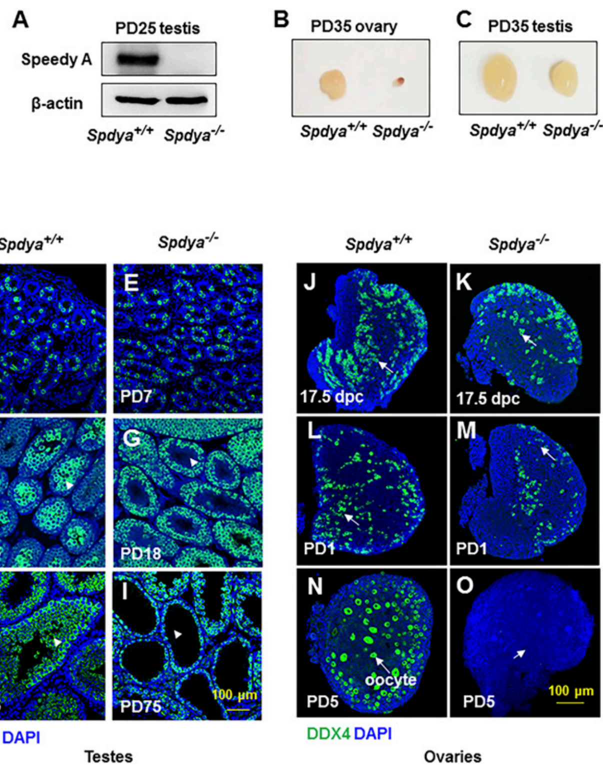

Fig. 3. Loss of Speedy A leads to depletion of male and female germ cells. (*A*) Western blot showing that Speedy A was deleted in PD25 testes. β-Actin was used as the loading control, and 40-μg lysate from PD25 *Spdya*$^{+/+}$ and *Spdya*$^{-/-}$ testes was loaded in each lane. (*B*) Ovaries from PD35 *Spdya*$^{+/+}$ and *Spdya*$^{-/-}$ female mice. (*C*) Testes from PD35 *Spdya*$^{+/+}$ and *Spdya*$^{-/-}$ male mice. (*D* and *E*) At PD7, the number of spermatogonia was comparable between *Spdya*$^{+/+}$ and *Spdya*$^{-/-}$ testes as indicated by DDX4 staining. (*F* and *G*) By PD18, the number of spermatogonia was comparable between *Spdya*$^{+/+}$ (*F*) and *Spdya*$^{-/-}$ testes (*G*), but some seminiferous tubules showed depletion of spermatocytes in *Spdya*$^{-/-}$ testes (*G*, arrowhead). (*H* and *I*) By PD75, most of the *Spdya*$^{-/-}$ seminiferous tubes were depleted of spermatocytes (arrowhead). (*J* and *K*) At 17.5 dpc, the numbers of oocytes were comparable between *Spdya*$^{+/+}$ (*J*) and *Spdya*$^{-/-}$ ovaries (*K*). (*L* and *M*) The number of oocytes was much lower in the *Spdya*$^{-/-}$ ovary (*M*) compared with the *Spdya*$^{+/+}$ ovary at PD1 (*L*). (*N* and *O*) At PD5, the *Spdya*$^{-/-}$ ovaries were depleted of oocytes (*O*, arrow) compared with the *Spdya*$^{+/+}$ ovary (*N*).

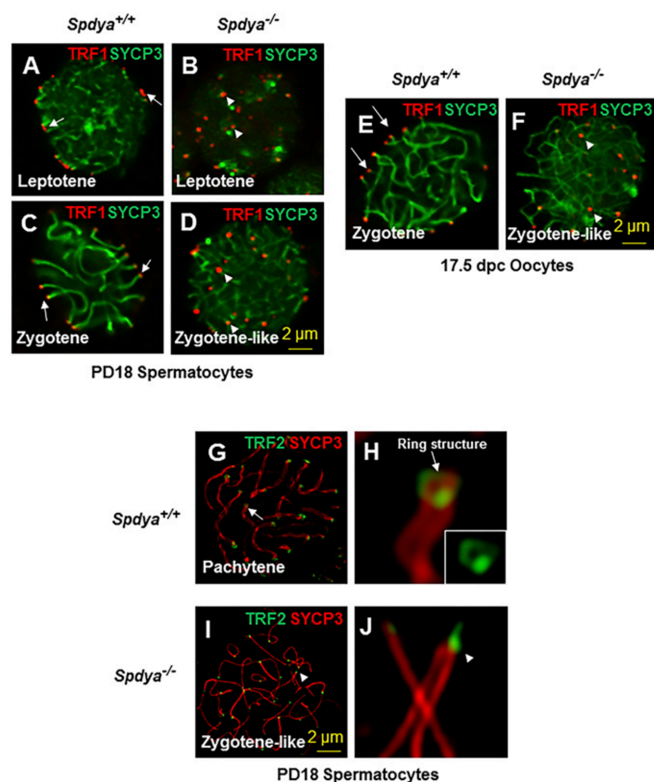

Fig. 4. Speedy A is required for telomere attachment to the NE and for telomere cap exchange. Structurally preserved PD18 spermatocytes were used to analyze telomere attachment to the NE. Telomeres were stained with TRF1. (*A–D*) In *Spdya*$^{+/+}$ spermatocytes, all telomeres were on the NE (arrows), whereas in *Spdya*$^{-/-}$ spermatocytes, telomeres were observed inside the nucleus (arrowheads). (*E* and *F*) Structurally preserved 17.5-dpc oocytes were used for analyzing telomere attachment to the NE. In *Spdya*$^{+/+}$ zygotene oocytes, all telomeres were on the NE (arrows). However, in *Spdya*$^{-/-}$ zygotene-like oocytes, telomeres were inside the nucleus (arrowheads). (*G–J*) Superresolution microscopy images of PD18 spermatocytes showing the telomere cap exchange. Telomeres were stained with TRF2. (*G* and *H*) A representative *Spdya*$^{+/+}$ pachytene spermatocyte showing the shelterin ring structure (arrows and *Inset*). (magnification: *H* and *J*, 10× *G* and *I*) (*I* and *J*) A representative *Spdya*$^{-/-}$ zygotene-like spermatocyte showing the absence of the shelterin ring structure (arrowheads).

break (DSB) marker γH2AX was restricted to the unsynapsed regions of the XY body (Fig. S4*C*, arrow), and the chiasmata marker MutL Homolog 1 (MLH1) appeared on recombination foci (Fig. S4*E*, arrow) (25–27). In contrast, in zygotene-like *Spdya*$^{-/-}$ spermatocytes, SYCP1 was partially loaded along the synaptonemal axes (Fig. S4*B*, arrowhead), γH2AX remained on the chromosomes (Fig. S4*D*, arrowheads), and MLH1 foci were absent (Fig. S4*F*, arrowhead). These data suggest that homologous synapsis, DSB repair, and homologous recombination are largely abolished in *Spdya*$^{-/-}$ spermatocytes.

We further analyzed the telomeric cap exchange that takes place in pachytene-stage germ cells (5). In wild-type spermatocytes, we observed that TRF2 (telomeric repeat-binding factor 2), a shelterin protein (28, 29), was disassociated from the end of the chromosome axis and formed a ring structure, indicating that cap exchange had occurred (Fig. 4 *G* and *H*, arrows and *Inset*). However, TRF2 did not form a ring structure in *Spdya*$^{-/-}$ cells (Fig. 4 *I* and *J*, arrowheads), suggesting that the cap-exchange process was abolished in the mutant. These data further confirmed that *Spdya*$^{-/-}$ spermatocytes did not progress beyond the pachytene stage.

In both *Spdya*$^{+/+}$ and *Spdya*$^{-/-}$ pachytene cells, TERB1 and MAJIN could be observed on a few telomeres that were attached to the NE (Fig. S4 *G–J*, arrows). However, the LINC complex component SUN1, which is localized to telomeres in *Spdya*$^{+/+}$ spermatocytes (Fig. S4*K*, arrow), was observed as a polarized cap along the NE in *Spdya*$^{-/-}$ spermatocytes (Fig. S4*L*, arrow). These results confirmed that Speedy A is essential for tethering telomeres to the NE during prophase.

Speedy A–Cdk2 Binding Facilitates the Initial Formation of the Telomere Complex Independent of Cdk2 Activation. Loss of Speedy A prevented the increase of p39 Cdk2 in PD17 *Spdya*$^{-/-}$ testes (Fig. S5*A*), and the kinase activity of Cdk2 was reduced in *Spdya*$^{-/-}$ testes extracts (Fig. S5*B*). By immunoprecipitation, we showed that Speedy A indeed interacts with Cdk2 in the testis (Fig. S5*C*). Moreover, using superresolution microscopy, we found that during meiotic prophase I, Speedy A appears on the NE before Cdk2, implying that Speedy A might recruit Cdk2 to the NE (Fig. S5 *D–O*). Furthermore, staining of *Spdya*$^{-/-}$ leptotene cells showed that Cdk2 was not localized to telomeres in the absence of Speedy A (Fig. S5 *P–R*, arrowheads).

In vitro studies have suggested that Speedy A's Ringo domain (amino acids 67–199) is essential for Cdk2 binding and that its C terminus (amino acids 200–310) promotes Cdk2 activation, but its N terminus (amino acids 1–66) has no known function (14). Using a kinase assay, we confirmed that the C terminus of Speedy A is essential for Cdk2 activation (Fig. S6).

To study how Speedy A is localized to telomeres, we expressed GFP-tagged fragments of Speedy A protein in wild-type mouse testes by electroporation (30) (illustrated in Fig. 5*A*). Western blot results demonstrated that these Speedy A protein fragments were correctly expressed, as shown in 293T cells (Fig. S7*A*, asterisks). Full-length Speedy A protein (amino acids 1–310) was localized to telomeres (Fig. S7 *B–D*, arrows). Moreover, the Speedy A fragment lacking the C terminus (amino acids 1–199) (Fig. S7 *E–G*, arrows) and the fragment consisting of the Ringo domain plus 3 aa of the N terminus (amino acids 64–199) (Fig. S7 *K–M*, arrows) could also localize to telomeres. In contrast, the fragment consisting of only the Ringo domain (amino acids 67–199) (Fig. S7 *H–J*, arrows) failed to localize to telomeres. These results suggested that the amino acids 64–199 region of Speedy A is a prerequisite for Speedy A to localize to telomeres, whereas the Cdk-activating C terminus (amino acids 200–310) is dispensable for the binding of Speedy A onto telomeres.

We next electroporated the GFP-tagged Speedy A fragments into live *Spdya*$^{-/-}$ testes. The full-length Speedy A protein (amino acids 1–310) rescued the telomere attachment defect as characterized by fully recovered TRF1 signal on the NE in structurally preserved spermatocytes (Fig. 5 *B–D*, arrows). Remarkably, we observed that Speedy A–ΔC (amino acids 1–199) could also rescue the telomere attachment defect in *Spdya*$^{-/-}$ spermatocytes and could colocalize with TRF1 (Fig. 5 *E–G*, arrows). Furthermore, Speedy A consisting of the Ringo domain plus 3 aa of the N terminus (amino acids 64–199) was also sufficient to localize to telomeres and rescue the attachment phenotype in *Spdya*$^{-/-}$ spermatocytes (Fig. 5 *K–M*, arrows). In contrast, the Speedy A fragment consisting of only the Ringo domain (amino acids 67–199) failed to localize to telomeres in *Spdya*$^{-/-}$ spermatocytes, and the internal telomere phenotype did not change upon electroporation of this fragment (Fig. 5 *H–J*, arrows). These results suggest that the amino acid 64–199 fragment of Speedy A contains the functional domain that facilitates telomere attachment to the NE. This domain is conserved among species and covers the distal portion of the N terminus and the Ringo domain (14, 16), and we call this domain the "telomere localization domain."

Localization of Cdk2 on Telomeres Depends on Its Interaction with Speedy A. To determine if the binding of Cdk2 to Speedy A is of any importance for the localization of Cdk2 to the telomere–NE complex, we expressed GFP-tagged wild-type and mutated p39 Cdk2,

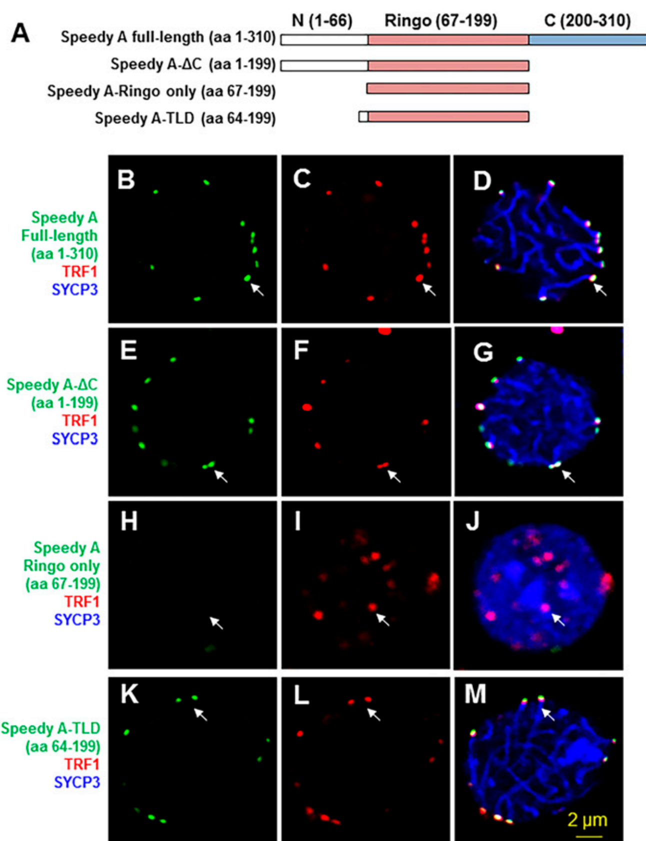

Fig. 5. Speedy A mediates the formation of the telomere complex independent of Cdk2 activation. (*A*) Illustration of different fractions of Speedy A protein. Amino acids 1–66 were defined as the N terminus, amino acids 67–199 were defined as the conserved Ringo domain, and amino acids 200–310 were defined as the C terminus of Speedy A. The TLD was defined as amino acids 64–199. Different forms of Speedy A protein were cloned into a pCAG-GFP vector and ectopically expressed by electroporation into PD18 *Spdya*$^{-/-}$ testes. (*B*–*D*) Representative images of immunostaining for GFP, TRF1, and SYCP3, showing that in vivo expression of full-length Speedy A protein in *Spdya*$^{-/-}$ testes rescued the telomere attachment defects (arrows). (*E*–*G*) In *Spdya*$^{-/-}$ testes, expression of Speedy A–ΔC protein could rescue the telomere attachment defects (arrows). (*H*–*J*) Expression of Speedy A–Ringo only protein could not rescue the telomere attachment defect in *Spdya*$^{-/-}$ spermatocytes (arrows). (*K*–*M*) In vivo expression of the Speedy A–TLD protein in *Spdya*$^{-/-}$ testes rescued the telomere attachment defects (arrows).

where its Lysine^{33} (Lys^{33}), Arginine^{50} (Arg^{50}), and Arginine^{150} (Arg^{150}) residues were separately mutated to Alanine (Ala), by electroporation into wild-type mouse testes. A previous in vitro study has reported that the Lys^{33}, Arg^{50}, and Arg^{150} residues of Cdk2 are required for Cdk2's binding to XRINGO, the *Xenopus* homolog of mouse Speedy A (18). Western blot results showed that these mutant Cdk2 forms can all be expressed in 293T cells (Fig. S8*A*), but they cannot bind or only weakly bind to Speedy A in 293T cells (Fig. S8*B*).

After electroporation into mouse testes, we found that p39 Cdk2 was localized to the telomeres (Fig. S8 *C*–*E*, arrows) and on the XY body (Fig. S8 *C* and *E*, arrowheads), as previously reported (9). In contrast, the p39 Cdk2 mutants Lys^{33}Ala, Arg^{50}Ala, and Arg^{150}Ala failed to localize to telomeres (Fig. S8 *F*–*N*, arrows). These results suggest that Cdk2 binding to Speedy A is indispensable for Cdk2's localization to telomeres.

Discussion

Mammalian Speedy A, the most conserved member of the mammalian Speedy family, is highly expressed in mouse and human testes, but its expression has also been detected in various other human tissues and cell lines (14). For example, human Speedy A (Spy1) levels are tightly regulated during the proliferation of mammary progenitor cells, and are enriched in malignant human glioma cells, suggesting important roles for Speedy A in somatic cell cycle regulation in humans (31, 32). The Cdk-binding Ringo domain of Speedy A is 98% identical in mice and humans (14). In vitro studies on mouse Speedy A have shown that although the Ringo domain is essential for Cdk2 binding, activation of Cdk2 is through the C terminus of Speedy A (33).

In this study, we have found that in mice, Speedy A is specifically expressed in male and female germ cells at meiotic prophase I and is localized to the telomeres that have attached to the NE. Deletion of Speedy A in mice causes meiotic prophase I arrest, leading to male and female infertility. In *Spdya*$^{-/-}$ germ cells, telomeres could not efficiently attach to the NE in meiotic prophase I, and were thus arrested at a zygotene-like stage before the occurrence of characteristic pachytene events, such as telomeric cap exchange, homologous synapsis, and recombination.

A very recent study has also reported a similar phenotype in RingoA (i.e., Speedy A) knockout mice (19). However, in the present study we have further identified a TLD (amino acids 64–199) on Speedy A that covers the distal N terminus and the Ringo domain. It seems that this TLD of Speedy A is sufficient for mediating the initial formation of the telomere complex. Surprisingly, the TLD does not include the Cdk2-activation domain of Speedy A, implying that Cdk2 activation by Speedy A might not be necessary for mediating the initial telomere attachment to the NE in meiotic prophase I. On the other hand, the Speedy A fragment consisting of only the Cdk-binding Ringo domain without the distal N-terminal region was unable to localize to telomeres or rescue the phenotype in *Spdya*$^{-/-}$ spermatocytes. Therefore, we believe that the TLD of Speedy A plays a fundamental role in mediating the initial formation of the telomere–NE complex.

The involvement of the Cdk-binding Ringo domain indicates that not only Speedy A, but also Cdk2, are likely to function as structural components of the telomere complex. Indeed, Cdk2 proteins carrying mutations in key residues for binding to Speedy A are not localized on telomeres, indicating that binding to Speedy A is a prerequisite for Cdk2's telomere localization. It is possible that the primary role of Speedy A during telomere attachment is to act as a structural protein that anchors Cdk2 on telomeres. We propose that the interdependent binding between Speedy A and Cdk2 is of great importance for the initial telomere–NE complex assembly during early meiotic prophase I. It is likely that after Cdk2 is initially recruited onto telomeres by Speedy A, its kinase activity is further activated.

The kinase activity of Cdk2 on telomeres can be activated by Speedy A, but it is likely that other cyclins also activate the telomeric Cdk2, which in turn regulates telomere-led chromosome movements on the NE, possibly through phosphorylation of SUN1 (19, 34). It has been reported that E-type cyclins (E1 and E2) also form complexes with Cdk2 in mouse spermatocytes, and the knockout mice for *Ccne1* and *Ccne2* partially mimic the phenotypes seen in *Cdk2*$^{-/-}$ spermatocytes, suggesting the involvement of canonical cyclins in the activation of Cdk2 during meiosis (35, 36).

In recent years, several meiosis-specific structural units that mediate chromosome attachment on the telomeric end have been identified in mice, including SUN1, KASH5, TERB1/2, and MAJIN (4–6, 8). Our present study suggests that Speedy A, which was previously known solely as a Cdk activator, has a structural function in the formation of the telomere complex during meiotic prophase I in mammals. The binding of Cdk2 to Speedy A might function as the initial structural component for the assembly the telomere–NE complex. Our study thus extends the function of Speedy A and Cdk2 from just being cell cycle

kinases to now having roles as telomere component proteins during meiotic prophase I.

Materials and Methods

Detailed methods are described in *SI Materials and Methods*.

Animals. The bacterial artificial chromosome (BAC) DNA used to generate the *Spdya* targeting vector was obtained from a 129/SV mouse BAC library (Invitrogen; Bac-RP23-456J14). Exon 2 of the *Spdya* gene was targeted and flanked by a *loxP* site at its 5′ end and a *FRT-Neo-FRT-loxP* cassette on its 3′ end using bacterial recombineering, as previously reported (37). The *Spdya* targeting vector was linearized with PvuI and introduced into CJ7 ES cells by a standard electroporation method. G418 and FIAU double-resistant ES cell clones were selected and analyzed using Southern blot hybridization of StuI/PacI-digested genomic DNA. Four independently targeted ES cell clones were injected into C57BL/6 blastocysts to produce the chimeras. Mice with the $Spdya^{loxP}$ allele of C57BL/6J background were crossed with *Zp3-Cre* transgenic mice of C57BL/6J background (23, 24) to delete exon 2 and generate $Spdya^{+/-}$ mice.

Mice were housed under controlled environmental conditions with free access to water and food. Illumination was on between 6:00 AM and 6:00 PM. Polyclonal antibodies were raised as described in *Supporting Information*. Experimental protocols were approved by the regional ethical committee of the University of Gothenburg, Sweden, and by the Institutional Animal Care and Use Committee at the Biological Research Centre animal facility at Biopolis, Singapore (for mice: #140927; for rabbits: #150998).

Purification of Male Germ Cells. The cell purification of male germ cells was performed as described previously (20). The cell type and purity in each fraction were assessed based on their diameters and morphological characteristics under a light microscope.

Preparation of Female Germ Cells by FACS. The embryonic gonads were harvested from 11.5-, 14.5-, 15.5-, 17.5-, 18.5-, and 19.5-dpc embryos. Gonads were cleaned of nongonadal tissues and digested for 30 min at 37 °C with 40 U/mL collagenase. After pipetting, the GFP^{+} female germ cells were sorted by FACS in a BD Biosciences FACS AriaII cytometer. The isolated female germ cells were confirmed by their GFP fluorescence.

Exogenous Expression of *Spdya* cDNAs in Testes. Different fragments of *Spdya* were cloned into the pCAG-GFP vector (Addgene #11150). Plasmid DNA was injected into live mouse testes, and the exogenous expression was achieved by electroporation, as previously described (30). Spermatocyte cells expressing the exogenous genes were detected by immunostaining of GFP.

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