with increasing amounts of DRAP1 protein inhibited FoxH1 binding, whereas incubation with nonspecific proteins or with Dr1 had no effect (fig. S3). Finally, we assessed binding of a FoxH1-Smad2-Smad4 transcription factor complex (activin-responsive factor, ARF) (*25, 26*) to an activin/Nodal-response element in nuclear extracts from activin-treated cells. We found that addition of DRAP1 alone, or DRAP1 together with Dr1, could effectively inhibit ARF binding, whereas Dr1 alone had no detectable effect (Fig. 4F) (fig. S3). These results indicate that DRAP1, independently of Dr1, can effectively compete for FoxH1 binding to DNA.

Our loss-of-function analysis has revealed the earliest essential role for *Drap1* in embryogenesis, in repressing the activity of the Nodal signaling pathway. Although these findings do not preclude multiple subsequent functions for *Drap1*, they show that *Drap1* is not essential for numerous patterning and differentiation events at pregastrulation stages. On the basis of our protein interaction data, we propose that DRAP1 regulates Nodal signaling in vivo through an interaction between DRAP1 and FoxH1 that precludes FoxH1-Smad2-Smad4 complex binding to its cognate DNA targets. Notably, this model implies that DRAP1-mediated repression is not universally exerted by forming a complex with Dr1 and TBP, in agreement with earlier suggestions (*11, 13*).

These findings suggest that a normal function of DRAP1 is to down-modulate the transcriptional response to Nodal signaling, particularly by attenuation of its positive feedback loop. Such a mechanism is likely to be essential for Nodal, which can function as a long-range morphogenetic signal (*27*). First, *Drap1* might function in nascent mesoderm to allow specification of distinct mesoderm fates in response to differing levels of inducing signal. Second, *Drap1* might function in epiblast cells to buffer the response to mesoderm-inducing signals and maintain prospective ectoderm unresponsive to low levels of mesoderm-inducing signals. Thus, *Drap1* may represent a key component of a mechanism for limiting the spatial or temporal extent of the response to a potent morphogenetic signal.

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29. We thank R. Behringer, H. Hamada, G. Hannon, A. Joyner, R. Krumlauf, E. Lai, F. Liu, R. Lovell-Badge, G. Martin, A. McMahon, P. Paddison, J. Rossant, M. Whitman, and C. Wright for gifts of reagents and probes, N. Desai and L. Wang for technical assistance, and C. Abate-Shen for helpful discussions and comments on the manuscript. We are particularly grateful to C. Abate-Shen and L. Vales for invaluable advice on protein interaction assays and to E. Robertson for generously providing *Nodal-lacZ* mice. Supported by awards from the American Heart Association (J.D.) and the Leukemia and Lymphoma Society (J.D.), and by a postdoctoral fellowship (Y.Z.) and grants from the NIH (D.R., M.M.S.). D.R. is an Investigator of the Howard Hughes Medical Institute.

Supporting Online Material

www.sciencemag.org/cgi/content/full/298/5600/1996/DC1

Materials and Methods

Figs. S1 to S3

30 April 2002; accepted 24 October 2002

Regulation of Spermatogenesis by Testis-Specific, Cytoplasmic Poly(A) Polymerase TPAP

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Spermatogenesis is a highly specialized process of cellular differentiation to produce spermatozoa. This differentiation process accompanies morphological changes that are controlled by a number of genes expressed in a stage-specific manner during spermatogenesis. Here we show that in mice, the absence of a testis-specific, cytoplasmic polyadenylate [poly(A)] polymerase, TPAP, results in the arrest of spermiogenesis. TPAP-deficient mice display impaired expression of haploid-specific genes that are required for the morphogenesis of germ cells. The TPAP deficiency also causes incomplete elongation of poly(A) tails of particular transcription factor messenger RNAs. Although the overall cellular level of the transcription factor TAF10 is unaffected, TAF10 is insufficiently transported into the nucleus of germ cells. We propose that TPAP governs germ cell morphogenesis by modulating specific transcription factors at posttranscriptional and posttranslational levels.

Poly(A) tails of eukaryotic mRNAs are implicated in various aspects of mRNA metabolism, including transport into the cytoplasm, stability, and translational control (*1, 2*). Thus, the control of poly(A) tail length is one of the posttranscriptional regulators of gene expression. Spermatogenesis—differentiation of male germ cells—is a specialized developmental process, which is precisely regulated at the transcriptional, posttranscriptional, and translational levels (*3, 4*). In previous work, we identified a testis-specific, cytoplasmic poly(A) polymerase, TPAP (PAPβ), as a candidate molecule involved in the additional extension of poly(A) tails of preexisting mRNAs in haploid germ cells, because this gene is expressed predominantly in round spermatids (*5*).

To elucidate the role of TPAP in spermatogenesis, we produced mutant mice lacking the functional TPAP gene (*Tpap*$^{-/-}$), using homologous recombination in embryonic stem cells (*6*) (fig. S1). Analysis of testicular RNA from *Tpap*$^{-/-}$ mice revealed the absence of TPAP mRNA, and protein extracts of the mutant mouse testis completely lacked 70-kD TPAP (*6*) (fig. S1). *Tpap*$^{-/-}$ male and female mice were normal in health condition, size, and behavior. However, *Tpap*$^{-/-}$ males were

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infertile, despite the normal formation of copulation plugs in females mated. Essentially similar results were obtained in both mouse lines derived from two independent embryonic stem cell clones. *Tpap*$^{-/-}$ females showed normal fertility and produced normal average litter sizes (8.2 ± 1.4 offspring, n = 13 litters), and *Tpap*$^{+/-}$ male mice also exhibited normal fertility.

Testicular tissues of 3-month-old *Tpap*$^{-/-}$ mice showed a 45% reduction in weight (63.1 ± 7.8 mg, n = 28 testes), as compared with that of the tissues of wild-type controls (113.3 ± 10.1 mg, n = 22 mice) (*6*) (fig. S2). Histochemical analysis revealed that the diameter of seminiferous tubules in *Tpap*$^{-/-}$ mice was 15 to 20% smaller than that in wild-type mice (*6*) (fig. S2). Although round spermatids in the seminiferous tubules of the mutant mice differentiated until step 7, they failed to undergo morphological development to elongating spermatids. Spermatogenesis in *Tpap*$^{-/-}$ mice was also distinguished from that in wild-type mice by the presence of multinucleated giant cells corresponding to apoptotic cells (*7*) and the presence of abnormal cells containing vacuolated cytoplasm and/or nucleus with an increased chromosome density (Fig. 1, A and B). TUNEL (terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate-biotin nick end labeling) assay (*6*) showed a large number of apoptotic cells at the stages of spermatocytes, spermatids, and possibly spermatogonia in some seminiferous tubules of 3-month-old *Tpap*$^{-/-}$ mice, which were barely detectable in wild-type mice (Fig. 1, C and D). The 3-week-old testes containing spermatocytes as the most predominant germ cells revealed no substantial difference between *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ mice, whereas both spermiogenic arrest and an increase of apoptotic cells were found in the 5-week-old *Tpap*$^{-/-}$ testis (*6*) (fig. S2). These results indicate that the deficiency of TPAP [as well as that of CREM (*7*, *8*), TRF2 (*9*, *10*), and MIWI (*11*)] elicits the spermatid arrest at the first wave of spermatogenesis. Thus, we speculate that the increased apoptosis in germ cells of the *Tpap*$^{-/-}$ testis before the stage of round spermatids is possibly due to indirect effects of the spermiogenic arrest. The apoptotic signals may be transduced to the earlier stages of germ cells by the spermiogenic arrest. Our data show that TPAP is essential for spermatid differentiation in late spermiogenesis. As expected, no sperm was found in the epididymis and seminal fluids of *Tpap*$^{-/-}$ mice (Fig. 1, E and F), which accounts for the infertility of the mutant mice.

To examine whether the absence of TPAP

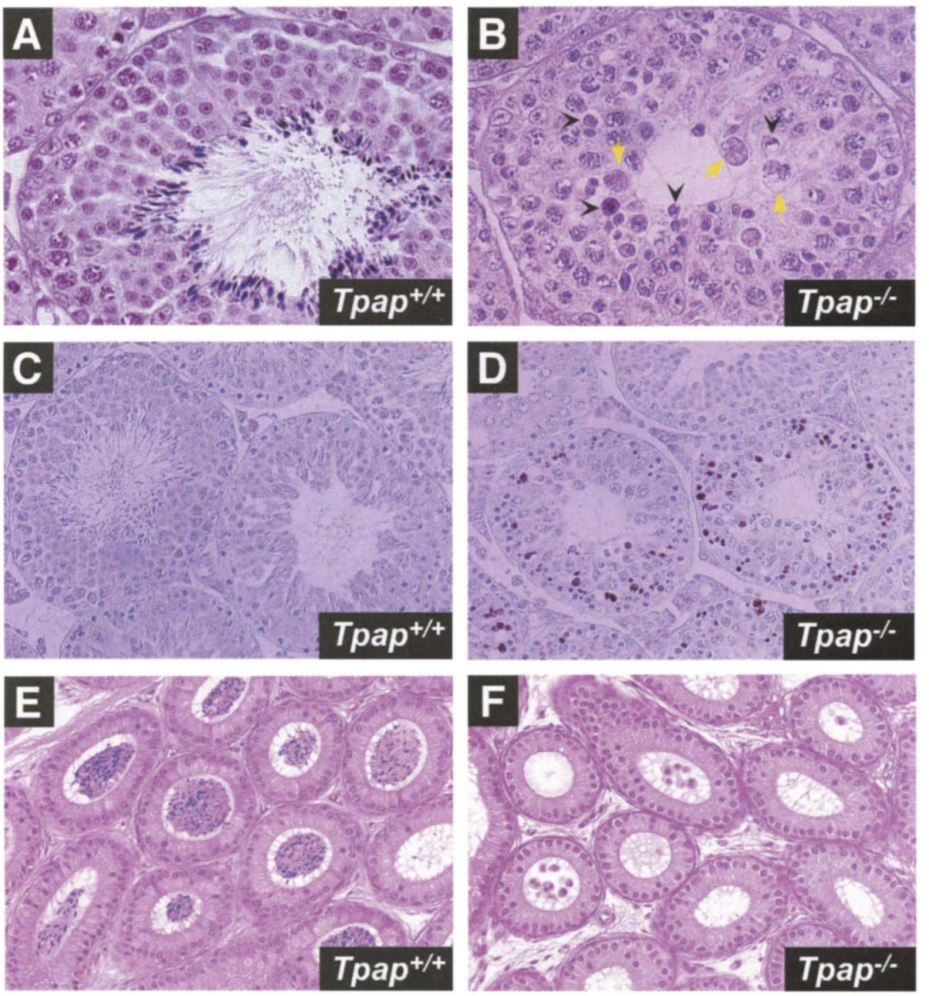

Fig. 1. Spermiogenesis arrest and germ cell apoptosis in TPAP-deficient mice. Histochemical analyses of testicular sections from 3-month-old (**A**) *Tpap*$^{+/+}$ and (**B**) *Tpap*$^{-/-}$ mice are shown (magnification, ×100). Arrows and arrowheads in (B) represent multinucleated giant cells corresponding to apoptotic cells and to abnormal cells containing vacuolated cytoplasm and/or nucleus with an increased chromosome density, respectively. (**C** and **D**) Increase of germ cell apoptosis in *Tpap*$^{-/-}$ testis. Apoptotic cells in seminiferous tubules of *Tpap*$^{+/+}$ (C) and *Tpap*$^{-/-}$ mice (D) were intensively stained by the in situ TUNEL method (*6*) (magnification, ×50). (**E** and **F**) Absence of sperm in the epididymis of *Tpap*$^{-/-}$ mice (magnification, ×50). The epididymal tubules were filled with sperm in *Tpap*$^{+/+}$ mice (**E**), whereas in *Tpap*$^{-/-}$ mice (F) they contained only a trace amount of cells, not corresponding to sperm.

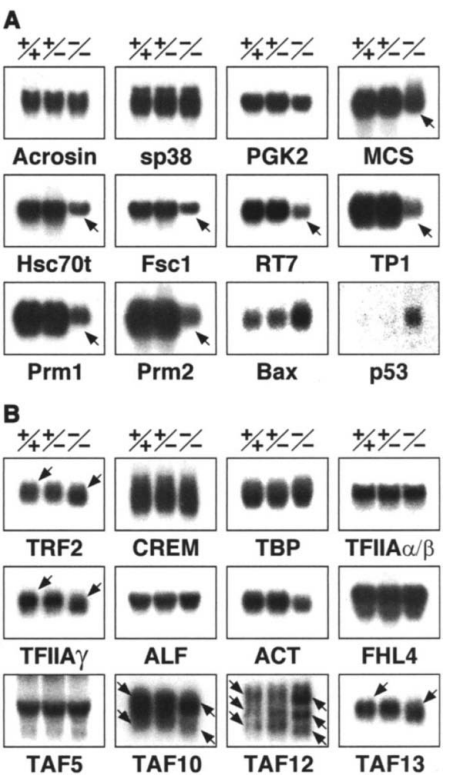

Fig. 2. Expression of testis-specific genes and transcription factor genes in TPAP-deficient mice. (**A**) Expression of 10 testis-specific and 2 apoptosis-inducing genes in *Tpap*$^{+/+}$, *Tpap*$^{+/-}$, and *Tpap*$^{-/-}$ mice. Northern blot analysis of total RNA (10 μg) from the testes of 3-month-old mice was carried out with ^{32}P-labeled DNA fragments as probes. Arrows indicate the absence of partially deadenylated, smaller forms of mRNA in *Tpap*$^{-/-}$ mice. (**B**) Expression of transcription factor genes in TPAP-deficient mouse testes. The total RNA (10 μg) from the testes of 3-month-old mice was subjected to Northern blot analysis.

affects the expression of other genes during spermatogenesis, we performed Northern blot analysis of testicular RNAs (Fig. 2A). Two genes coding for testis (sperm)–specific proteins (*6*), acrosin and sp38, were normally expressed in *Tpap*$^{-/-}$ testes, whereas the expression level of the phosphoglycerate kinase–2 (PGK2) gene in *Tpap*$^{-/-}$ testes was ~60% that in the *Tpap*$^{+/+}$ or *Tpap*$^{+/-}$ testes. Expression of these three genes starts during meiosis and continues in early round spermatids, and the transcripts are drastically diminished in elongating spermatids, except that PGK2 mRNA persists until the stages of late spermatids. Seven other genes encoding mitochondrial capsule selenoprotein MCS, heat shock protein Hsc70t, sperm fibrous sheath component Fsc1, sperm outer dense fiber protein RT7 (Odf1), transition protein TP1, and protamines (Prms) 1 and 2, which are expressed exclusively in round spermatids, showed a noticeable reduction in expression in *Tpap*$^{-/-}$ testes. Because the translational activation of these mRNAs accompanies shortening of poly(A) tails in elongating spermatids (*12*), the lack of the partially deadenylated, smaller forms of mRNAs (Fig. 2A, arrows) verifies the absence of elongating spermatids in *Tpap*$^{-/-}$ testes (Fig. 1B). Moreover, expression of the Bax and p53 genes as apoptosis-inducing genes was severalfold up-regulated in *Tpap*$^{-/-}$ testes (Fig. 2A), which may partly explain the increased apoptosis (Fig. 1D). These results demonstrate that the TPAP deficiency links to the transcriptional down-regulation of the haploid-specific genes involved in the morphogenesis of male germ cells, including flagellar formation and nuclear elongation. Indeed, the expression levels of the haploid-specific genes in round spermatids of *Tpap*$^{-/-}$ mice were 7 to 56% those of *Tpap*$^{+/+}$ mice, despite the equal expression of three genes encoding cytoskeletal actin, PGK2, and a haploid-specific coactivator of CREM, ACT, in round spermatids between *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ mice (*6*) (fig. S3).

Because *Tpap*$^{-/-}$ mice exhibit a phenotype similar to that of male mice lacking a TATA-binding protein (TBP)–related factor 2 (TRF2/TRP/TLF/TLP) (*9*, *10*), we hypothesized that reduced expression of haploid-specific genes in *Tpap*$^{-/-}$ mice (Fig. 2A) may be explained by a possible abnormality of TRF2 or other transcription factors. Thus, we examined gene expression of 12 transcription factors in *Tpap*$^{-/-}$ testes, including TRF2 (*9*, *10*), CREM (*7*, *8*), and general factors involved in RNA polymerase II–mediated transcription (*13*, *14*). No significant difference in gene expression was found among *Tpap*$^{+/+}$, *Tpap*$^{+/-}$, and *Tpap*$^{-/-}$ mice, except that ACT expression in *Tpap*$^{-/-}$ testes was ~50% that in *Tpap*$^{+/+}$ or *Tpap*$^{+/-}$ testes, presumably owing to the absence of elongating spermatids (Fig. 2B). However, the sizes of mRNAs for TRF2, TFIIAγ, TAF10, TAF12, and a testis-specific form of TAF13 in *Tpap*$^{-/-}$ testes were 50 to 100 nucleotides (nt) smaller than those in *Tpap*$^{+/+}$ and *Tpap*$^{+/-}$ testes (Fig. 2B, arrows). These data imply that TPAP acts on cytoplasmic polyadenylation of preexisting mRNAs for specific transcription factor genes.

To examine the above possibility, we analyzed the sizes of mRNA poly(A) tails by Northern blotting after deadenylation of mRNAs by ribonuclease H (RNase H) in vitro (*5*, *15*) (Fig. 3A) or by a polymerase chain reaction–based poly(A) test (*6*) (fig. S4). The poly(A) length in sp38 mRNA was identical in pachytene spermatocytes or round spermatids between *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ mice. In mRNAs coding for TRF2, TFIIAγ, TAF10, TAF12, and TAF13, the poly(A) tails all underwent incomplete elongation of 50 to 100 nt solely in round spermatids of *Tpap*$^{-/-}$ mice, although both poly(A) lengths in pachytene spermatocytes and expression levels in pachytene spermatocytes and round spermatids were similar in *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ mice. The poly(A) lengths of TBP and TAF15 mRNAs in round spermatids were identical in *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ mice (*16*). Therefore, we conclude that mRNAs of particular transcription factors, including TRF2, TFIIAγ, TAF10, TAF12, and TAF13,

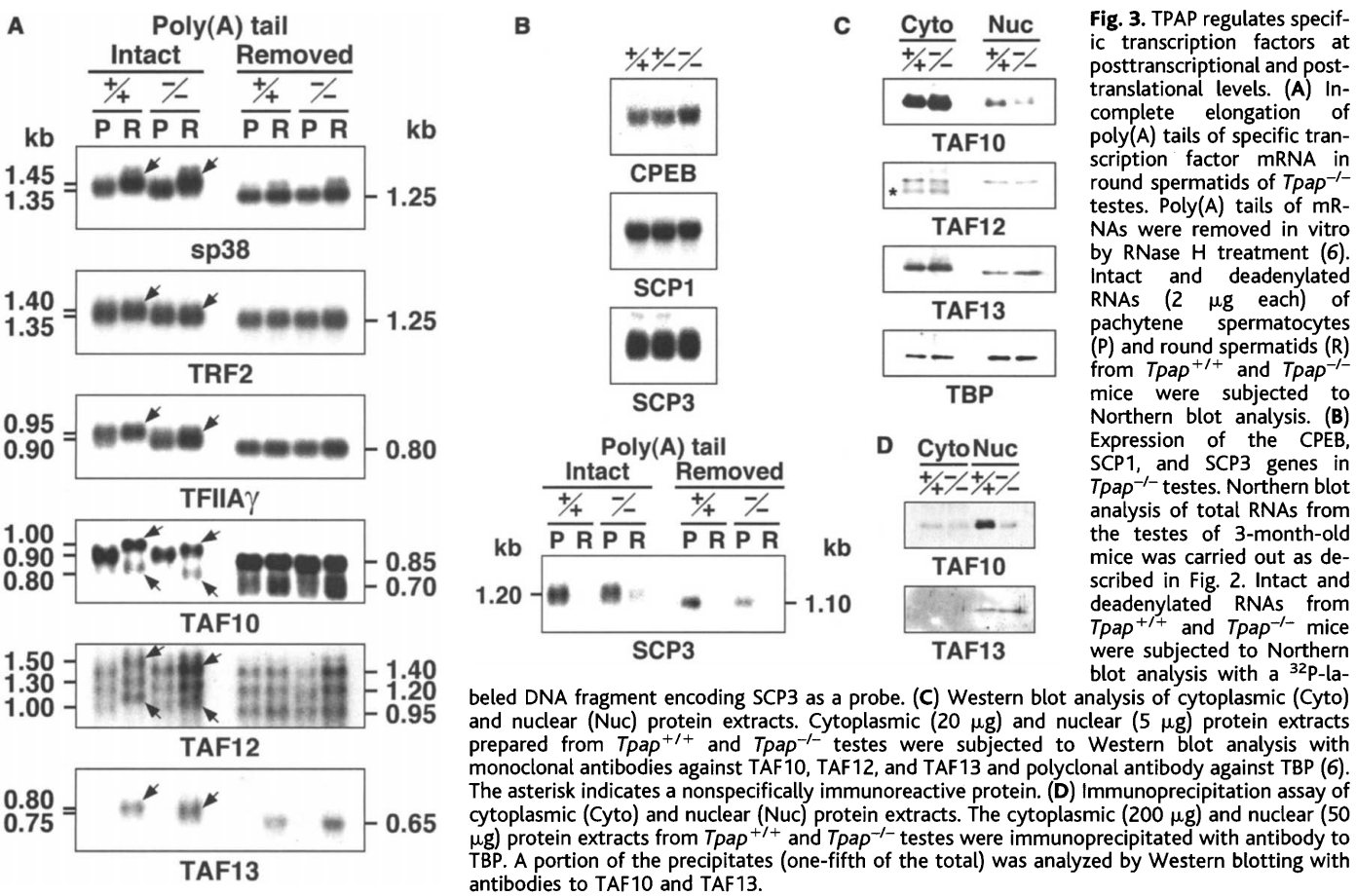

Fig. 3. TPAP regulates specific transcription factors at posttranscriptional and post-translational levels. (**A**) Incomplete elongation of poly(A) tails of specific transcription factor mRNA in round spermatids of *Tpap*$^{-/-}$ testes. Poly(A) tails of mRNAs were removed in vitro by RNase H treatment (*6*). Intact and deadenylated RNAs (2 μg each) of pachytene spermatocytes (P) and round spermatids (R) from *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ mice were subjected to Northern blot analysis. (**B**) Expression of the CPEB, SCP1, and SCP3 genes in *Tpap*$^{-/-}$ testes. Northern blot analysis of total RNAs from the testes of 3-month-old mice was carried out as described in Fig. 2. Intact and deadenylated RNAs from *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ mice were subjected to Northern blot analysis with a ^{32}P-labeled DNA fragment encoding SCP3 as a probe. (**C**) Western blot analysis of cytoplasmic (Cyto) and nuclear (Nuc) protein extracts. Cytoplasmic (20 μg) and nuclear (5 μg) protein extracts prepared from *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ testes were subjected to Western blot analysis with monoclonal antibodies against TAF10, TAF12, and TAF13 and polyclonal antibody against TBP (*6*). The asterisk indicates a nonspecifically immunoreactive protein. (**D**) Immunoprecipitation assay of cytoplasmic (Cyto) and nuclear (Nuc) protein extracts. The cytoplasmic (200 μg) and nuclear (50 μg) protein extracts from *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ testes were immunoprecipitated with antibody to TBP. A portion of the precipitates (one-fifth of the total) was analyzed by Western blotting with antibodies to TAF10 and TAF13.

are the target molecules that undergo the additional poly(A) extension by TPAP in round spermatids.

It has been demonstrated that the deficiency of cytoplasmic polyadenylation element–binding protein (CPEB) results in the arrests of oogenesis and spermatogenesis at the pachytene stages (*17*). Although expression of two cytoplasmic polyadenylation element–containing mRNAs coding for the synaptonemal complex proteins SCP1 and SCP3 are unaffected by the CPEB deficiency, the poly(A) tails are reduced in length, and the protein products are missing in CPEB-deficient mice. In this study, the expression levels and sizes of CPEB, SCP1, and SCP3 mRNAs in *Tpap*$^{-/-}$ testes were similar to those in *Tpap*$^{+/+}$ and *Tpap*$^{+/-}$ testes (Fig. 3B). Moreover, no significant difference of the poly(A) tail size of SCP3 mRNA in pachytene spermatocytes was found between *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ mice. These data suggest that CPEB is not involved in the TPAP-mediated cytoplasmic polyadenylation. The null mutation of the TPAP gene does not affect the size or expression level of mRNAs encoding ovary-specific zona pellucida 1 (ZP1) and ZP2 (*6*) (fig. S3), verifying the testis-specific function of TPAP.

Westen blot analysis of cytoplasmic and nuclear protein extracts from testicular tissues revealed that the level of TAF10 was reduced only in the nuclear fraction of *Tpap*$^{-/-}$ testes, whereas TBP, TAF12, TAF13, and TRF2 (*16*) were equally present in the cytoplasmic or nuclear fractions of *Tpap*$^{+/+}$ and *Tpap*$^{-/-}$ testes (Fig. 3C). The specific reduction of TAF10 was verified by immunoprecipitation analysis of the nuclear extracts with antibody to TBP (Fig. 3D). These data demonstrate that TPAP affects the transport of at least TAF10 into the nucleus, possibly by additional polyadenylation-dependent translational activation of dormant mRNA encoding a transporter protein or possibly by TPAP itself. Moreover, the poly(A) tails of TAF10, TAF12, and TAF13 mRNAs are unlikely to contribute to stability and translational control, because the levels of these three mRNAs and cytoplasmic proteins in *Tpap*$^{-/-}$ testes are comparable to those in *Tpap*$^{+/+}$ testes, despite the incomplete elongation of poly(A) tails (Fig. 3, A and C).

Several TAFs, including TAF10, as a component of the TFIID complex have been demonstrated to be dispensable for general RNA polymerase II–mediated transcription and to be essential for selective expression of specific genes (*14*, *18*–*22*). Because the TFIID complex containing TAF10 is severely impaired in *Tpap*$^{-/-}$ testes by insufficient transport of TAF10 into the nucleus (Fig. 3, C and D), it is conceivable that TAF10 may play an important role in the expression of a subset of haploid-specific genes, possibly as a CREM coactivator (*23*), required for the morphogenetic program during spermatogenesis. However, a small amount of TAF10, which probably forms the functional TFIID complex, seems to be still present in the nucleus of round spermatids in *Tpap*$^{-/-}$ mice (Fig. 3, C and D). Even if this is so, round spermatids appear to be incapable of producing enough mRNA to advance cell morphogenesis, because gene transcription (RNA synthesis) ceases around step-10 spermatids in the mouse (*24*). Our study shows a direct link between the deficiency of a cytoplasmic poly(A) polymerase, TPAP, and the arrest of mouse spermiogenesis, providing information on the regulation of haploid-specific genes by cytoplasmic polyadenylation in male germ cells.

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25. We thank L. Tora, I. Davidson, and T. Tamura for providing antibodies. This study was partly supported by Grant-in-Aids for Scientific Research on Priority Areas (A) and (B), Scientific Research (A), and Exploratory Research from the Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science and Technology in Japan.

Supporting Online Material

www.sciencemag.org/cgi/content/full/298/5600/1999/DC1
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3 June 2002; accepted 16 October 2002

A Role for the Protease Falcipain 1 in Host Cell Invasion by the Human Malaria Parasite

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Cysteine proteases of *Plasmodium falciparum* are required for survival of the malaria parasite, yet their specific cellular functions remain unclear. We used a chemical proteomic screen with a small-molecule probe to characterize the predominant cysteine proteases throughout the parasite life cycle. Only one protease, falcipain 1, was active during the invasive merozoite stage. Falcipain 1–specific inhibitors, identified by screening of chemical libraries, blocked parasite invasion of host erythrocytes, yet had no effect on normal parasite processes such as hemoglobin degradation. These results demonstrate a specific role for falcipain 1 in host cell invasion and establish a potential new target for antimalarial therapeutics.

Malaria is a devastating disease that affects 300 to 500 million people and kills about 2 million people per year. Currently, quinolines and antifolates are the most common drugs for disease prevention and cure. However, multidrug resistance is a major issue, highlighting the need for new antimalarial drugs to combat this parasite. Proteases represent one of the largest families of potential therapeutic targets, and cysteine proteases have been shown to be essential for the survival of several human parasites (*1*–*3*). Cysteine proteases have been specifically implicated in several cellular functions during the *P. falciparum* life cycle, including hemoglobin degradation (*4*–*5*), cleavage of red blood cell ankyrin to

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