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Cyclin T2 Is Essential for Mouse Embryogenesis▽†

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The positive transcription elongation factor b (P-TEFb) is essential for the elongation of transcription and cotranscriptional processing by RNA polymerase II. In mammals, it contains predominantly the C-type cyclin cyclin T1 (CycT1) or CycT2 and cyclin-dependent kinase 9 (Cdk9). To determine if these cyclins have redundant functions or affect distinct sets of genes, we genetically inactivated the CycT2 gene (*Ccnt2*) using the β-galactosidase–neomycin gene (β-*geo*) gene trap technology in the mouse. Visualizing β-galactosidase during mouse embryogenesis revealed that CycT2 is expressed abundantly during embryogenesis and throughout the organism in the adult. This finding was reflected in the expression of CycT2 in all adult tissues and organs. However, despite numerous matings of heterozygous mice, we observed no CycT2$^{-/-}$ embryos, pups, or adult mice. This early lethality could have resulted from decreased expression of critical genes, which were revealed by short interfering RNAs against CycT2 in embryonic stem cells. Thus, CycT1 and CycT2 are not redundant, and these different P-TEFb complexes regulate subsets of distinct genes that are important for embryonic development.

Eukaryotic transcription by RNA polymerase II (RNAPII) is regulated at several distinct steps, which include initiation, promoter clearance, elongation, and cotranscriptional processing of primary transcripts (19, 25, 27). Of these, elongation is regulated by the positive transcription elongation factor b (P-TEFb), which contains predominantly the C-type cyclin cyclin T1 (CycT1) or CycT2 and cyclin-dependent kinase 9 (Cdk9). All these different P-TEFb complexes phosphorylate serines at position 2 (S2) in the C-terminal domain (CTD) of RNAPII, as well as components of the negative transcription elongation factor, which contains minimally the DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole) sensitivity-inducing factor (DSIF) and the negative elongation factor. These posttranslational modifications exchange basal transcription factors for splicing and polyadenylation machineries on RNAPII, as well as modify DSIF for productive elongation (25).

Although these P-TEFb complexes can phosphorylate the CTD and lead to transcriptional elongation when recruited to RNAPII via heterologous nucleic acid-tethering systems, it is not clear whether they have redundant or unique functions in cells (18, 33). Thus far, CycT1, which is the most abundant of these cyclins, has been implicated as the coactivator of the transcriptional transactivator Tat from human immunodeficiency virus, RelA from NF-κB, class II transactivator, the protooncogene c-*myc*, several members of the steroid hormone receptor family, and the autoimmune regulator AIRE (3, 8, 14–16, 24, 29, 36). Moreover, Runx1, which is the active repressor of CD4 expression in double-negative thymocytes, decoys CycT1 away from the CD4 promoter, thus keeping the engaged RNAPII from elongating on this gene (13). On the other hand, CycT2 has been implicated as the coactivator of MyoD, pRb, and ENL (23, 31, 32). However, except for Tat, it has not been ruled out formally that these two cyclins cannot substitute for one another.

Besides these specific interactions, little is known about the roles of different P-TEFb complexes during development and in the adult organism. Thus far, only the genetic inactivation of CycT1 has been reported, but with CycT1$^{-/-}$ mice still expressing small amounts of the wild-type (WT) protein, only minor immunological defects were observed (24). Moreover, in *Caenorhabditis elegans*, the genetic inactivation of CycT1 or CycT2 alone had no effect, whereas inhibiting both cyclins led to the same severe phenotype as the genetic inactivation of RNAPII itself (28). Given these findings, we wanted to determine if these different P-TEFb complexes played redundant or unique roles in mammals. To this end, we genetically inactivated CycT2, which led to an embryonic-lethal phenotype. In this report, we present our findings with our CycT2$^{-/-}$ mice and stem cells.

MATERIALS AND METHODS

Generation of CycT2$^{+/-}$ mice. Embryonic stem (ES) cells (clone W048F02-04304) bearing a β-galactosidase–neomycin (β-geo) trap in the CycT2 gene (CycT2.β-geo), were obtained from the German Gene Trap Consortium (30). Morula-ES cell aggregations were carried out by the Mouse Genetics Core at the University of California at San Francisco (UCSF). They were injected into superovulated C57BL/6 morulas, and chimeric mice were generated. Chimeric males were then bred with C57BL/6 females, and heterozygous animals were identified by PCR genotyping. Staging of embryos for LacZ staining was also performed. After the breeding was set up, females were examined for vaginal plugs, and this developmental stage was set as embryonic day 0.5 (E0.5). Midgestation and preimplantation embryos at various stages were obtained by dissecting the uterine horn or the oviducts. The mice were maintained in accordance with UCSF regulations for the humane use of animals in research.

Genotyping. To distinguish WT and trapped (TR) CycT2.β-geo genes, one common forward primer (WTf, 5′-CCAAGTGATCGCCTCCAAGTTATCAG G-3′) and two specific reverse primers (TRr, 5′-ATGCCCAGCCAAAATCTT TCTGGCCTC-3′, and WTr, 5′-GGATGGTAGTAGGAATCTCTACCAGGT-3′) were designed. Direct sequencing revealed the expected sequences of these

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PCR amplicons. They also validated the integration site of β-geo within intron 7 of the CycT2 gene. Genomic DNA was isolated from mouse tails with the REDExtract-N-Amp tissue PCR kit (Sigma, St. Louis, MO).

Western blotting. For detection of the fusion protein between the N terminus of CycT2 and the β-Geo protein in donor ES cells (W048F02-04304) or for the detection of decreased expression of CycT1 and CycT2 after RNA interference, ES cells were collected in lysis buffer (10 mM Tris-HCl, pH 8.0, 25 mM EDTA, 1% sodium dodecyl sulfate), sonicated, and cleared by centrifugation at 10,000 × *g* for 10 min at 4°C. Concentrations of total proteins were determined with the TMB Substrate Kit (Pierce, Rockford, IL). Lysates were diluted to the same concentration, mixed with Laemmli sample buffer, and boiled for 5 min. Western blotting was performed with anti-β-galactosidase (Z378A; Promega, Madison, WI) or anti-CycT1, -CycT2, and -Cdk9 (sc-10750, sc-12421, and sc-484; Santa Cruz Biotechnology, Santa Cruz, CA).

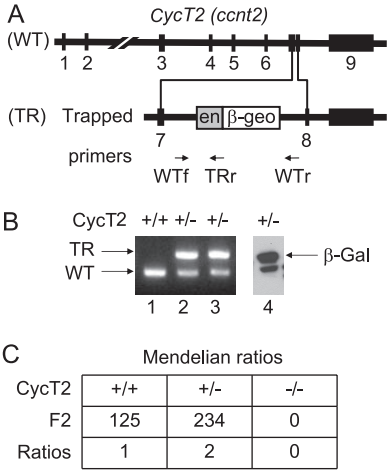
For the assessment of protein levels of subunits of P-TEFb in diverse mouse organs, the heart, brain, lung, skeletal muscle, spleen, kidney, thymus, liver, testis, and pancreas were isolated from WT mice, lysed on ice in lysis buffer, sonicated, and cleared by centrifugation at 10,000 × *g* for 10 min at 4°C. Concentrations of total proteins were determined with the TMB Substrate Kit (Pierce). Lysates were diluted to the same concentration, mixed with Laemmli sample buffer, and boiled for 5 min. Western blotting analyses were carried out with anti-CycT1, -CycT2, and -Cdk9 antibodies (sc-10750, sc-12421, and sc-484; Santa Cruz Biotechnology).

LacZ staining. Embryos at a given developmental stage were removed from the uteruses of pregnant heterozygous female mice, after which supporting and extraembryonic tissues were dissected. Whole embryos were washed in cold phosphate-buffered saline and immediately transferred to 0.2% glutaraldehyde in phosphate buffer (23 mM $NaH_2PO_4 \cdot H_2O$, 72 mM Na_2HPO_4, 5 mM EGTA, and 2 mM $MgCl_2$) for 15 min on ice. The embryos were then washed three times with washing buffer (23 mM $NaH_2PO_4 \cdot H_2O$, 72 mM Na_2HPO_4, 2 mM $MgCl_2$, 0.01% deoxycholate, and 0.02% NP-40) for 15 min on ice. Finally, the embryos were transferred to staining solution [washing buffer supplemented with 5 mM $K_4Fe(CN)_6 \cdot 3H_2O$, 5 mM $K_3Fe(CN)_6$, and 1 mg/ml X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)] and incubated at 30°C overnight.

Knockdown of CycT1 and CycT2 and microarray analyses. Feeder-free mouse E14 ES cells were maintained in the presence of LIF (Millipore). Cells in 6-cm plates were transfected with 200 pmol of individual short interfering RNAs (siRNAs) in duplicate with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Control siRNA contained an RNA duplex that did not match any sequence from the mouse genome (Integrated DNA Technologies, San Diego, CA). The siRNA duplex against CycT1 contained 5′-UUCCGAAUACGUUUCAGCCUGCUUGGA-3′ (sense) and 5′-AAGGC UUAUGCAAAGUCGGACGAAC-3′ (antisense) sequences. Three siRNAs from Santa Cruz Biotechnology targeted different regions of mouse CycT2 mRNA. Forty-eight hours after the transfection, the cells were harvested. Half of the cells were lysed with protein-loading buffer. We determined the concentrations of cell lysates with the bicinchoninic acid protein assay kit (Pierce). Equal amounts of protein were loaded for Western blotting. Total RNA was also extracted from the remaining cells with Trizol (Invitrogen), followed by RNeasy column purification (Qiagen). The concentration and quality of RNA were determined with an Agilent 2100 bioanalyzer, and all the purified RNA samples had scores of more than 9.80. Total RNA from each siRNA knockdown was amplified with an Illumina TotalPrep RNA Amplification kit (Ambion). The amplified RNA was examined with an Agilent 2100 bioanalyzer before microarray analysis to ensure that similar amplification efficiencies were achieved. Microarray analysis with MouseWG-6 v2.0 chips (Illumina) was carried out with the help of the UCSF Microarray Core. Data were extracted with the BeadStudio Gene Expression Module (Illumina), and gene ontology analysis was carried out using DAVID (http://david.abcc.ncifcrf.gov/) and grouped based on the Kyoto Encyclopedia of Genes and Genomes pathway.

RESULTS

Generation and characterization of CycT2$^{+/-}$ mice. To gain more insight into the physiological function of CycT2, we utilized mouse ES cells (W048F02-04304) with a β-*geo* gene trap insertion (pT1ATGbetageo) in intron 7 from the German Gene Trap Consortium (Fig. 1A) (30). After implantation into mouse blastocysts, we achieved stable germ line transmission of our transgene (Fig. 1B). Indeed, the trap could be detected easily using primers to intron 7 (WTf) and β-geo (TRr) sequences (Fig. 1A). This insertion created a fusion protein between the N terminus of CycT2 and the β-Geo protein. We detected this fusion protein, which consisted of 235 and 1,323 residues from CycT2 and β-Geo (170 kDa), respectively, with anti β-galactosidase antibodies by Western blotting (Fig. 1B, right). Of note, the fusion protein created a loss-of-function allele, which contained only the N terminus of CycT2 but lacked the middle and C-terminal regions of the protein. Importantly, in the absence of complete cyclin boxes, this truncated CycT2 protein neither binds nor exerts a dominant-negative phenotype on Cdk9 (17). Indeed, we demonstrated previously that these missing sequences were essential for the function of this cyclin (17).



C

Mendelian ratios

CycT2	+/+	+/-	-/-
F2	125	234	0
Ratios	1	2	0

FIG. 1. Analysis of CycT2$^{+/-}$ ES cells and mice. (A) Schematic representation of WT and TR (CycT2.β-geo) alleles. The integration of the gene trap vector containing part of intron 1 and exon 2 of the engrailed 2 (en) gene and β-galactosidase–neomycin gene (β-*geo*) into intron 7 inactivated the CycT2 gene (*Ccnt2*). The arrows under the scheme represent DNA primers WTf, TRr, and WTr, which were used in panel B. (B) Representative result of PCR genotyping and protein expression. (Left) Genomic DNA was isolated from E12.5 embryos and genotyped with two sets of primers—WTf and WTr, and WTf and TRr—which distinguished WT from TR alleles of CycT2. (Right) The fusion protein between CycT2 and β-Geo was detected in parental W048F02-04304 ES cells by Western blotting. The lower band represents a degradation product. β-Gal, β-galactosidase. (C) Ratios of genotypes from heterozygous breedings. A total of 125 WT (CycT2$^{+/+}$) and 234 heterozygous (CycT2$^{+/-}$) mice were obtained and genotyped. No homozygous (CycT2$^{-/-}$) mice were observed.

Although our WT and heterozygous CycT2$^{+/-}$ mice were healthy, appeared normal, and reproduced well, their mating did not generate any homozygous CycT2$^{-/-}$ mice (Fig. 1C). From analyzing 359 pups, we noted the Mendelian ratio of 1:2 for WT to CycT1$^{+/-}$ mice, which indicates that the two strains have equal fitness. However, the predicted ratio (or 25%) of homozygous CycT2$^{-/-}$ mice did not materialize. We conclude that our trapped allele does not encode a functional protein and that CycT2 is essential for mouse development.

CycT2 is expressed abundantly throughout mouse embryogenesis. To define further the function of CycT2 during mouse development, embryos at E7.5, E9.5, E10.5, and E14.5 or more from matings of heterozygous CycT2$^{+/-}$ mice were genotyped (Table 1). Importantly, no CycT2$^{-/-}$ embryos were detected in any given time period, suggesting that CycT2 might play an

TABLE 1. Genotyping of mutant CycT2 embryos and pups

Embryo stage	No. of embryos screened/genotyped[a]		
	CycT2$^{+/+}$	CycT2$^{+/-}$	CycT2$^{-/-}$
>E15	4	11	0
E14.5	1	2	0
E10.5	7	9	0
E9.5	4	7	0
E7.5	11	14	0
Blastocyst (E3.5)	11	28	0
Morula	1	2	0
4-cell	2	4	0

[a] Total numbers of embryos screened/genotyped from all litters examined.

important role even prior to implantation. To access this period of mouse development, E3.5 aggregates, which represent the blastocyst stage, were isolated from pregnant females. Again, no CycT2$^{-/-}$ embryonic tissue was found, supporting an important role for CycT2 in early mouse development (Table 1).

CycT2 is expressed ubiquitously during mouse embryogenesis. To elucidate the spatiotemporal expression of CycT2 in developing mouse embryos, LacZ staining was monitored in E7.5, E8.5, E9.5, E11.5, and E14.5 embryos. Since the CycT2 promoter directed the expression of the hybrid CycT2.β-Geo protein (Fig. 1A), this staining reflects the transcription of CycT2 at any given developmental stage. As presented in Fig. 2, β-galactosidase was expressed in the embryonic region (E6.5) (data not presented), the embryonic ectoderm (E7.5), the forming brain and neural tube (E8.5/9.5), and throughout the forming tissues and organs (E11.5 and E14.5) (Fig. 2). This staining suggests further that this P-TEFb complex (CycT2-Cdk9) plays an important role throughout mouse embryogenesis.

CycT2 is expressed in all tissues and organs of the adult mouse. To extend these findings to the adult organism, we prepared lysates from various mouse tissues and organs. Western blotting was performed with anti-CycT1, -CycT2, and -Cdk9 antibodies (Fig. 3). Since previous studies had revealed that CycT2 is the coactivator of MyoD (32), it was not surprising that we found high levels of expression in skeletal muscle (Fig. 3). Significant levels of CycT2 were also found in the heart, brain, kidney, liver, testis, and pancreas, followed by lesser amounts in the lung, spleen, and thymus (Fig. 3, middle). Although CycT2 is spliced differentially to produce CycT2a and CycT2b, our Western blots did not differentiate between them. In addition, levels of Cdk9 did not reflect those of CycT2 (Fig. 3, bottom), which suggests that CycT1 is the predominant cyclin in all P-TEFb complexes in the organism. Importantly, this pattern of expression parallels that found in a previous study (2). In contrast, since CycT1 is found abundantly in hematopoietic cells, its highest levels were observed in the lung, spleen, and thymus (Fig. 3, top) (13, 20, 38). Interestingly, both cyclins were expressed abundantly in organs such as the testis, which contain many proliferating cells.

P-TEFb complexes containing CycT1 and CycT2 perform redundant and nonredundant functions in ES cells. Since the genetic inactivation of CycT2 had such a dramatic effect on mouse development, we decided to study the functions of CycT1 and CycT2 in ES cells. These cells form the inner cell

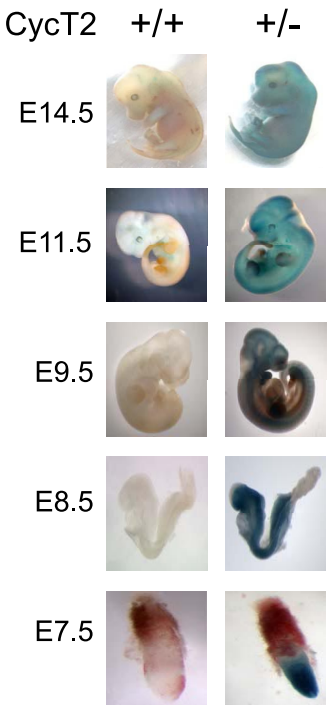


FIG. 2. Expression of CycT2 during mouse midgestation and early embryogenesis. LacZ staining of heterozygous embryos was used to visualize the spatiotemporal expression of CycT2.β-Geo, which reflects transcription from the Cyc T2 promoter. WT (+/+) and heterozygous (+/−) mouse embryos were isolated at the indicated embryonic days after conception, fixed with glutaraldehyde, and stained with X-Gal. CycT2 was expressed in all forming organs and supporting tissues in E10.5 to E14.5 embryos. Interestingly, the expression of CycT2 was mostly localized to the embryonic ectoderm and the forming brain and neural tube in E7.5 and E8.5/E9.5 embryos, respectively.

mass of blastocysts and can differentiate into various cell types. We used specific siRNAs to decrease the levels of CycT1 and CycT2 (Fig. 4A). We analyzed RNA expression profiles after only 48 h so as to minimize secondary effects by transcription factors, whose expression depends on these different P-TEFb complexes. By densitometry, better than 90% reduction of both cyclins was achieved, and specific siRNAs for one cyclin did not affect the expression of the other (Fig. 4A, lanes 2 and

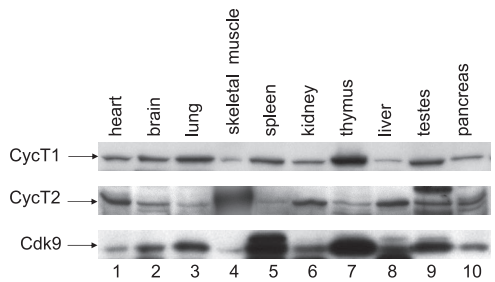


FIG. 3. CycT2 is expressed in all tissues and organs in the adult mouse. Expression patterns of subunits of different P-TEFb complexes in tissues and organs from 3-month-old mice were probed with appropriate antibodies by Western blotting. Presented are data with anti-CycT1, -CycT2, and -Cdk9 antibodies.

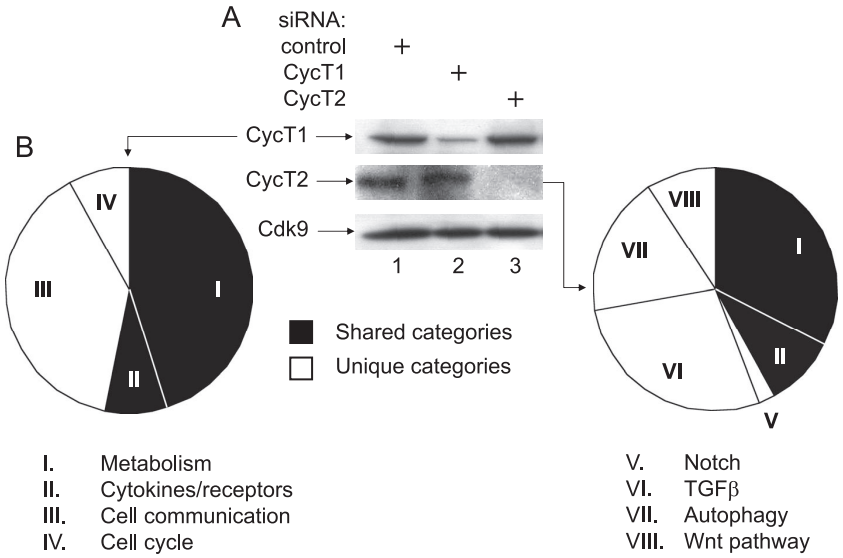


FIG. 4. Genes regulated by CycT1 and CycT2 in mouse ES cells. (A) Knockdown of CycT1 and CycT2 in ES cells. Specific siRNAs were used to decrease the expression of CycT1 and CycT2 in ES cells. Western blotting with antibodies against CycT1, CycT2, and Cdk9 detected the levels of these proteins. The arrows on the left point to specific proteins. (B) Mouse whole-genome RNA expression profiles between ES cells with siRNA species against CycT1 and CycT2. Forty-nine and 43 differentially expressed genes are depicted by pie charts for CycT1 and CycT2 knockdowns, respectively. Their expression differed more than 1.5-fold between control and CycT1 and CycT2 knockdown ES cells. Ontologic analyses were carried out using DAVID (http://david.abcc.ncifcrf.gov/) and grouped based on the Kyoto Encyclopedia of Genes and Genomes pathway. The black and white slices denote shared and unique categories of proteins, respectively. The supplemental material provides a list of genes that showed more than 1.5-fold reduction in their mRNA levels when CycT1 or CycT2 was knocked down in a descending order of reduction.

3). Levels of Cdk9 were reduced slightly, which reflects the almost equivalent levels of CycT1 and CycT2 in these cells. Similar downregulation of Cdk9 in vivo or in cultured cell lines was also reported by other researchers (24, 37).

Global gene expression profiles were examined with mouse whole-genome microarrays. If the functions of CycT1 and CycT2 were redundant, the expression of a majority of genes should not be altered. Out of ~8,600 genes expressed in ES cells (with signals twofold above background levels), only 59 or 76 genes demonstrated a 1.5-fold reduction in the amounts of mRNA when CycT1 or CycT2 was knocked down, respectively (see the supplemental material). Gene ontology analyses demonstrated that these knockdowns affected a distinct set of cellular genes and pathways (Fig. 4B; see the supplemental material). For CycT1 knockdown, 22 of 49 annotated genes were involved in fatty acid and glucose metabolism (45%). For CycT2 knockdowns, 14 out of 43 genes affected purine and glucose metabolism (32%) (see the supplemental material). Interestingly, although glucose metabolism was affected in both cases, the genes that were targeted were different. Knockdown of CycT1, but not CycT2, also affected genes involved in cell communication (39%), while knockdown of CycT2, but not CycT1, affected pathways important for early embryogenesis, including transforming growth factor β (TGF-β), Notch, Wnt signaling, and autophagy (58%) (Fig. 4B; see the supplemental material). From all these data, we conclude that different P-TEFb complexes are mostly redundant but that there is a set of genes that are specifically under the control of CycT1 or CycT2, some of which are also critical for the early development of the mouse embryo.

DISCUSSION

In this report, we analyzed the function of CycT2 for the organism. Since we found no CycT2$^{-/-}$ embryos or blastocysts, its genetic inactivation must have led to preimplantation lethality. Nevertheless, the nature of the β-geo trap allowed us to follow the expression of CycT2 during embryogenesis. We found it expressed in all organs, which was confirmed with tissues from adult mice by Western blotting. Finally, we found that CycT1 and CycT2 are also required for the expression of distinct and important genes in ES cells. We conclude that different cyclins involved with P-TEFb serve many redundant and some nonredundant functions in the embryogenesis and early development of mammals.

Since these cyclins are redundant in *C. elegans*, it was surprising that genetic ablation of CycT2 led to embryonic lethality in mice. In *C. elegans*, genetic inactivation of CycT1 or CycT2 had no apparent phenotype (28). In contrast, their combined ablation had the same phenotype as the genetic inactivation of RNAPII, which resulted in early embryonic lethality. In addition, mice with greatly reduced levels of CycT1 are viable, albeit with several immunological defects (24). However, given their nonoverlapping critical target genes, as evidenced by our siRNA experiments (Fig. 4B), we expect that the complete genetic inactivation of CycT1 would also lead to an embryonic-lethal phenotype. Thus, both major P-TEFb complexes appear to be essential for the development and viability of mammals.

It is most likely that the lack of CycT2 directly caused this preimplantation lethality. When comprehensive analyses of expressed sequence tags were carried out in different develop-

mental stages of female gametogenesis, i.e., in oocytes prior to meiotic maturation, fully grown eggs, and two-cell embryos, an intriguing expression pattern of P-TEFb transcripts was revealed (11). Importantly, CycT1 and CycT2 mRNA species were found in the fully grown egg, but not in the two-cell embryo. Since, substantial amounts of stored RNA are destroyed shortly after fertilization (12, 26), it is likely that CycT1 and CycT2 transcripts are also lost at this stage. However, the expression of these cyclins must commence soon thereafter, as S2 in the CTD of RNAPII is heavily phosphorylated during zygotic gene activation, when transcription becomes a requirement for further development (4). However, with CycT2$^{-/-}$ eggs, once maternal P-TEFb complexes are depleted and no new CycT2 is synthesized, this change in the transcriptional program could have lethal consequences. As a result, no blastocysts should be observed and all further embryonic development should stop.

Transcription of most genes by RNAPII requires P-TEFb (7). The best-characterized example is with human immunodeficiency virus, where the transcriptional transactivator Tat binds CycT1 (and not CycT2) on the transactivation response RNA stem-loop, which recruits and/or repositions Cdk9 to modify RNAPII for elongation and cotranscriptional processing of viral transcripts (35). Subsequent studies demonstrated that other activators also recruit P-TEFb via their interactions with CycT1 or CycT2 (3, 6, 10, 15–17). Among them, ENL binds CycT2 (23). Of note, attempts to inactivate the ENL gene also found no ENL$^{-/-}$ embryos at E8.5 or earlier (9), which might provide another clue to the demise of our CycT2$^{-/-}$ mice. Further candidates might have been revealed by our microarray data. We chose mouse ES cells, not only because of early embryonic lethality of our CycT2$^{-/-}$ blastocysts, but because most developmental genes are expressed in these cells. Indeed, we found a subset of genes that were differentially regulated by P-TEFb complexes, which contained CycT1 or CycT2. Since the levels of transcripts encoding Oct-4, Nanog, and Sox2, which maintain stem cells, were unaltered, the observed effects with our siRNA species were not secondary to ES cell differentiation. In addition, our transfected cells appeared morphologically normal and stained positive for alkaline phosphatase. Since siRNA against CycT1 did not affect levels of CycT2, and vice versa, these effects were also cyclin specific. Importantly, levels of CycT1 and CycT2 were decreased substantially. We conclude that CycT1 and CycT2 serve redundant and nonredundant functions in mammals and that some of their target genes are essential for early embryonic development.

Which additional target genes could lead to this phenotype? Gene ontology analyses revealed that a reduction in CycT2 affected mostly TGF-β and Wnt signaling pathways, as well as autophagy. The most affected genes were those for Lefty 1 and Lefty 2, members of the TGF-β superfamily. Of note, the expression of Lefty is positively regulated by Wnt signaling in ES cells, which was also affected by siRNA against CycT2 (5). Importantly, both arms are critical components of early development and differentiation, as is autophagy. For example, Lefty proteins are highly expressed in the inner cell mass and trophoectoderm during embryogenesis (1). Whereas there are abnormalities in left-right axis formation in Lefty 1-deficient mice, excessive mesoderm is formed in Lefty 2-deficient mice (21, 22). Moreover, during the transition from oocytes to embryos, maternal proteins are rapidly degraded and replaced by zygotic proteins. This process is mediated by the ubiquitin-proteasomal system and autophagy. Interestingly, autophagy-defective oocytes fail to develop beyond the four- and eight-cell stages, and its zygotic mRNA components can be detected as early as the two-cell stage (34). Thus, CycT2 and genes regulated by this form of P-TEFb are critically important for early developmental pathways in mice.

In summary, CycT2 is essential for mouse embryogenesis. Although most genes appear to be regulated by either or both cyclins, there are sets of genes that are specifically under the control of CycT1 or CycT2. Further studies will be needed to examine these direct targets of CycT2 during development. In addition, conditional knockouts will be instructive to further analyze their physiological functions in the adult organism.

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