A naturally occurring point substitution in *Cdc25A*, and not *Fv2/Stk*, is associated with altered cell-cycle status of early erythroid progenitor cells

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The *Friend virus susceptibility gene 2 (Fv2)* controls the polyclonal expansion of infected cells that occurs early during Friend erythroleukemia virus infection. *Fv2* has recently been shown to encode a truncated form of the *Stk* receptor tyrosine kinase (Sf-Stk). This observation, coupled with earlier work, suggested that Sf-Stk drives the expansion of infected cells by forming a complex with the Friend virus envelope glycoprotein, gp55, and the erythropoietin receptor. *Fv2* has also been implicated in the control of cell cycling in early erythroid progenitors (erythroid blast-forming units [BFU-Es]). Mouse strains that are homozygous for the resistant allele of *Fv2 (Fv2^{rr})* have few actively cycling BFU-Es. In this report, we demonstrate that the control of BFU-E cycling is encoded by a gene linked to, but distinct from, *Fv2*, and suggest that this gene is the dual-specific protein phosphatase Cdc25A, which regulates the G1- to S-phase transition of the cell cycle. We show that a naturally occurring allele of Cdc25A, which increases Cdc25A phosphatase activity and promotes cell-cycle progression, segregates in mouse strains that exhibit high levels of BFU-E cell cycling. In wild-type mice, this allele of Cdc25A does not overtly affect erythropoiesis; however, when this allele is combined with a mutation of the Kit receptor (*Kit^{WV}*), the anemia of the mice is enhanced. Furthermore, overexpression of Cdc25A in bone marrow cells causes a defect in the BFU-E colony formation. These results suggest that proper regulation of the cell cycle through Cdc25A is required for normal erythropoiesis. (Blood. 2002;100:3804-3811)

Introduction

Friend-virus–induced erythroleukemia progresses through 2 characteristic stages. Initially, there is a polyclonal expansion of infected cells, followed by the acquisition of further mutations and the emergence of a malignant clone.[1,2] The specificity of Friend virus for the erythroid lineage and the characteristic progression of the Friend erythroleukemia has allowed for the identification of a number of host genes that regulate leukemogenesis after Friend virus infection. One of these host genes, the *Friend virus susceptibility gene 2 (Fv2)*, was first identified by Lilly[3] more than 30 years ago as a gene that controlled the expansion of infected cells during the early stage of the disease. Mice homozygous for the resistant allele of *Fv2* (*Fv2^{rr}*) are resistant to Friend disease. Even though the virus infects early erythroid progenitors (erythroid blast-forming units [BFU-Es]) that are target cells for Friend virus, the infected cells fail to expand.[4] Two hypotheses have been put forward to explain the mechanism of *Fv2* action. First, it has been proposed that *Fv2* regulates complex formation between the erythropoietin receptor (EpoR) and the virally encoded glycoprotein, gp55, and thereby regulates a mitogenic signal to infected erythroid cells.[5,6] Second, *Fv2* has been proposed to regulate cell-cycle progression of BFU-E. Mice homozygous for the resistant allele of *Fv2*, *Fv2^{rr}*, have few BFU-Es that are actively cycling as measured by their resistance to killing by [^{3}H]thymidine and hydroxyurea.[7] In contrast, the BFU-Es from mice homozygous or heterozygous for the sensitive allele of *Fv2* are actively cycling. Because retroviral infection requires that the target cell be actively cycling for productive infection, the cell-cycling differences between *Fv2*-sensitive and -resistant mice might explain the resistance to erythroleukemia mediated by Fv2.[1,8]

Recently, the *Fv2* locus was shown to encode a naturally occurring truncated form of Mst1r (formerly the Stk receptor tyrosine kinase), known as short-form Stk or Sf-Stk.[9] The identification of the Sf-Stk receptor as Fv2 is consistent with the possibility that Fv2 functions as a component of the gp55/EpoR complex. However, the genetic linkage mapping that was used to identify Sf-Stk as *Fv2* was based solely on scoring the expansion of infected cells in the spleen of infected mice and did not analyze the cell-cycle status of BFU-E.[9,10] Although it is clear that Sf-Stk is essential for the expansion of infected cells early during Friend erythroleukemia, it is unclear whether the expression of Sf-Stk in *Fv2^{ss}* is responsible for the observed differences in cell cycling between *Fv2^{rr}* and *Fv2^{ss}* mice.

In this report we show that the control of BFU-E cell cycling is encoded by a gene linked to, but distinct from, *Mst1r/Fv2*. We identify a naturally occurring mutation in the cell-cycle regulatory protein phosphatase Cdc25A that segregates with the increased cell cycling of BFU-E observed in *Fv2^{ss}* mice. This point mutation in the noncatalytic amino-terminal domain of Cdc25A results in increased phosphatase activity. Furthermore, primary mouse embryo fibroblast cultures derived from mice carrying the Cdc25A mutation exhibited increased growth rates and entered S phase on average 1 to 2 hours earlier than congenic control cells, which

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correlated with an earlier activation of cyclin-dependent kinase 2 (CDK2) activity. Although inbred strains of mice that carry the mutant Cdc25A allele exhibited no gross defects in erythropoiesis, when this allele was combined with a mutation in the Kit receptor (Kit^{Wv}), the anemia of Kit^{Wv}/Kit^{Wv} mice was significantly enhanced. Cell-cycle control and, in particular, the role of Cdc25A in the regulation of erythropoiesis was further demonstrated by the observation that overexpression of either the wild-type or mutant allele of Cdc25A in bone marrow cells had a profound effect on the formation of BFU-E. Based on these results, we propose that an activating mutation in Cdc25A causes the alterations in BFU-E cycling observed in $Fv2^{ss}$ mice and that proper cell-cycle control mediated through Cdc25A is required for normal erythropoiesis.

Materials and methods

Testing BFU-E cycling by hydroxyurea sensitivity

C57BL/6J, BALB/cJ, and 129P3 (formerly 129/J) mice were purchased from JAX (Bar Harbor, ME). At 10 weeks of age, 5 mice from each strain were injected with hydroxyurea (1 mg/g body weight) in 250 μL phosphate-buffered saline (PBS), and 5 control mice from each strain were injected with 250 μL PBS. One hour after injection, the mice were killed and bone marrow cells were harvested. Then 1×10^5 cells were plated in Methocult with recombinant cytokines (StemCell Technologies, Vancouver, BC, Canada) for BFU-E assays, and 1×10^4 cells were plated for erythroid colony-forming unit (CFU-E) assays. Triplicate assays were performed for each mouse. BFU-Es were counted after 7 days, and CFU-Es were counted after 2 days. BFU-Es and CFU-Es were identified using acid benzidine staining. Percentage BFU-E or CFU-E killed by hydroxyurea was calculated as described previously.[7]

Infection of mice with Friend virus

Three 10-week-old C57BL/6J, BALB/cJ, and 129P3 mice were injected with NB tropic FV-P as previously described.[10] Fourteen days after infection, the mice were killed and their spleens were removed, weighed, and compared with the average-weight spleens of 2 uninfected controls.

Isolation of RNA and reverse transcription–polymerase chain reaction cloning and sequencing of Cdc25A alleles from C57BL/6J and BALB/cJ mice

RNA was isolated from bone marrow and kidneys of C57BL/6J and BALB/cJ mice using Trizol (Gibco-BRL, Grand Island, NY) reagent. cDNA was generated from total RNA using random hexamers as primers and Superscript Reverse Transcriptase (Gibco-BRL). The coding region of Cdc25A was amplified from cDNA samples using primers corresponding 5′ to the translation start site and 3′ just downstream of the TGA stop codon (5′ primer, 5′-GGGATCCCATGGAACTGGGCCCGAG-3′; 3′ primer, 5′-GAAGCTCAGGCTGCTACCATT-3′). Multiple clones of full-length polymerase chain reaction (PCR) fragments that were derived from C57BL/6J and BALB/cJ kidney and bone marrow cDNA were sequenced.

PCR assay to detect the $Cdc25A^{His128Gln}$ allele

PCR primers that flanked the mutation in codon 128 were generated so that the presence of the CAC histidine codon would generate an *Alw*44I restriction site (5′ primer, 5′-AGCCACTCTGATTCTCTAGTGCA-3′; 3′ primer, 5′-GGTCGATGAGGTGAAAGGTGTC-3′; underlined nucleotides in the 5′ primer are mismatches that generate the *Alw*44I site in the $Cdc25A^{128His}$ allele). DNA was isolated from F2 progeny of the Fv2 mapping backcross and Fv2 congenic strains, as previously described.[10] DNA from inbred strains was purchased from the Jackson Laboratory Mouse DNA repository (Bar Harbor, ME). PCR was performed as previously described.[10] Amplification conditions for these primers were 1 minute at 94°C, 2 minutes at 62°C, and 1 minute at 72°C for 40 cycles and 7 minutes at 72°C for 1 cycle. Two microliters from a 20-μL PCR reaction was cut with *Alw*44I in a total reaction volume of 20 μL according to the manufacturer's instructions. The *Alw*44I digests were run on a 10% acrylamide (19:1) gel.

Expression of GST-$Cdc25A^{128Gln}$ and GST-$Cdc25A^{128His}$ and analysis of phosphatase activity

A clone of human CDC25A fused in-frame to glutathione S-transferase (GST) was obtained from Dr Helen Piwnica-Worms (Washington University, St Louis, MO). The His128Gln mutation was introduced into this clone using the Chameleon Double-Stranded, Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. The GST-$Cdc25A^{His128Gln}$, GST-$Cdc25A^{WT}$, and GST proteins were produced as previously described.[11] To determine the activity of these protein phosphatases, bacterially expressed GST-$CDC25A^{128His}$, GST-$CDC25A^{128Gln}$, or GST alone were incubated in a final volume of 200 μL with 40 mM para-nitrophenyl-phosphate (pNPP), 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA (ethylenediaminetetraacetic acid), and 1 mM dithiothreitol (DTT) for the indicated time at 30°C. Reactions were stopped by the addition of 1 mL of 0.2 M NaOH, and absorbance at 410 nm was measured.

Cell-cycle analysis of the mouse embryo fibroblast cultures

Mouse embryo fibroblast (MEF) cultures were derived from C57BL/6J embryos and B6.C-$H7^b$/By embryos harvested at day 12.5 of embryogenesis.[12] Growth curves were generated by plating 1×10^3 cells/well in 24-well plates. Cells were trypsinized and counted using a Coulter counter at all indicated points in triplicate, and the data presented are representative of 3 independent experiments using MEF cultures derived from independent embryos. Cell-cycle progression was analyzed by starving confluent MEF cultures in low serum (0.1% fetal calf serum [FCS]) for 24 hours. Cells were then trypsinized, split 1:2, and replated in media containing 10% FCS. At the indicated times, cells were trypsinized and harvested and then resuspended in 1 mL ice-cold PBS and fixed with 70% EtOH. Fixed cells were resuspended in 100 μL of 150 mM NaCl, 100 mM Tris-HCl, pH 7.6, and 900 μL 0.1% Na-citrate, pH 7.6, 10 mM NaCl, was added. Cells were stained by first adding 20 μL RNase A (1 mg/mL) and then adding 20 μL propidium iodide (PI; 1 mg/mL). Cells were stained for 20 minutes at 25°C, and the PI staining was determined by flow cytometry. The percentage of cells in S phase was calculated using the Multicycle (Phoenix Flow Systems, San Diego, CA) program.

Determination of CDK2 histone H1 kinase activity

H1 kinase assays were performed as described.[13,14] In brief, MEF cultures were grown to confluence in Dulbecco modified Eagle medium (DMEM) and 5% FCS. Cells were trypsinized, split 1:3, and replated in media containing 0.1% FCS for 24 hours. The low-serum media were removed, and cells were cultured for the indicated times in media containing 5% serum. For kinase assays, cells were trypsinized at the indicated times, washed once with ice-cold PBS, and resuspended in 1 mL NP40 lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 0.5% NP40, 50 mM NaF, 1 mM sodium orthovanadate, and 1× protease inhibitors [Complete Protease Inhibitor Cocktail; Roche Diagnostics, Indianapolis, IN]). Lysates were sonicated (3 pulses of 5 seconds each on maximum setting) and then incubated for 30 minutes on ice. They were then centrifuged at 13 000 rpm for 15 minutes at 4°C to remove insoluble material. CDK2 was immunoprecipitated using anti-CDK2 antibody (M2) from Santa Cruz Biotechnology (Santa Cruz, CA) and Protein A Sepharose beads (Sigma-Aldrich, St Louis, MO) for 4 to 8 hours at 4°C. H1 kinase assays were performed as described.[14]

Generation and analysis of Kit^{WV}/Kit^{WV};$Cdc25A^{His128Gln}$ mice

C57BL/6J-Kit^{WV}/+ mice were crossed with *B6.C*-H7b/*By* (*$Cdc25A^{His128Gln}$/^{His128Gln}*) mice. F1 mice that were Kit^{WV}/+ were intercrossed. Kit^{WV}/Kit^{WV} F2 progeny were identified by coat color, and their survival times and hematocrit levels were measured.

Infection of bone marrow cells with Cdc25A^{WT} and Cdc25A^{His128Gln} retroviruses and determination of BFU-E colony-forming ability of infected cells

Human Cdc25A^{128His} and Cdc25A^{128Gln} were cloned in pMSCV-neo as *Bam*H1–*Eco*R1 fragments to generate pMSCV-Cdc25A^{WT} and pMSCV-Cdc25A^{His128Gln}. Recombinant virus was generated as previously described.[15] Bone marrow was isolated from the femur of an adult BALB/cJ mouse and was resuspended in 750 μL Iscove modified Dulbecco medium (IMDM) and 5% FCS. An equal volume of bone marrow cells (250 μL) was incubated with each viral supernatant supplemented with 10 ng/mL stem cell factor (SCF), 10 ng/mL interleukin-3 (IL-3; both from PeproTech, Rocky Hill, NJ), and 8 μg/mL polybrene (5 mL total volume; Sigma-Aldrich) overnight at 37°C. The next morning, 1×10^5 infected cells were plated in Methocult media (Stem Cell Technologies) containing 3 U/mL EPO, 100 ng/mL SCF, and 1 mg/mL G418 (0.719 mg/mL active ingredient; all 3 a gift from D. Wojchowski, Pennsylvania State University) in triplicate for each virus. After 7 days, BFU-Es were stained in situ using acid benzidine stain.

Results

Control of BFU-E cell cycling is genetically distinct from Fv2-mediated control of Friend erythroleukemia

Cloning of the *Fv2* locus demonstrated that Sf-Stk is required for the expansion of infected cells early during the progression of Friend erythroleukemia.[9] However, the cloning of *Fv2* was based on linkage mapping that used the expansion of infected cells in the spleens of infected mice as a phenotypic readout; thus, the role of Sf-Stk in the regulation of BFU-E cell cycling has not been investigated.[9,10] We set out to test whether Sf-Stk was involved in regulating BFU-E cell cycling by testing whether sensitivity to Friend erythroleukemia and altered BFU-E cell cycling were genetically separable.

The original analysis of the differences in BFU-E cell cycling between *Fv2^{rr}* and *Fv2^{ss}* mice was performed using congenic mice that were bred based on their sensitivity to Friend erythroleukemia.[7] Thus, if the gene controlling BFU-E cell-cycle status was closely linked to *Fv2*, it would have been present in the congenic region, and the cosegregation of BFU-E cell cycling with SF-Stk/Fv2 status in these mice would not have constituted proof of genetic identity. To address this problem, we analyzed a variety of inbred mouse strains for their susceptibility to Friend erythroleukemia and for the cycling status of BFU-E. Because inbred strains were not developed based on their susceptibility to Friend virus, we reasoned that we might identify a strain where the 2 traits were separable. We used C57BL/6J mice as the *Fv2^{rr}* and BFU-E out-of-cycle control strain and BALB/cJ mice as the *Fv2^{ss}* and BFU-E actively cycling control strain. The initial strain tested was 129P3. Although 129P3 mice have been shown to be *Fv2^{ss}*,[16] we first confirmed the susceptibility of all 3 strains to Friend erythroleukemia. Ten-week-old 129P3, C57BL/6J, and BALB/cJ mice were infected with the NB–tropic polycythemia-inducing strain of Friend virus. Fourteen days later the mice were assayed for susceptibility by comparing the spleen weights of infected mice with those of uninfected controls (Table 1). As expected, C57BL/6J mice were resistant to Friend virus, as exhibited by the limited 1.4-fold increase in spleen size. In contrast, BALB/cJ mice were completely sensitive to Friend virus (20.2-fold increase in spleen size). We also observed sensitivity in the 129P3 mice; however, the expansion of cells in the spleen was not as extensive as that seen in BALB/cJ mice (4.0-fold increase in 129P3 compared with 20.2-fold in the BALB/cJ). Although the increase in spleen size was significantly less than that observed in the BALB/cJ mice, spleens from 129P3 mice were clearly infected as evidenced by macroscopic spleen foci present on some spleens (data not shown).

Table 1. Analysis of hydroxyurea sensitivity and susceptibility to Friend erythroleukemia in C57BL/6J, BALB/cJ, and 129P3 mice

Strain	Genotype	Killed by hydroxyurea, %	Friend virus susceptibility (increase in spleen weight)
C57BL/6J	Fv2^{rr}	16.2 ± 0.3	Resistant (1.4×)
BALB/cJ	Fv2^{ss}	66.5 ± 0.2	Sensitive (20.2×)
129P3	Fv2^{ss}	12.4 ± 0.4	Sensitive (4.0×)

For all strains, more than 75% of CFU-Es were killed by hydroxyurea treatment. Ten mice of each strain (5 injected with hydroxyurea, 5 uninjected controls) were used to calculate the percentage of BFU-Es killed by hydroxyurea.[7] As a control, the percentage of CFU-Es killed by hydroxyurea was also measured. Friend virus sensitivity was measured by examining spleen weight 2 weeks after infection with Friend virus.

The percentage of actively cycling BFU-E was measured in the 3 strains by determining the sensitivity of BFU-E to killing by hydroxyurea (Table 1). As expected, BFU-Es from C57BL/6J mice were resistant to killing by hydroxyurea, whereas BALB/cJ BFU-Es were sensitive to hydroxyurea (16.2% killed in C57BL/6J vs 66.5% killed in BALB/cJ). In contrast, 129P3 mice sensitive to Friend erythroleukemia were resistant to hydroxyurea (12.4% of BFU-E killed by hydroxyurea). These results clearly demonstrate that susceptibility to Friend erythroleukemia and control BFU-E cell cycling are genetically separable.

Point mutation in Cdc25A segregates with Fv2^{ss} mouse strains that exhibit altered cell cycling of BFU-E

We reasoned that the gene that controls BFU-E cycling must be closely linked to *Fv2* because *Fv2^{ss}* congenic strains exhibit increased BFU-E cycling.[7] Thus, genes that mapped near the *Fv2* locus on mouse chromosome 9 or the homologous region on human chromosome 3 would be candidates for the gene that regulates BFU-E cycling. One ideal candidate gene that mapped to this region in human and mouse is *Cdc25A*.[17,18] *Cdc25A* is a member of the Cdc25 family of dual-specific protein phosphatases that regulate cell-cycle transitions by dephosphorylating and activating CDKs.[19] Cdc25A functions during the G1- to S-phase transition of the cell cycle. The primary targets for Cdc25A are the CDK2-cyclin E and CDK2-cyclin A complexes, which act to drive the cell from G1 to S phase.[19]

If *Cdc25A* is the *Fv2*-linked gene that regulates BFU-E cell cycling, then different alleles of *Cdc25A* should be present in strains that differ in BFU-E cell-cycle status. To determine whether differences in the coding sequence of *Cdc25A* were responsible for altered BFU-E cycling, we made full-length Cdc25A cDNA clones from RNA isolated from either C57BL/6J (BFU-E out-of-cycle) or BALB/cJ (BFU-E in-cycle) mice. Sequencing of the clones revealed that the coding sequence of the BALB/cJ *Cdc25A* gene contained a C to A change in codon 128 that converts a CAC histidine codon (Cdc25A^{128His}) to a CAA glutamine codon (Cdc25A^{128Gln}). This mutation lies in the amino-terminal noncatalytic domain of Cdc25A near the end of a block of 24 amino acids that are absolutely conserved in mouse, human, and rat. Comparison of the sequence of this region in human and rat with sequence from BALB/cJ and C57BL/6J revealed that the C57BL/6J allele, Cdc25A^{128His}, is conserved in human and rat, suggesting that this allele may be the "wild-type" allele (Figure 1).[20,21]

Using this mutation, we developed a PCR-based assay to distinguish between the 2 alleles. We mapped *Cdc25A* relative to

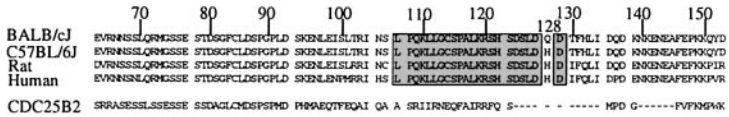

Figure 1. Alignment of the amino acid sequence of Cdc25A from BALB/cJ, C57BL/6J, rat, and human. The dark box indicates a domain of 23 amino acids that are identical in mouse, human,[20] and rat Cdc25A.[21] The position of codon 128 is highlighted within this domain. Below, a portion of the sequence of CDC25B2 is aligned with Cdc25A to demonstrate that the region containing the codon 128 is deleted in CDC25B2.[41]

Fv2 using a (C3HeB/FeJ × C57BL/6J)F1 × C57BL/6J backcross panel.[10] *Cdc25A* mapped approximately 2 cM distal to *Fv2* (Figure 2A). At the same time, we scored the presence of this mutation in a panel of DNA samples representing 22 inbred or congenic mouse strains tested for susceptibility to Friend erythroleukemia, including 10 strains that exhibited differences in BFU-E cell-cycle status as measured by sensitivity to hydroxyurea or [^{3}H]thymidine.[7] The 6 strains in which BFU-E were out of cycle all contained the Cdc25A^{128His} allele, whereas the 5 strains in which most BFU-Es were in cycle all contained the Cdc25A^{128Gln} allele (Figure 2B). Thus, the *Cdc25A* mutation segregated perfectly with differences in BFU-E cell-cycle status, raising the possibility that the *Cdc25A^{128Gln}* mutation is responsible for the differences in BFU-E cycling rather than *Fv2* itself.

Cdc25A^{His128Gln} mutation results in increased phosphatase activity

Cdc25A is a positive regulator of the cell cycle. Overexpression of Cdc25A can lead to cell proliferation and transformation.[22,23] Thus, mutations that increase Cdc25A activity might lead to increased cell proliferation. Cdc25A and the other family members, Cdc25B and C, are modular proteins. The carboxy-terminal portion of the protein contains the phosphatase domain, and the amino-terminal domain is thought to regulate the activity of the enzyme. Truncation of the amino-terminal domain results in a 3- to 10-fold increase in phosphatase activity, suggesting that this domain may directly inhibit phosphatase activity.[24,25] We sought to determine whether the His128Gln mutation, which lies in the amino-terminal regulatory domain, affected the phosphatase activity of Cdc25A. The complete coding domain of human CDC25A^{128His} and CDC25A^{128Gln} were fused in-frame with GST, and the fusion proteins were produced in *Escherichia coli*. Phosphatase activity of purified fusion proteins was determined by measuring the conversion of para-nitrophenyl phosphate to para-nitrophenol using absorbance at 410 nm. In 3 separate experiments, CDC25A^{128Gln} consistently demonstrated an increase in phosphatase activity (Figure 3). These results suggest that a point mutation in the amino-terminal domain of Cdc25A may alter the conformation of the protein, resulting in increased phosphatase activity.

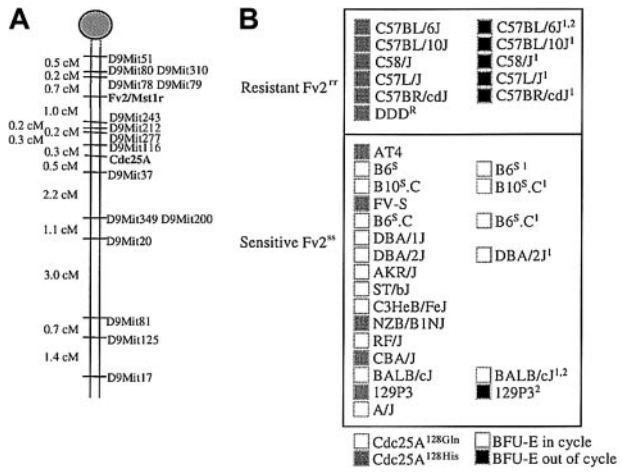

Figure 2. Linkage mapping of Cdc25A on mouse chromosome 9 and comparison of the segregation of BFU-E cell-cycle status and the 2 alleles of *Cdc25A* in inbred and congenic mouse strains. (A) Genetic linkage map of the region of mouse chromosome 9 containing *Fv2/Stk* and *Cdc25A*. Map distances between adjacent loci are given in cM at the left. The centromere is at the top. The linkage map was generated using a C3HheB/FeJ × C57BL/6J)F1 × C57BL/6J backcross panel of 425 animals.[10] The linkage map was constructed using MAPMAKER/EXP 3.0.[57] (B) Segregation of BFU-E cell-cycle status and the Cdc25A^{128His} and Cdc25A^{128Gln} alleles in inbred and congenic strains. Inbred and congenic strains were scored for the Cdc25A alleles by PCR. Results are presented in the left-side boxes. BFU-E cycling status of these strains has been determined by resistance to [^{3}H]thymidine or hydroxyurea and is shown in the right-side boxes. At the top are strains shown previously to be *Fv2^{rr}*, and at the bottom are strains shown previously to be *Fv2^{ss}*. For cell cycling status, strains marked 1 were analyzed by Suzuki and Axelrad[7] and strains marked 2 were investigated in this report. B10^s.C indicates B10.C-*H7^b*(47N)/Sn; B6^s.C, B6.C-H7^b/By.

Cells expressing Cdc25A^{His128Gln} exhibit an accelerated cell cycle

The expression of Cdc25A is not limited to erythroid progenitors and is thought to be expressed by all dividing cells. Thus, a mutation that increases Cdc25A phosphatase activity might affect the cell-cycle parameters of many cell types. To determine whether differences in *Cdc25A* alleles affected cell-cycle parameters in a cell population more amenable to experimental analysis than BFU-E, we derived primary MEF cultures from C57BL/6J and the congenic B6.C-*H7^b*/By, which is congenic for the H7 histocompatability locus that is linked to *Fv2* and *Cdc25A*. B6.C-*H7^b*/By mice carry the Cdc25A^{128Gln} allele, whereas the C57BL/6J MEFs express the Cdc25A^{128His} allele. Early-passage MEFs of each genotype were plated out, and the cultures were allowed to expand over a 6-day period. The B6.C-*H7^b*/By (Cdc25A^{128Gln}) MEFs consistently expanded at a faster rate than the C57BL/6J(Cdc25A^{128His}) MEFs (Figure 4A). These data support the idea that the increased phosphatase activity of Cdc25A^{128Gln} is associated with an increase in cell cycling.

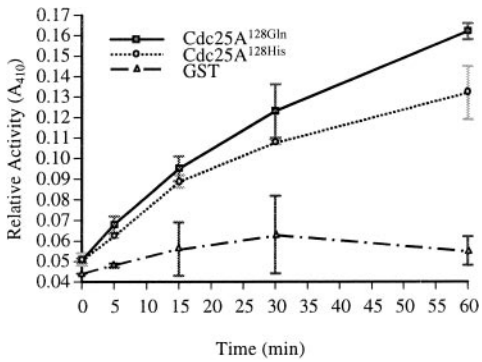

Figure 3. Analysis of phosphatase activity of GST-Cdc25A^{128His} and GST-Cdc25A^{128Gln} fusion proteins. GST fusion proteins were purified and tested for phosphatase activity. Equivalent amounts of GST-CDC25A^{128His}, GST-CDC25A^{128Gln}, and GST were incubated with 40 mM pNPP for the indicated times (see "Materials and methods"). Reactions were stopped, and absorbance at 410 nm was measured. The graph is representative of 3 independent experiments. Each time point was done in triplicate. Data are averages ± SD.

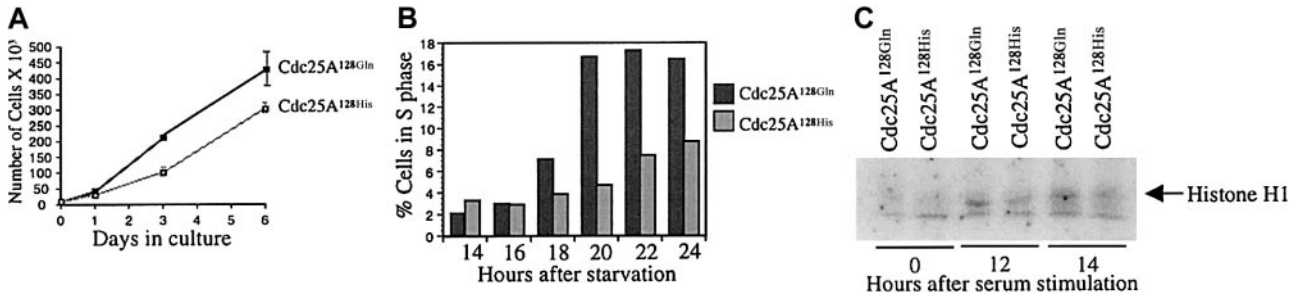

Figure 4. Cdc25A^{128Gln} alters the growth characteristics of mouse embryo fibroblasts (MEFs). MEF cultures were established from C57BL/6J embryos (Cdc25A^{128His}) and the congenic strain, B6.C-*H7^{b}*/By, embryos (Cdc25A^{128Gln}). (A) Growth analysis of MEF cultures. Initially, 1×10^3 cells were plated out. At the indicated times, cells were counted. Procedures at each time point were performed in duplicate. The graph is representative of 3 independent experiments, each using independently derived MEF cultures. Data are averages ± SD. (B) Analysis of S-phase progression in MEF cultures. Cells were grown to confluence and starved at low serum for 24 hours to synchronize the cells in G0/G1. Cells were then split into new media containing serum and grown for the indicated times. Cells were harvested, fixed, and stained with PI. Stained cells were analyzed by flow cytometry, and the percentage of cells in S phase was calculated by using the Multicycle (Phoenix Flow Systems) program. The graph is representative of 3 independent experiments using 3 independently derived MEF cultures for each allele. (C) Analysis of CDK2 H1 kinase activity in MEF cultures. Cells were grown in low-serum media to synchronize cells in G0/G1. Cells were then transferred to new media containing serum and grown for the indicated times. The cells were lysed, and CDK2 was immunoprecipitated. Histone H1 kinase activity was measured.[13,14] The gel is representative of 2 independent experiments using 2 different independently derived MEF cultures for each allele.

Cdc25A functions during the G1- to S-phase transition of the cell cycle, acting as a positive regulator of this process.[19] Thus, Cdc25A alleles with increased phosphatase activity might be expected to shorten the G1 phase and to hasten the entry into S phase of the cell cycle. To test this hypothesis, we used the MEF cultures derived from congenic mouse strains. The cultures were synchronized in G0/G1 using low-serum culture conditions and were transferred to serum-containing medium. The progression of cells into S phase was measured by staining of the cells with PI, followed by flow cytometry to determine DNA content. Between 18 and 24 hours after replating in serum-containing media, the proportion of Cdc25A^{128Gln} cells in S phase was significantly higher than the corresponding fraction from cells carrying the Cdc25A^{128His} allele (Figure 4B). Cdc25A positively regulates the G1- to S-phase transition by dephosphorylating CDK2-cyclin A/E complexes, which leads to an activation of CDK2 activity. Given that MEFs expressing Cdc25A^{128Gln} enter S phase more rapidly than those expressing Cdc25A^{128His}, we tested whether the decrease in G1- to S-phase transition time correlated with an earlier activation of CDK2 in Cdc25A^{128Gln} cells. MEF cells were synchronized in G0/G1 by culturing in low serum and then were switched to serum-containing medium to induce cell-cycle progression. CDK2 was immunoprecipitated, and histone H1 kinase activity was measured. At 12 and 14 hours following the switch to serum-containing media, Cdc25A^{128Gln} cells exhibited increased CDK2 activity when compared with Cdc25A^{128His} cells (Figure 4C). Taken together, these data suggest that the increase in phosphatase activity of the Cdc25A^{128Gln} allele results in a decreased overall cell-cycle time and an increased proliferation rate, correlating with a more rapid activation of CDK2 activity.

Cdc25A^{His128Gln} enhances the anemia of W^{V}/W^{V} mice

The original observations that demonstrated differences in BFU-E cycling suggest that Cdc25A^{His128Gln} might have an effect on erythropoiesis.[7] However, no significant differences in the number of BFU-Es or any other blood parameters have been observed between C57BL/6J mice and BALB/cJ mice. Thus, any effects of the increased BFU-E cycling must be compensated for by other mechanisms. As with other leaky mutant alleles, the effects of Cdc25A^{His128Gln} on erythropoiesis may not be apparent except under sensitized conditions. To explore this possibility, we crossed B6.C-*H7b*/By (Cdc25A^{His128Gln}) mice with C57BL/6J mice carrying a mutation in the Kit receptor tyrosine kinase (*Kit^{Wv}*). Mice homozygous for the *Kit^{Wv}* allele of Kit are viable but severely anemic.[26,27] F2 progeny were generated that were homozygous for *Kit^{Wv}*, and the effects of the different Cdc25A alleles were determined. Mice that were doubly homozygous for *Kit^{Wv}* and Cdc25A^{His128Gln} exhibited significantly lower hematocrits than *Kit^{Wv}/Kit^{Wv}* mice that were either heterozygous or homozygous for the Cdc25A^{128His} allele (Table 2). Furthermore, the percentage of pups living longer than 4 weeks was dramatically reduced in the *Kit^{Wv}/Kit^{Wv};* Cdc25A$^{His128Gln/His128Gln}$ mice than in *Kit^{Wv}/Kit^{Wv}* mice heterozygous or homozygous for Cdc25A^{128His} (Table 2). Thus, in mice with severe anemia, the increase in Cdc25A activity was associated with enhanced defects in erythropoiesis.

Overexpression of Cdc25A^{128His} or Cdc25A^{128Gln} inhibits BFU-E colony formation

The enhanced anemia observed in *Kit^{Wv}/Kit^{Wv}* mice that were also homozygous for the Cdc25A^{128Gln} allele suggested that, under sensitized conditions, alterations in cell-cycle regulation lead to defects in erythropoiesis. Work in *Schizosaccharomyces pombe* demonstrated that the overexpression of Cdc25 accelerates the cell cycle and induces cells to enter the S phase prematurely, which is similar to what happens to the phenotype exhibited by the Cdc25A^{128Gln} MEFs.[34] We next tested whether alteration of the cell cycle by overexpression of Cdc25A^{128His} or Cdc25A^{128Gln} could affect the ability of bone marrow cells to form BFU-E colonies. Cdc25A^{128His} and Cdc25A^{128Gln} were cloned to the pMSCV-neo retroviral vector, and these constructs were used to generate a virus that was used to infect bone marrow cells. Infected cells were

Table 2. Analysis of the effect of the Cdc25A alleles on the anemia and survival of Kit$^{Wv/Wv}$ mice

Genotype	Hematocrit, %	Survival longer than 4 wk, %
W^{v}/W^{v};Cdc25A^{128His}/Cdc25A^{128His}	38.1 ± 4.2	80
W^{v}/W^{v};Cdc25A^{128His}/Cdc25A^{128Gln}	38.5 ± 3.0	67
W^{v}/W^{v};Cdc25A^{128Gln}/Cdc25A^{128Gln}	30.5 ± 2.2*	25

*$P < .01$.

W^{v}/W^{v};Cdc25A^{128His}/Cdc25A^{128His}, *W^{v}/W^{v};Cdc25A^{128His}/Cdc25A^{128Gln}*, and *W^{v}/W^{v};Cdc25A^{128Gln}/Cdc25A^{128Gln}* mice were generated as described. Hematocrit levels were measured at 3 weeks of age. The *Cdc25A^{128Gln}* allele had no effect on the mild anemia of *Kit^{Wv}* heterozygotes.

plated in methylcellulose media containing EPO, SCF, and G418, which allowed for the selection of infected cells. Overall, BFU-E colonies were more sensitive than other myeloid colonies (culture colony-forming units [CFU-Cs]). Infection with Cdc25A^{128His} virus resulted in a significant reduction in BFU-E (50% of control, $P <$ .01), whereas the total number of CFU-Cs was not significantly different (Figure 5). In contrast, infection with Cdc25A^{128Gln} resulted in an almost total loss of BFU-E and CFU-C (Figure 5). This loss of colony-forming ability was not caused by low Cdc25A^{128Gln} viral titer. Control experiments using MEF cells that measured neo^{R} colony formation showed that the titers of the MSCV-neo control, MSCV-Cdc25A^{128His}, and MSCV-Cdc25A^{128Gln} viruses were approximately equal (data not shown). These results suggest that BFU-Es are more sensitive to alterations in cell-cycle progression than other myeloid colony-forming cells and that the overexpression of Cdc25A^{128Gln}, a mild activating allele of Cdc25A, results in inhibition of erythroid and myeloid differentiation.

Discussion

Previous work on the *Fv2* locus suggested 2 potential mechanisms for the resistance to Friend erythroleukemia in *Fv2^{rr}* mice. One model postulated that *Fv2* was a component of the EpoR signaling complex that interferes with the interaction of virally encoded gp55 with the EpoR in *Fv2^{rr}* mice or that promotes the interaction in *Fv2^{ss}* mice.[5,9] The second model suggested that *Fv2* regulates cell cycling of BFU-E, the target cell for Friend virus, such that in *Fv2^{rr}* mice too few target cells actively cycle for productive infection.[1,7] Although the cloning of *Fv2* demonstrated that it encoded Sf-Stk, a truncated form of the Mst1r receptor tyrosine kinase,[9] which supports the first model, this finding does not rule out a role for Sf-Stk in the regulation of BFU-E cell-cycle status. In this report, we have demonstrated that altered cell cycling and susceptibility to erythroleukemia are governed by distinct genes. Furthermore, we have identified a mutation in a gene closely linked to *Fv2/Sf-Stk*, *Cdc25A*, which segregates in mouse strains that exhibit altered BFU-E cycling.

Cdc25A is a positive regulator of cell-cycle progression and plays a key role in the transition to the S phase.[19] The activity of Cdc25A is tightly regulated at several levels. Expression of the *Cdc25A* gene is inhibited by the Rb/E2F complexes and is induced by both c-Myc and E2F.[28-33] Alterations in Cdc25 expression can have profound consequences on the cell cycle. Overexpression of Cdc25 in *S pombe* induces cells to divide at a faster rate, resulting in a small cell or "wee" phenotype.[34] In mammalian cells, overexpression of Cdc25A results in an accelerated G1- to S-phase transition.[22,35] Both observations are consistent with our findings that the increased phosphatase activity of the Cdc25A^{His128Gln} allele causes a shortened G1 phase and a faster expansion of cells in culture.

How might the His128Gln mutation affect the enzymatic activity of Cdc25A? In general, the enzymatic activity of the Cdc25 family appears to be regulated through its amino-terminal regulatory domain. This domain contains several phosphorylation sites for different kinases.[19,36] Phosphorylation of these sites can positively and negatively regulate Cdc25. Phosphorylation of Cdc25A by cyclin E/CDK2 activates phosphatase activity and represents a positive feedback loop for activation of the enzyme.[37] In contrast, the amino-terminus of Cdc25A is phosphorylated by either Chk1 or Chk2 kinase in response to DNA damage, which leads to ubiquitination and degradation by the proteosome.[38,39] Phosphorylation by Chk2 occurs at Ser123, which is located in the conserved block of 24 amino acids that contains codon 128.[40] Thus, the His128Gln mutation might alter the conformation of the protein and thereby affect the phosphorylation of this site, which in turn could affect the stability and the activity of Cdc25A. In the human homologue of CDC25B, a naturally occurring splice variant, CDC25B2, deletes this conserved region (Figure 1). This variant has increased phosphatase activity when used to rescue the temperature-sensitive allele of *S pombe Cdc25*.[41] These results, coupled with our observations presented here, suggest that this region of the amino-terminal domain plays a key role in regulating Cdc25A activity in vivo.

The His128Gln mutation in the germline of certain mouse strains causes only a modest increase in phosphatase activity. This small increase in phosphatase activity may be constrained by the fact that normal mouse development must still occur in animals expressing Cdc25A^{His128Gln}. As discussed above, the overexpression of Cdc25 in *S pombe* induces premature entry into the cell cycle.[34] In a single-cell organism such as *S pombe*, this disregard for maintaining cell size requirements can lead to an increase in aberrant mitoses.[34] In the context of a multicellular organism, high levels of Cdc25A phosphatase activity are incompatible with normal growth and development, as demonstrated by the loss of BFU-E and CFU-C when bone marrow cells are infected with a Cdc25A^{128Gln} virus. Thus, the Cdc25A^{128Gln} allele exhibits only a modest increase in phosphatase activity and, with endogenous expression levels, is compatible with normal development. However, when placed in a sensitized background such as in the *Kit^{Wv}/Kit^{Wv}* anemic background or when overexpressed in bone marrow cells, this allele causes a significant defect in the development of erythroid and myeloid progenitors.

Analysis of tumorigenesis in humans and mice has suggested that Cdc25A and other Cdc25 family members play a role in unregulated cell growth. Overexpression of CDC25A has been observed in several human tumors and is often associated with more aggressive tumors.[42-46] Furthermore, overexpression of Cdc25A or Cdc25B has been shown to increase susceptibility to tumors in animal models and can transform cells in culture.[23,47,48]

The central role of Cdc25A in promoting the G1- to S-phase transition is underscored by the observation that Cdc25A is an essential target of the G1- to S- and the intra–S-phase checkpoints.[49,50] In response to DNA damage, CDC25A is rapidly degraded, and S-phase progression is blocked.[39] This response is

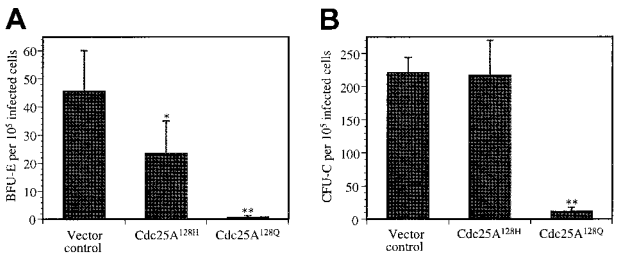

Figure 5. Overexpression of Cdc25A^{128His} or Cdc25A^{128Gln} decreases the ability of bone marrow cells to form BFU-E. Bone marrow cells were infected with retroviruses expressing Cdc25A^{128His}, Cdc25A^{128Gln}, or vector control and were plated in methylcellulose media containing EPO, SCF, and G418 to select for infected cells. Colonies were scored 7 days after plating. (A) Overexpression of Cdc25A^{128His} or Cdc25A^{128Gln} significantly decreases the number of BFU-E compared with vector control. *$P <$ .01; **$P <$ .001. (B) Overexpression of Cdc25A^{128Gln} significantly decreases the number of CFU-C. All non–BFU-E colonies were scored as CFU-C. **$P <$ .001. Each bar represents the average of 3 independent experiments.

regulated by the Ataxia telangiectasia–mutated (ATM)/ATM-related (ATR) kinases that signal through a Chk1/2 kinase intermediate.[50] Chk1/Chk2 phosphorylates Cdc25A, targeting it for degradation. Using *Xenopus* extracts to reconstitute this checkpoint in vitro, it was shown that the addition of exogenous Cdc25A can override the checkpoint and drive the extract into S phase.[51] These results suggest that increased levels of Cdc25A or potentially increased activity of Cdc25A could drive cells through cell-cycle checkpoints, resulting in further mutations. This prediction is borne out in some tumors in humans, when the overexpression of Cdc25A is associated with more aggressive tumors.[42,52,53] We have not analyzed whether the progression to leukemia in mice expressing Cdc25A^{His128Gln} is more rapid than in mice expressing the wild-type version. However, the expansion of infected cells in the spleen during the first 4 days after Friend virus infection occurs 2.7-fold faster in BALB/cJ (Cdc25A^{128Gln}) mice than in 129P3 (Cdc25A^{128His}) mice (R.F.P., unpublished observations, June 1996). Taken together, the observations in human tumors, the role of CDC25A in checkpoint control, and our observations that Cdc25A^{128Gln}-infected cells expand more rapidly, one might predict that Cdc25A^{His128Gln} would decrease the time necessary to develop leukemia after Friend virus infection.

In wild-type mice, differences in BFU-E cell-cycling status appear to cause no overt erythropoietic phenotypes.[7] It appears that the erythropoietic compartment is sufficiently plastic to compensate for any deficiencies caused by the alteration in cell cycling. However, as is the case with other weak mutant alleles, the phenotypic effects of the Cdc25A^{128Gln} mutation become manifest only when placed on a sensitized background of mutations, as in *Kit^{Wv}/Kit^{Wv}*. How might alterations in cell cycling affect erythropoiesis in these mice? The Kit receptor signaling pathway plays a key role in the proliferation and survival of erythroid progenitors.[26] The *Kit^{Wv}* allele results in a decrease in kinase activity (approximately 15% of wild-type) and is functionally analogous to cells being starved of SCF.[54] If Cdc25A^{His128Gln} drives cells into the cell cycle prematurely, erythroid progenitors may die because they lack sufficient Kit/SCF signal to keep them alive. This explanation seems less likely because the overexpression of Cdc25A^{128His} in bone marrow cells resulted in a similar decrease (50%) in BFU-E colonies regardless of whether a high concentration of SCF (100 ng/mL; Figure 5) or a low concentration of SCF (10 ng/mL; R.F.P., unpublished observations, January 2002) was used. Thus, in these experiments, low levels of Kit receptor signaling did not result in cells more sensitive to alterations in the cell cycle. Alternatively, erythroid differentiation requires progenitor cells to exit the cell cycle and terminally differentiate.[55,56] Cdc25A^{His128Gln} in the *Kit^{Wv}* cross or overexpression of either Cdc25A allele in bone marrow cells could interfere with the cell-cycle exit and disrupt normal differentiation.

In summary, we have described a naturally occurring mutation in *Cdc25A* that segregates with altered BFU-E cell-cycle status. These results demonstrate that the differences in BFU-E cell cycling previously ascribed to *Fv2* are likely caused by this allele of Cdc25A. This mutation, in the amino-terminal regulatory domain of Cdc25A, has a moderate but discernible effect on Cdc25A phosphatase activity and a significant effect on cell-cycle status. Although this mutation has no discernible phenotypic consequences under normal conditions, the effects are manifest when the mutation is placed on the sensitized *Kit^{Wv}/Kit^{Wv}* mutant background or when either Cdc25A^{128His} or Cdc25A^{128Gln} are overexpressed in bone marrow cells. These results suggest that proper cell-cycle control is required for normal erythropoietic differentiation.

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References

1. Ben-David Y, Bernstein A. Friend virus-induced erythroleukemia and the multistage nature of cancer. Cell. 1991;66:831-834.
2. Ney P, D'Andrea A. Friend erythroleukemia revisited. Blood. 2000;96:3675-3680.
3. Lilly F. *Fv2*: identification and location of a second gene governing the spleen focus response to Friend leukemia virus in mice. J Natl Cancer Inst. 1970;45:163-169.
4. Van der Gaag H, Axelrad A. Friend virus replication in normal and immunosuppressed C57BL/6 mice. Virology. 1990;177:837-839.
5. Hoatlin M, Kabat D. Host-range control of a retroviral disease: Friend erythroleukemia. Trends Microbiol. 1995;3:51-57.
6. Majumdar M, Cho C, Fox M, et al. Mutations in the env gene of Friend spleen focus forming virus overcome Fv2R-mediated resistance to Friend erythroleukemia virus. J Virol. 1992;66:3652-3660.
7. Suzuki S, Axelrad A. *Fv-2* locus controls the proportion of erythropoietic progenitor cells (BFU-E) synthesizing DNA in normal mice. Cell. 1980;19:225-236.
8. Varmus H, Padgett T, Heasley S, Simon G, Bishop J. Cellular functions are required for the synthesis and integration of avian sarcoma virus specific DNA. Cell. 1977;11:307-319.
9. Persons D, Paulson R, Loyd M, et al. Fv2 encodes a truncated form of the Stk receptor tyrosine kinase. Nat Genet. 1999;23:159-165.
10. Paulson R, Bernstein A. A genetic linkage map of the mouse chromosome 9 region encompassing the Friend virus susceptibility gene 2 (*Fv2*). Mamm Genome. 1998;9:381-384.
11. van der Geer P, Wiley S, Lai V, et al. A conserved amino-terminal Shc domain binds to phosphotyrosine motifs in activated receptors and phosphopeptides. Curr Biol. 1995;5:404-412.
12. Cheng A, Saxton T, Sakai R, et al. Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. Cell. 1998;95:793-803.
13. Brooks G. Cyclin-dependent kinases and cyclin-dependent kinase inhibitors: detection methods and activity measurements. Methods Mol Biol. 2001;124:161-170.
14. Tsubari M, Taipale J, Tiihonen E, Keski-Oja J, Laiho M. Hepatocyte growth factor releases mink epithelial cells from transforming growth factor beta1-induced growth arrest by restoring Cdk6 expression and cyclin E-associated Cdk2 activity. Mol Cell Biol. 1999;19:3654-3663.
15. Finkelstein L, Ney P, Liu Q, Paulson R, Correll P. Sf-Stk kinase activity and the Grb2 binding site are required for EPO-independent growth of primary erythroblasts infected with Friend virus. Oncogene. 2002;21:3562-3570.
16. Lilly F, Pincus T. Genetic control of murine viral leukemogenesis. Adv Cancer Res. 1973;17:231-277.
17. Lock L, Wickramasinghe D, Ernst M, et al. The Cdc25 genes map to mouse chromosomes 2, 9, and 18. Mamm Genome. 1996;7:771-772.
18. Demetrick D, Beach D. Chromosome mapping of human CDC25A and CDC25B phosphatases. Genomics. 1993;18:144-147.
19. Nilsson I, Hoffmann I. Cell cycle regulation by the Cdc25 phosphatase family. Prog Cell Cycle Res. 2000;4:107-114.
20. Galaktionov K, Beach D. Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins. Cell. 1991;67:1181-1194.
21. Jinno S, Suto K, Nagata A, et al. Cdc25A is a novel phosphatase functioning early in the cell cycle. EMBO J. 1994;13:1549-1556.
22. Blomberg I, Hoffmann I. Ectopic expression of Cdc25A accelerates the G(1)/S transition and leads to premature activation of cyclin E- and cyclin A-dependent kinases. Mol Cell Biol. 1999;19:6183-6194.
23. Galaktionov K, Lee A, Eckstein J, et al. CDC25 phosphatases as potential human oncogenes. Science. 1995;269:1575-1577.
24. Xu X, Burke S. Roles of active site residues and the NH2-terminal domain in the catalysis and substrate binding of human Cdc25. J Biol Chem. 1996;271:5118-5124.
25. Fauman E, Cogswell J, Lovejoy B, et al. Crystal structure of the catalytic domain of the human cell cycle control phosphatase, Cdc25A. Cell. 1998;93:617-625.

26. Paulson R, Bernstein A. Receptor tyrosine kinases and the regulation of hematopoiesis. Semin Immunol. 1995;7:267-277.
27. Lyon M, Rasten S, Brown S. Genetic variants and strains of the laboratory mouse. Oxford, United Kingdom: Oxford University Press; 1996.
28. Chen X, Prywes R. Serum-induced expression of the cdc25A gene by relief of E2F-mediated repression. Mol Cell Biol. 1999;19:4695-4702.
29. Galaktionov K, Chen X, Beach D. Cdc25 cell-cycle phosphatase as a target of c-*myc*. Nature. 1996;382:511-517.
30. Paskind M, Johnston C, Epstein P, et al. Structure and promoter activity of the mouse CDC25A gene. Mamm Genome. 2000;11:1063-1069.
31. Iavarone A, Massague J. Repression of the CDK activator Cdc25A and cell-cycle arrest by cytokine TGF-beta in cells lacking the CDK inhibitor p15. Nature. 1997;387:417-422.
32. Iavarone A, Massague J. E2F and histone deacetylase mediate transforming growth factor beta repression of cdc25A during keratinocyte cell cycle arrest. Mol Cell Biol. 1999;19:916-922.
33. Tiefenbrun N, Melamed D, Levy N, et al. Alpha interferon suppresses the cyclin D3 and cdc25A genes, leading to a reversible G0-like arrest. Mol Cell Biol. 1996;16:3934-3944.
34. Enoch T, Nurse P. Mutation of fission yeast cell cycle control genes abolishes dependence of mitosis on DNA replication. Cell. 1990;60:665-673.
35. Sexl V, Diehl J, Sherr C, et al. A rate limiting function of cdc25A for S phase entry inversely correlates with tyrosine dephosphorylation of Cdk2. Oncogene. 1999;18:573-582.
36. Wegener S, Hampe W, Herrmann D, Schaller H. Alternative splicing in the regulatory region of the human phosphatases CDC25A and CDC25C. Eur J Cell Biol. 2000;79:810-815.
37. Hoffmann I, Draetta G, Karsenti E. Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition. EMBO J. 1994;13:4302-4310.
38. Bernardi R, Liebermann D, Hoffman B. Cdc25A stability is controlled by the ubiquitin-proteasome pathway during cell cycle progression and terminal differentiation. Oncogene. 2000;19:2447-2454.
39. Mailand N, Falck J, Lukas C, et al. Rapid destruction of human Cdc25A in response to DNA damage. Science. 2000;288:1425-1429.
40. Falck J, Mailand N, Syljuasen R, Bartek J, Lukas J. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature. 2001;410:842-847.
41. Baldin V, Cans C, Superti-Furga G, Ducommun B. Alternative splicing of human CDC25B tyrosine phosphatase: possible implications for growth control? Oncogene. 1997;14:2485-2495.
42. Broggini M, Buraggi G, Brenna A, et al. Cell cycle-related phosphatases CDC25A and B expression correlates with survival in ovarian cancer patients. Anticancer Res. 2000;20:4835-4840.
43. Cangi M, Cukor B, Soung P, et al. Role of the Cdc25A phosphatase in human breast cancer. J Clin Invest. 2000;106:753-761.
44. Dixon D, Moyana T, King M. Elevated expression of the cdc25A protein phosphatase in colon cancer. Exp Cell Res. 1998;240:236-243.
45. Gasparotto D, Maestro R, Piccinin S, et al. Overexpression of CDC25A and CDC25B in head and neck cancers. Cancer Res. 1997;57:2366-2368.
46. Wu W, Fan Y, Kemp B, Walsh G, Mao L. Overexpression of cdc25A and cdc25B is frequent in primary non-small cell lung cancer but is not associated with overexpression of c-*myc*. Cancer Res. 1998;58:4082-4085.
47. Ma Z, Chua S, DeMayo F, Tsai S. Induction of mammary gland hyperplasia in transgenic mice over-expressing human Cdc25B. Oncogene. 1999;18:4564-4576.
48. Yao Y, Slosberg E, Wang L, et al. Increased susceptibility to carcinogen-induced mammary tumors in MMTV-Cdc25B transgenic mice. Oncogene. 1999;18:5159-5166.
49. Bartek J, Lukas J. Pathways governing G1/S transition and their response to DNA damage. FEBS Lett. 2001;490:117-122.
50. Bartek J, Lukas J. Mammalian G1- and S-phase checkpoints in response to DNA damage. Curr Opin Cell Biol. 2001;13:738-747.
51. Costanzo V, Robertson K, Ying C, et al. Reconstitution of an ATM-dependent checkpoint that inhibits chromosomal DNA replication following DNA damage. Mol Cell. 2000;6:649-659.
52. Evans K. Overexpression of CDC25A associated with poor prognosis in breast cancer [abstract]. Mol Med Today. 2000;6:459.
53. Takemasa I, Yamamoto H, Sekimoto M, et al. Overexpression of CDC25B phosphatase as a novel marker of poor prognosis of human colorectal carcinoma. Cancer Res. 2000;60:3043-3050.
54. Nocka K, Tan J, Chiu E, et al. Molecular bases of dominant negative and loss of function mutations at the murine c-kit/white spotting locus: W37, Wv, W41and W. EMBO J. 1990;9:1805-1813.
55. Humbert P, Rogers C, Ganiatsas S, et al. E2F4 is essential for normal erythrocyte maturation and neonatal viability. Mol Cell. 2000;6:281-291.
56. Whyatt D, Karis A, Harkes I, et al. The level of the tissue-specific factor GATA-1 affects the cell-cycle machinery. Genes Funct. 1997;1:11-24.
57. Lander E, Green P, Abrahamson J, et al. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics. 1987;1:174-181.