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 (Stk/Stk). This observation, coupled with earlier work, suggested that Stk/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]).

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. 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 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 (Fv2rr) 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. 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. 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]).

Second, Fv2 has been proposed to regulate cell-cycle progression of BFU-E. Mice homozygous for the resistant allele of Fv2 (Fv2rr), have few BFU-Es that are actively cycling as measured by their cycling in early erythroid progenitors (erythroid blast-forming units [BFU-Es]).

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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 (KitWv), the anemia of KitWv/KitWv 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 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 infected with Friend virus from MBP (Bar Harbor, ME). At 10 weeks of age, 5 mice from each strain were infected with Friend virus. Testing BFU-E cycling by hydroxyurea sensitivity was performed 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′-GGGATCCCATCCGAGAAGTCCGAG-3′; 3′ primer, 5′-GGAAGCTGAGGTGTTAGGAGTTCG-3′). Multiple clones of full-length polymerase chain reaction (PCR) fragments were sequenced from C57BL/6J and BALB/cJ kidney and bone marrow cDNA were sequenced.

PCR assay to detect the Cdc25AHis128Gln allele

PCR primers that flanked the mutation in codon 128 were generated so that the presence of the CAC histidine codon would generate an Alw44I restriction site 5′-ACCACCTGGTTTCTACTGCGA-3′; 5′ primer, 5′-GGTCTAGGATGTTAGGAGTTGC-3′; underlined nucleotides in the 5′ primer are mismatches that generate the Alw44I site in the Cdc25AHis128Gln 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 Alw44I in a total reaction volume of 20 μL according to the manufacturer’s instructions. The Alw44I digests were run on a 10% acrylamide (19:1) gel.

Expression of GST-Cdc25AHis128Gln and GST-Cdc25AHis128He 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-Cdc25AHis128Gln, GST-Cdc25AWT, and GST proteins were produced as previously described.11 To determine the activity of these protein phosphatases, bacterially expressed GST-Cdc25AHis128Gln, GST-Cdc25AHis128He, 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-H7b/By embryos harvested at day 12.5 of embryogenesis.5 Growth curves were generated by plating 1 × 105 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 staining 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. 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 KitWV/KitWV;Cdc25AHis128Gln mice

C57BL/6J mice carrying the KitWV allele were crossed with B6.C-H7b/By (Cdc25AHis128Gln/ 
H7bHis128Gln) mice. F1 mice that were KitWV +/KitWV were intercrossed. KitWV/KitWV F2 progeny were identified by coat color, and their survival times and hematocrit levels were measured.
Infection of bone marrow cells with Cdc25A<sup>WT</sup> and Cdc25A<sup>His128Gln</sup> retroviruses and determination of BFU-E colony-forming ability of infected cells

Human Cdc25A<sup>WT</sup> and Cdc25A<sup>His128Gln</sup> were cloned in pMSCV-neo as BamH1-EcoRI fragments to generate pMSCV-Cdc25A<sup>WT</sup> and pMSCV-Cdc25A<sup>His128Gln</sup>. Recombinant virus was generated as previously described. 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 μL total volume; Sigma-Aldrich) overnight at 37°C. The next morning, 1 × 10<sup>5</sup> 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. 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. We set out to test whether Sf-Stk was involved in regulating BFU-E cell cycling, then different alleles of Fv2 would be 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 altered BFU-E cell cycling were genetically separable.

Point mutation in Cdc25A segregates with Fv2<sup>ss</sup> 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<sup>ss</sup> congenic strains exhibit increased BFU-E cycling. 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<sup>17,18</sup> Cdc25A is a member of the Cdc25 family of dual-specific protein phosphatases that regulate cell-cycle transitions by dephosphorylating and activating CDKs<sup>19</sup>. 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.<sup>19</sup>

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<sup>128His</sup>) to a CAA glutamine codon (Cdc25A<sup>128Gln</sup>). 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<sup>128His</sup>, is conserved in human and rat, suggesting that this allele may be the “wild-type” allele (Figure 1).<sup>20,21</sup>

Using this mutation, we developed a PCR-based assay to distinguish between the 2 alleles. We mapped Cdc25A relative to
Fv2 using a (C3HeB/FeJ × C57BL/6J)F1 × C57BL/6J backcross panel. 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 [3H]thymidine. The 6 strains in which BFU-E were out of cycle all contained the Cdc25A128His allele, whereas the 5 strains in which most BFU-Es were in cycle all contained the Cdc25A128Gln allele (Figure 2B). Thus, the Cdc25A mutation segregated perfectly with differences in BFU-E cell-cycle status, raising the possibility that the Cdc25A128Gln mutation is responsible for the differences in BFU-E cycling rather than Fv2 itself.

Cdc25A128Gln 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. 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.

Cells expressing Cdc25A128Gln 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 affect 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-H7b/By, which is congenic for the H7 histocompatibility locus that is linked to Fv2 and Cdc25A. B6.C-H7b/By mice carry the Cdc25A128Gln allele, whereas the C57BL/6J MEFs express the Cdc25A128His 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-H7b/By (Cdc25A128Gln) MEFs consistently expanded at a faster rate than the C57BL/6J (Cdc25A128His) MEFs (Figure 4A). These data support the idea that the increased phosphatase activity of Cdc25A128Gln is associated with an increase in cell cycling.
Cdc25A functions during the G1- to S-phase transition of the cell cycle, acting as a positive regulator of this process. 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 expressing Cdc25A 128His, we tested whether the decrease in the increase in BFU-E cycling must be compensated for by other mechanisms. As with other leaky mutant alleles, the effects of Cdc25A 128Gln on erythropoiesis may not be apparent except under sensitized conditions. To explore this possibility, we crossed B6.C-H7/HbBy (Cdc25A 128Gln) mice with C57BL/6J mice carrying a mutation in the Kit receptor tyrosine kinase (Kit<sup>W<inf>v</inf></sup>). Mice homozygous for the Kit<sup>W<inf>v</inf></sup> allele of Kit are viable but severely anemic. F2 progeny were generated that were homozygous for Kit<sup>W<inf>v</inf></sup>, and the effects of the different Cdc25A alleles were determined. Mice that were doubly homozygous for Kit<sup>W<inf>v</inf></sup> and Cdc25A 128Gln exhibited significantly lower hematocrits than Kit<sup>W<inf>v</inf></sup>/Kit<sup>W<inf>v</inf></sup> mice that were either heterozygous or homozygous for Cdc25A 128Gln (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<sup>W<inf>v</inf></sup>/Kit<sup>W<inf>v</inf></sup> 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. We next tested whether alteration of the cell cycle by overexpression of Cdc25A 128Gln or Cdc25A 128His 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<sup>W<inf>v</inf></sup>/Kit<sup>W<inf>v</inf></sup> mice**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hematocrit, %</th>
<th>Survival longer than 4 wk, %</th>
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<tbody>
<tr>
<td>W/W; Cdc25A&lt;sup&gt;128His&lt;/sup&gt;/Cdc25A&lt;sup&gt;128His&lt;/sup&gt;</td>
<td>38.1 ± 4.2</td>
<td>80</td>
</tr>
<tr>
<td>W/W; Cdc25A&lt;sup&gt;128Gln&lt;/sup&gt;/Cdc25A&lt;sup&gt;128Gln&lt;/sup&gt;</td>
<td>38.5 ± 3.0</td>
<td>67</td>
</tr>
<tr>
<td>W/W; Cdc25A&lt;sup&gt;128His&lt;/sup&gt;/Cdc25A&lt;sup&gt;128Gln&lt;/sup&gt;</td>
<td>30.5 ± 2.2</td>
<td>25</td>
</tr>
</tbody>
</table>

*<sup>P</sup> < .01.*

W/W; Cdc25A<sup>128His</sup>/Cdc25A<sup>128Gln</sup>, W/W; Cdc25A<sup>128Gln</sup>/Cdc25A<sup>128Gln</sup>, and W/W; Cdc25A<sup>128His</sup>/Cdc25A<sup>128Gln</sup> mice were generated as described. Hematocrit levels were measured at 3 weeks of age. The Cdc25A<sup>128His</sup> allele had no effect on the mild anemia of Kit<sup>W<inf>v</inf></sup>/Kit<sup>W<inf>v</inf></sup> 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 Cdc25A128His 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 Cdc25A128Gln 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 Cdc25A128Gln viral titer. Control experiments using ME cells that measured neo \( ^{8} \) colony formation showed that the titers of the MSCV-neo control, MSCV-Cdc25A128His, and MSCV-Cdc25A128Gln 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 Cdc25A128Gln, a mildly 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\(^{+}\) 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\(^{+}\) mice or that promotes the interaction in Fv2\(^{-}\) 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\(^{+}\) 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 Mst1 receptor tyrosine kinase,\(^{5} \) 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 play a key role in the transition to the S phase.\(^{19,36} \) 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 region of Cdc25A.\(^{41} \) 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 Cdc25A128His.\(^{128,40} \) As discussed above, the overexpression of Cdc25A 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 Cdc25A128Gln virus. Thus, the Cdc25A128Gln 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\(^{+}\)/Kit\(^{-}\) 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.\(^{39,50} \) In response to DNA damage, CDC25A is rapidly degraded, and S-phase progression is blocked.\(^{39} \) This response is
regulated by the Ataxia telangiectasia–mutated (ATM)/ATM-related (ATR) kinases that signal through a Chk1/2 kinase intermediate. 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. 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. Cdc25A128Gln -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. 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 Cdc25A128Gln mutation become manifest only when placed on a sensitized background of mutations, as in KitWv/KitWv. 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. The KitWv allele results in a decrease in kinase activity (approximately 15% of wild-type) and is functionally analogous to cells being starved of SCF. If Cdc25AHis128Gln 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 Cdc25A128His 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. Cdc25A128Gln in the KitWv 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 KitWv/KitWv mutant background or when either Cdc25A128His or Cdc25A128Gln are overexpressed in bone marrow cells. These results suggest that proper cell-cycle control is required for normal erythropoietic differentiation.

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