Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9540–9544, October 1995
Medical Sciences

Signaling by *ABL* oncogenes through cyclin D1

DANIEL E. H. AFAR*, JAMI MCLAUGHLIN*, CHARLES J. SHERR†‡, OWEN N. WITTE*§¶, AND MARTINE F. ROUSSEL†

*Department of Microbiology and Molecular Genetics, §Molecular Biology Institute and Howard Hughes Medical Institute, University of California, Los Angeles, CA 90095; and †Department of Tumor Cell Biology and ‡Howard Hughes Medical Institute, St. Jude Children's Research Hospital, Memphis, TN 38105

Communicated by Paul D. Boyer, University of California, Los Angeles, CA, July 17, 1995

ABSTRACT **Oncogenic signals induce cellular proliferation by deregulating the cell division cycle. Cyclin D1, a regulator of G_1-phase progression, acts synergistically with *ABL* oncogenes in transforming fibroblasts and hematopoietic cells in culture. Synergy with v-*Abl* depended on a motif in cyclin D1 that mediates its binding to the retinoblastoma protein, suggesting that *ABL* oncogenes in part mediate their mitogenic effects via a retinoblastoma protein-dependent pathway. Overexpression of cyclin D1, but not cyclin E, rescued a signaling-defective src-homology 2 (SH2) domain mutant of *BCR–ABL* for transformation of cells in culture and malignant tumor formation *in vivo*. These results demonstrate that cyclin D1 can provide essential signals for malignant transformation in concert with an activated tyrosine kinase.**

Progression through a late G_1-phase restriction point defines the time in the cell division cycle when cells lose their dependency on mitogens and commit to DNA synthesis (1). This transition is controlled by D and E type cyclins and their cyclin-dependent kinases (Cdks), whose phosphorylation of critical substrates facilitates entry into S phase (2). D type cyclins, induced as part of the delayed early response to growth factor stimulation (3, 4), assemble with Cdk4 and Cdk6 (5–7) to form holoenzymes whose activities are first detected in mid-G_1 phase and increase as cells approach the G_1/S boundary (6, 7). The activity of cyclin D-dependent kinases is maintained in proliferating cells as long as mitogenic stimulation is present, suggesting that D type cyclins act as growth factor sensors (2). In contrast, cyclin E is expressed later in G_1 and combines with Cdk2 to maximally induce its periodic activity at the G_1/S transition (8, 9). Overexpression of cyclins D or E in rodent fibroblasts shortens G_1 phase and reduces the cells' dependency on mitogenic growth factors (10–12). The effects of G_1 cyclins on the duration of G_1 phase can be additive (13), and they likely regulate distinct transitions (13–15).

Cyclin D-dependent kinases have a distinct *in vitro* substrate preference for retinoblastoma protein (Rb) vs. histone H1 (5–7), which in part reflects the ability of D type cyclins to bind to Rb directly (16–18). Inhibition of cyclin D1 (11, 19) or Cdk4 (20–22, 57) activity *in vivo* prevents entry into S phase, but only in cells that express a functional Rb (21–25, 57). Moreover, cyclin D mutants disrupted in a Leu-Xaa-Cys-Xaa-Glu (LXCXE) motif that is necessary for their efficient binding to Rb are defective in enforcing S-phase entry when stably overexpressed in Rb-positive rodent fibroblasts or myeloid cells (26, 27). Therefore, Rb and possibly other Rb-regulated proteins appear to represent the critical physiologic substrates for cyclin D-dependent kinases. Although the cyclin E–Cdk2 complex might also contribute to Rb phosphorylation late in G_1 phase (14, 16), inhibition of cyclin E function in Rb-negative cells induces G_1 arrest (15), implying that cyclin E–Cdk2 complexes phosphorylate key substrates other than Rb.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Many oncogenes act during G_1 phase to deregulate the controls normally imposed by G_1 Cdks (28). The v-Abl and BCR–ABL proteins are cytoplasmic tyrosine kinases associated with leukemias in mice and humans. Chromosomal translocation-mediated fusion of *BCR* sequences upstream of the c-*ABL* second exon yields P210 BCR–ABL and P185 BCR–ABL, which are predominantly associated with human chronic myelogenous leukemia (CML) and acute lymphocytic leukemia, respectively (29). The murine v-*abl* gene is a result of recombination between *gag* sequences of mouse murine leukemia virus and c-*abl* (30). Both v-ABL and BCR–ABL exhibit elevated tyrosine kinase activity in comparison to c-Abl (31) and can transform fibroblasts *in vitro* (32) and hematopoietic cells *in vitro* (33) and *in vivo* (34–36). Transformed pre-B cells require v-Abl activity only during G_1 phase (37), suggesting that constitutively activated ABL kinases might mimic the effects of mitogen-induced signals on the assembly and activation of G_1 Cdks. By using genetic complementation of signaling-defective BCR–ABL mutants, we showed that BCR–ABL activates multiple signaling pathways for transformation (38). Herein we demonstrate that there is a functional cooperation between *ABL* oncogenes and cyclin D1 and that a signal dependent on the ABL src-homology 2 (SH2) domain functions in a cyclin D1-dependent pathway.

MATERIALS AND METHODS

Plasmid Construction and Generation of Virus Stocks. The different P185 BCR–ABL forms, v-abl, cyclin D1, D1 Δ21 (provided by M. Ewen, Dana–Farber Cancer Research Institute, Boston), and cyclin E (39) (provided by E. Lees, DNAX) were cloned into the retroviral vector pSRαMSVtkneo (40) by using a unique *Eco*RI site 3′ of the retroviral long terminal repeat (LTR). A modified version of this vector was used to generate constructs with P185 and cyclin D1 in cis, allowing the simultaneous expression of both genes from a single retrovirus (41). Cyclin D1 or the neomycin-resistance gene (neo gene) was inserted into the unique *Eco*RI site, and P185 was cloned into a unique *Xba* I site, 3′ of the herpes simplex virus thymidine kinase (tk) promoter.

Helper-free retroviruses were generated by transient cotransfection of 293T cells (42) (provided by D. Baltimore, Massachusetts Institute of Technology, Cambridge) with retroviral vectors and a Psi(−) ecotropic packaging vector (40). Supernatant from transfected 293T cells was collected 36–50 h after transfection. Viral titers were determined indirectly by measuring protein expression 48–72 h after infection of Rat-1 cells by using Western blot analysis or immunohistochemistry (40). Viral stocks were normalized to give equivalent protein expression for the various samples.

Soft Agar Transformation Assays. Soft agar transformation assays were performed as described (32, 40). Cells were

Abbreviations: Rb, retinoblastoma protein; SH2, src-homology 2; Cdk, cyclin-dependent kinase; CML, chronic myelogenous leukemia; LTR, long terminal repeat; neo gene, neomycin-resistance gene; tk, thymidine kinase; SCID, severe combined immunodeficiency.

¶To whom reprint requests should be addressed.

coinfected with retroviruses containing *ABL* oncogenes and either neo- or cyclin D1-containing retroviruses. Infected cells were grown for 48–72 h and then plated in soft agar at 1×10^4 cells per 6-cm dish. The samples were plated in duplicate in medium containing either 5% or 20% (vol/vol) fetal calf serum. Colonies equal to and larger than 0.5 mm in diameter were counted 2–3 weeks after plating.

Bone Marrow Transformation Assays and Tumor Challenges. Bone marrow was isolated from tibias and femurs of 4- to 8-week-old male BALB/c mice and infected with matched retroviral stocks. Cells were plated at 5×10^6 cells per 6-cm dish and the number of nonadherent pre-B cells were counted 10–14 days after infection (33). A culture was scored positive if it reached $\geq 1 \times 10^6$ cells per culture.

Transformed bone marrow cultures were selected to grow in the absence of stromal feeder layers for 6–8 weeks, and 1×10^6 feeder-independent lymphoid cells were injected bilaterally and subcutaneously into the flanks of severe combined immunodeficient (CB.17 SCID) mice (43). Mice were analyzed for tumor growth at local sites of injection and were examined for signs of metastatic growth of injected cells.

Western Blot Analysis. Western blot analysis was performed as described (40). Cells were lysed in boiling SDS containing sample buffer. Solubilized protein was resolved by SDS/PAGE, transferred to nitrocellulose filters, and probed with specific antibodies. Samples were analyzed by using monoclonal antibodies directed to ABL (pex 5), cyclin D1 (D1-72-13-G11) (6, 11), and cyclin E (HE12) (also provided by E. Lees).

RESULTS

To test for functional cooperation between *ABL* oncogenes and cyclin D1, v-*abl* and *BCR–ABL* (P185) were cointroduced with cyclin D1 by retroviral infection into NIH 3T3 cells and Rat-1 cells, respectively. High-titer retroviruses were generated in 293T cells by transient transfection (38, 40) and retroviral transfer was assessed by measuring protein expression with Western blot analysis (Fig. 1). The transforming potential of P185, in the presence or absence of cyclin D1, was assessed by using a soft agar colony formation assay (32, 40). The established criteria for a transformed phenotype include acidification of the medium and increases in colony size and number.

The parental fibroblasts did not form colonies and, in agreement with previous results (11), remained anchorage dependent even when cyclin D1 was overexpressed (Fig. 2). Expression of both v-Abl and P185 induced colony formation. Cells overexpressing cyclin D1 with v-Abl or P185 formed more numerous (3- to 4-fold more) and larger colonies (2–3 mm in diameter) than cells coexpressing *ABL* and neo genes. Acidification of the medium also appeared more rapidly (within 1–2 weeks) in the presence of cyclin D1 overexpression (compared to 3 weeks with neo).

The G_1 phase of the cell cycle is regulated by both D cyclins and cyclin E. To compare cyclins D1 and E for their ability to complement v-*abl*, both were overexpressed in NIH 3T3 cells with v-*abl* (Fig. 3*A*). In contrast to cyclin D1, overexpression of cyclin E failed to increase the transforming efficiency of v-Abl (Fig. 3*B*). Cyclin D1, but not cyclin E, can bind directly to Rb. This interaction depends on an N-terminal LXCXE motif (16–18), which D type cyclins share with a number of cellular and viral Rb-binding proteins (16, 17). A cyclin D1 mutant (Δ21) lacking sequences encoding this pentapeptide could not efficiently collaborate with v-Abl (Fig. 3*B*) even when expressed at levels equivalent to that of wild-type cyclin

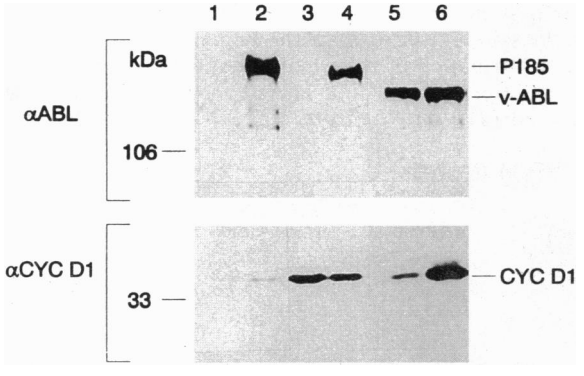


FIG. 1. Expression levels of ABL kinases and cyclin D1 in fibroblasts. Retroviruses encoding BCR–ABL, v-abl, cyclin D1, and neo were generated in 293T cells. Rat-1 cells were coinfected with P185 and either cyclin D1- or neo-encoding retroviruses. NIH 3T3 cells were coinfected with retroviruses containing v-*abl* and either cyclin D1 or neo. Cells were harvested 48–72 h after infection and were analyzed on a Western blot with monoclonal antibody to ABL (αABL; pex 5) and monoclonal antibody to cyclin D1 (αCYC D1). Lanes: 1, neo; 2, P185 and neo; 3, neo and cyclin D1; 4, P185 and cyclin D1; 5, v-Abl and neo; 6, v-Abl and cyclin D1.

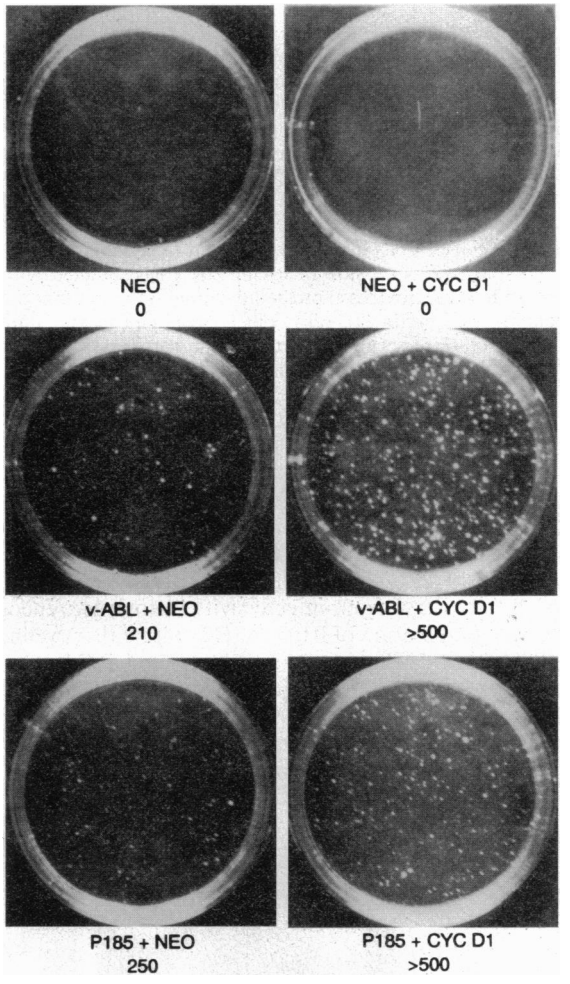
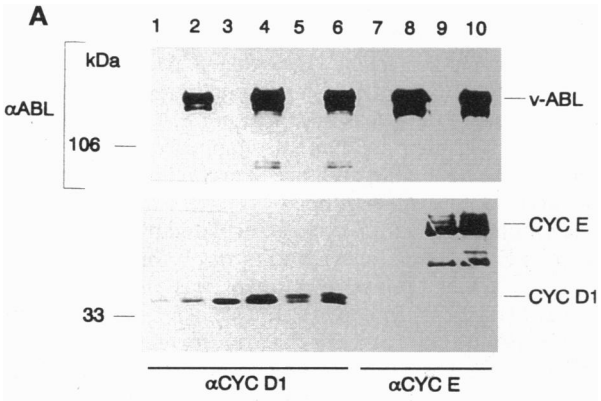


FIG. 2. Cooperation of cyclin D1 with ABL kinases in soft agar colony formation assays. Cells coinfected with retroviruses containing *ABL* oncogenes and cyclin D1 (or neo) were plated into soft agar in medium containing 20% fetal calf serum. Colonies equal to and larger than 0.5 mm in diameter were counted after 2–3 weeks of growth in soft agar. The number of colonies is indicated below each plate and represent the average from three experiments rounded off to two significant figures. Expression of the neo gene or neo plus cyclin D1 did not induce cells to form colonies in agar. Colonies formed by cells expressing the neo gene with P185 or v-*abl* ranged in size from 0.5 to 2 mm in diameter. Cells co-expressing cyclin D1 with either P185 or v-*abl* reached sizes up to 3 mm in diameter.



B

	Colonies, no.			
	NEO	CYC D1	D1Δ21	CYC E
NEO	0	0	0	0
v-ABL	100	380	80	90

FIG. 3. Comparison of transforming activities of v-Abl with cyclin E, cyclin D1, and an Rb-binding mutant of cyclin D1. NIH 3T3 cells were coinfected with retroviruses containing v-*abl* and human cyclin E (CYC E), mouse cyclin D1 (CYC D1), or cyclin D1 Δ21, which is deficient in Rb binding. (*A*) Western blot analysis was performed as described in Fig. 1. Cyclin E was detected by using a monoclonal antibody to human cyclin E (HE12). Lanes: 1, neo; 2, v-Abl and neo; 3, neo and cyclin D1; 4, v-Abl and cyclin D1; 5, neo and D1 Δ21; 6, v-Abl and D1 Δ21; 7, neo; 8, v-Abl and neo; 9, neo and cyclin E; 10, v-Abl and cyclin E. Cells infected with cyclin D1 retrovirus displayed a 3- to 5-fold overexpression of cyclin D1. Cells infected with the mutant cyclin D1 retrovirus expressed two times more mutant protein than endogenous cyclin D1. (*B*) Cells were plated in medium containing 5% fetal calf serum. The colony numbers represent averages of two experiments after 3 weeks in culture.

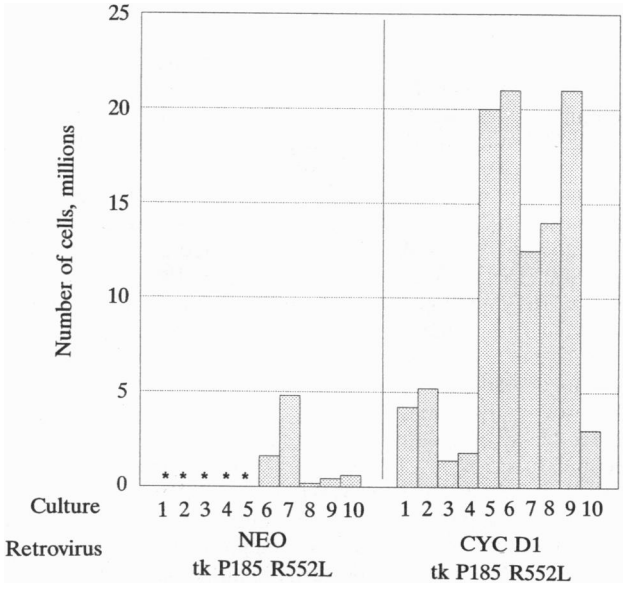


FIG. 5. Acceleration of P185-mediated bone marrow transformation by cyclin D1. Retroviruses were generated containing P185 R552L (FLVRES mutant) linked to either neo or cyclin D1 (NEO tkP185 R552L and CYC D1 tkP185 R552L, respectively). Cyclin D1 and neo were driven by the retroviral LTR, while expression of P185 R552L was controlled by the tk promoter. Primary mouse bone marrow was infected with retroviruses and plated in liquid culture. A total of 10 replicates of each sample from two experiments were monitored for pre-B-cell outgrowths. Transformed pre-B cells were counted at different time intervals. Cell counts from both experiments, taken after 20 days in culture, are shown. Cultures with cell numbers $>2 \times 10^7$ cells are confluent.
*Cultures with no cellular outgrowth.

D1 (Fig. 3*A*). This assay, therefore, distinguishes the functions of cyclin D1 and E and suggests that the synergy between v-Abl and cyclin D1 depends in part on an Rb-mediated pathway that is not influenced by cyclin E.

To determine whether cyclin D1 functions in a specific signaling pathway, we tested whether a transformation defective mutant of *BCR–ABL* could be complemented by G_1 cyclins. Rat-1 cells were co-infected with retroviruses encoding an SH2 deletion mutant of P185 (ΔSH2) with either cyclins D1 or E. The infected cell populations were plated in soft agar and were analyzed for colony formation 2–3 weeks later. The results show that cyclin D1, but not cyclin E, rescues P185ΔSH2 for transformation (Fig. 4). The colonies formed during the rescue acidified the medium and were similar in number and comparable in size (0.5–2 mm in diameter) to colonies formed by cells expressing wild-type P185. Similar results were obtained by using a transformation-defective P185 with a point mutation in the FLVRES motif of the SH2 domain (R552L) (38) (data not shown). These results suggest that cyclin D1 compensates for loss of signaling from the ABL SH2 domain.

The transforming activity of BCR–ABL is normally manifested in hematopoietic cells. BCR–ABL can transform primary mouse bone marrow *in vitro*, causing the emergence of immature B cells in a selective culture environment (33). The onset of pre-B-cell growth in culture is dependent on the strength of the transforming potential of the *BCR–ABL* allele (44). We sought to determine whether cyclin D1 can accelerate the transformation of bone marrow in culture by BCR–ABL mutants. Retroviruses that encode both genes in cis were generated (41, 45) to ensure that all infected cells express

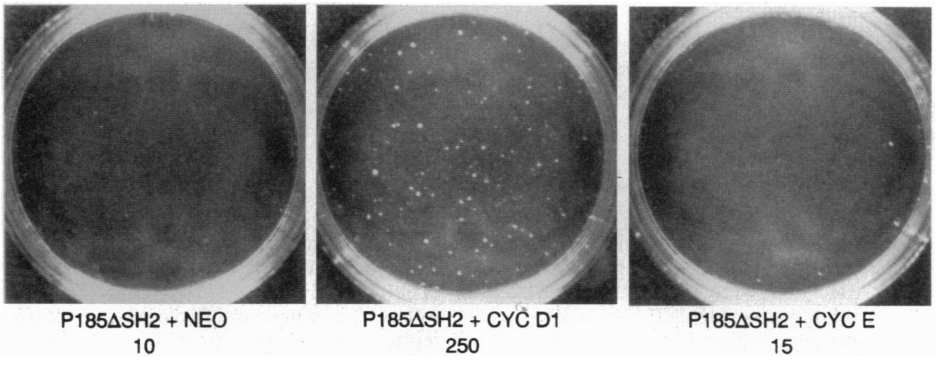


FIG. 4. Rescue of SH2 domain P185 mutant with cyclin D1. Rat-1 cells were coinfected with retroviruses encoding an SH2 domain deletion mutant of P185 (P185ΔSH2) and neo, cyclin D1, or cyclin E. Expression levels were determined as described in Fig. 1. Cells were plated in medium containing 5% fetal calf serum. The pictures were taken after 3 weeks in agar and the colony numbers, indicated below the plates, represent averages of two experiments. Cells expressing neo, cyclin D1, or cyclin E alone failed to form colonies in agar.

Table 1. Cyclin D1 rescues the FLVRES mutant of BCR–ABL for leukemogenesis

Cell line injected	No. tumors/no. total sites	Latency, days	Metastatic growth
neo tk P185 WT	6/6	8–14	Spleen, lymph nodes
cyc D1 tk P185 WT	6/6	8–14	Spleen, lymph nodes
neo tk P185 R552L	5/12	30–34	Spleen only
cyc D1 tk P185 R552L	12/14	8–14	Spleen, lymph nodes

Bone marrow cells were transformed with wild-type (WT) or FLVRES mutant (R552L) P185 in conjunction with neo or cyclin D1 (cyc D1) and were passaged independent of feeder cells for 8 weeks, and then 1×10^6 feeder-independent cells were injected subcutaneously per site into the flanks of SCID mice. Animals were monitored over a 5-week period. Those with tumors were analyzed for metastasis to spleen (S) and lymph nodes (L). Expression of BCR–ABL was verified in primary tumors and metastases by Western blot analysis (data not shown).

cyclin D1 and P185 simultaneously. Although the FLVRES mutant is capable of transforming primary mouse bone marrow, its transforming potential is attenuated when compared with wild-type P185 (43). The expression of P185 was controlled by the tk promoter, which permits a 10-fold lower expression compared to LTR-driven P185. This allows a more sensitive comparison of biological activity between wild-type and mutant P185. In two experiments, 5 of 10 cultures infected with retrovirus encoding neo and the FLVRES mutant failed to exhibit pre-B-cell transformation (Fig. 5). In contrast, all 10 cultures infected with retrovirus encoding cyclin D1 and the P185 FLVRES mutant exhibited transformation of pre-B cells. These cultures also reached saturation densities 3–5 days more rapidly.

Bone marrow cells transformed by wild-type P185 give rise to metastatic tumors with short latency when introduced into SCID mice. In contrast, cells expressing the FLVRES mutant are only weakly tumorigenic (43). To test whether cyclin D1 can complement the FLVRES mutant for tumorigenicity, bone marrow cells were transformed with wild-type P185 or the FLVRES mutant in conjunction with neo or cyclin D1. Expression of the P185 forms was controlled by the tk promoter, and expression of neo and cyclin D1 was regulated by the retroviral LTR. Transformed cells were passaged for 8 weeks independent of feeder cells and were injected subcutaneously into the flanks of SCID mice. All animals receiving cells expressing wild-type P185 with or without cyclin D1 developed large subcutaneous tumors within 8–14 days (Table 1). These animals also exhibited visibly enlarged spleens (two or three times larger than spleens of control animals) and lymph nodes (0.5 cm in diameter). Protein analysis of these tissues confirmed expression of BCR–ABL (data not shown). Cells expressing the FLVRES mutant gave rise to tumors in only 50% of the animals after 30–34 days. While the spleen appeared enlarged in these mice, metastasis to lymph nodes was not detected. Eighty-five percent of mice receiving cells coexpressing the FLVRES mutant with cyclin D1 recovered short latency of tumor formation (within 2 weeks) and full metastasis to spleen and lymph nodes. This result demonstrates that cyclin D1 can replace a defective SH2-mediated signal to gain full malignancy *in vivo*.

DISCUSSION

Activated ABL tyrosine kinases are potent oncogenes that transform cells via multiple pathways (38). Although these signaling pathways are partially redundant in hematopoietic cells, full malignant transformation *in vivo* and metastasis by BCR–ABL depends on a functional SH2 domain (43). Here we show that cyclin D1 can cooperate with *ABL* oncogenes to induce full *in vitro* and *in vivo* transformation by replacing an SH2 domain-dependent signal.

Like cyclin D1, *myc* can act synergistically with *ABL* oncogenes (32, 45) and can restore transformation by the FLVRES mutant of BCR–ABL (38). Although the simplest interpretation is that *myc* might normally be necessary to induce cyclin D1 expression (46), *myc* has also been reported to decrease cyclin D1 transcription under certain circumstances (47). Moreover, cyclin D1 may regulate Myc activity indirectly by triggering release of Rb-bound E2F, which in turn can transactivate the *myc* promoter (48). Although cyclin D1 has little transforming activity on its own, cyclin D1 and *myc* transgenes act synergistically to induce B-cell tumors *in vivo* (49, 50). This indicates that the two genes may not act in a linear pathway. Cyclin D1 and Myc also depend on one another's functions in rescuing mitogenic signaling by a partially defective colony-stimulating factor 1 receptor mutant (Y809F), with inhibition of the activity of either protein preventing complementation by the other (27). Therefore, cyclin D1 and Myc likely exert nonoverlapping functions, each of which is critical for mitogenesis.

Cyclin D1 is required during G_1 phase but not later in the cell cycle (3). It probably functions by targeting Cdk4 and Cdk6 to Rb, thereby triggering Rb inactivation by phosphorylation and affecting Rb's tethering to transcription factors, such as E2F (2). Inhibition of cyclin D1 function prevents S-phase entry in cells containing functional Rb (11, 19) but is without effect in Rb-negative cells (21–25, 57). Specific inhibitors of Cdk4 and Cdk6, such as $p16^{INK4a}$, can also arrest cells in G_1 phase and can prevent transformation of fibroblasts by Ras plus Myc, but not Ras plus E1A (20). Ink4 proteins also fail to block cell cycle progression in Rb-negative cells (21, 22, 57). These data argue that cyclin D1–Cdk complexes function upstream of Rb. Thus, Rb or Rb-regulated proteins may represent their critical physiologic targets. Our finding that a cyclin D1 mutant that fails to efficiently bind to Rb is also deficient in cooperating with v-Abl supports the concept that v-Abl can override Rb-mediated G_1 suppression via a cyclin D1-dependent mechanism. Cyclins D1 and D2 with deletions in the LXCXE motif were similarly deficient in rescuing signaling via mutant colony-stimulating factor 1 receptors (27). Cyclin E failed to function in either assay system, consistent with the idea that it regulates a different transition (15).

Cyclin D1 acts synergistically with *ABL* genes in B cells, where cyclins D2 and D3 are expressed and D1 normally is not (3, 4, 49–51). Therefore, we might expect that any D type cyclin would act synergistically with ABL in this setting. Feeder-independent BCR–ABL-transformed pre-B cells formed tumors in SCID mice. Cyclin D1 rescues the FLVRES mutant for short latency growth and full metastatic behavior, implicating cell cycle-independent effects of cyclin D1 in this assay.

BCR–ABL plays a role in the initiation and maintenance of CML. The terminal or blast-crisis phase of CML is an acute leukemia characterized by expansion of myeloid or lymphoid progenitor cells (52) and additional genetic alterations are necessary to trigger blast crisis. One possible mechanism may involve the cyclin D/Rb pathway. Deletions of Rb have been detected in patients exhibiting megakaryoblastic crisis in CML (53). Deletions in $p16^{INK4a}$ have been detected in certain tumor cell lines and human cancers (28) and occur in tumors that retain wild-type Rb (54, 55). In a recent study, 50% of patients with lymphoid blast crisis in CML exhibited homozygous

deletions of $p16^{INK4a}$ (56). In blocking its ability to inhibit Cdks, mutations or deletions in $p16^{INK4a}$ might mimic the effects of overexpressed D cyclins in hematopoietic cells.

We are grateful to Drs. Larry Zipursky, Charles Sawyers, Steve Smale, Doug Saffran, Ale Merino, Stefan Mahlmann, and Andrei Goga for critical review of the manuscript; to Jamie White for photography; and to Julia Shimaoka and Mandee Khu for preparation of the manuscript. Marie Havlik and James Johnson-White provided excellent technical assistance. We thank Dr. Emma Lees for providing constructs containing cyclin E and antibodies directed to cyclin E, Dr. Mark Ewen for providing cyclin D1 Δ21, and Dr. David Baltimore for providing 293T cells. This work was supported in part by Grants CA53867 from the National Institutes of Health (O.N.W.), CA56819 (M.F.R.), and CA47064 (C.J.S.) from the National Cancer Institute. D.E.H.A. is a postdoctoral fellow of the Medical Research Council of Canada. O.N.W. and C.J.S. are Investigators of the Howard Hughes Medical Institute.

1. Pardee, A. B. (1989) *Science* **246,** 603–608.
2. Sherr, C. J. (1994) *Cell* **79,** 551–555.
3. Matsushime, H., Roussel, M. F., Ashmun, R. A. & Sherr, C. J. (1991) *Cell* **65,** 701–713.
4. Ajchenbaum, F., Ando, K., DeCaprio, J. A. & Griffin, J. D. (1993) *J. Biol. Chem.* **268,** 4113–4119.
5. Matsushime, H., Ewen, M. E., Strom, D. K., Kato, J.-Y., Hanks, S. K., Roussel, M. F. & Sherr, C. J. (1992) *Cell* **71,** 323–334.
6. Matsushime, H., Quelle, D. E., Shurtleff, S. A., Shibuya, M., Sherr, C. J. & Kato, J.-Y. (1994) *Mol. Cell. Biol.* **14,** 2066–2076.
7. Meyerson, M. & Harlow, E. (1994) *Mol. Cell. Biol.* **14,** 2077–2086.
8. Dulic, V., Lees, E. & Reed, S. I. (1992) *Science* **257,** 1958–1961.
9. Koff, A., Giordano, A., Desai, D., Yamashita, K., Harper, J. W., Elledge, S., Nishimoto, T., Morgan, D. O., Franza, B. R. & Roberts, J. M. (1992) *Science* **257,** 1689–1694.
10. Ohtsubo, M. & Roberts, J. M. (1993) *Science* **259,** 1908–1912.
11. Quelle, D. E., Ashmun, R. A., Shurtleff, S. A., Kato, J.-Y., Bar-Sagi, D., Roussel, M. F. & Sherr, C. J. (1993) *Genes Dev.* **7,** 1559–1571.
12. Resnitzky, D., Gossen, M., Bujard, H. & Reed, S. I. (1994) *Mol. Cell. Biochem.* **14,** 1669–1679.
13. Resnitzky, D. & Reed, S. (1995) *Mol. Cell. Biol.* **15,** 3463–3469.
14. Hatakeyama, M., Brill, J. A., Fink, G. R. & Weinberg, R. A. (1994) *Genes Dev.* **8,** 1759–1771.
15. Ohtsubo, M., Theodoras, A., Schumacher, J., Roberts, J. M. & Pagano, M. (1995) *Mol. Cell. Biol.* **15,** 2612–2624.
16. Dowdy, S. F., Hinds, P. W., Louie, K., Reed, S. I., Arnold, A. & Weinberg, R. A. (1993) *Cell* **73,** 499–511.
17. Ewen, M. E., Sluss, H. K., Sherr, C. J., Matsushime, H., Kato, J.-A. & Livingston, D. M. (1993) *Cell* **73,** 487–497.
18. Kato, J., Matsushime, H., Hiebert, S. W., Ewen, M. E. & Sherr, C. J. (1993) *Genes Dev.* **7,** 331–342.
19. Baldin, V., Lukas, J., Marcote, M. J., Pagano, M. & Draetta, G. (1993) *Genes Dev.* **7,** 812–821.
20. Serrano, M., Gomez-Lahoz, E., DePinho, R. A., Beach, D. & Bar-Sagi, D. (1995) *Science* **267,** 249–252.
21. Lukas, J., Parry, D., Aagaard, L., Mann, D. J., Bartkova, J., Strauss, M., Peters, G. & Bartek, J. (1995) *Nature (London)* **375,** 503–506.
22. Medema, R. H., Herrera, R. E., Lam, F. & Weinberg, R. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 6289–6293.
23. Tam, S. W., Theodoras, A. M., Shay, J. W., Draetta, G. & Pagano, M. (1994) *Oncogene* **9,** 2663–2674.
24. Lukas, J., Muller, H., Bartkova, J., Spitkovsky, D., Kjerulff, A. A., Jansen-Durr, P., Strauss, M. & Bartek, J. (1994) *J. Cell Biol.* **125,** 625–638.
25. Lukas, J., Bartkova, J., Rohde, M., Strauss, M. & Bartek, J. (1995) *Mol. Cell. Biol.* **15,** 2600–2611.
26. Kato, J. & Sherr, C. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11513–11517.
27. Roussel, M. F., Theodoras, A. M., Pagano, M. & Sherr, C. J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 6837–6841.
28. Hunter, T. & Pines, J. (1994) *Cell* **79,** 573–582.
29. Kurzrock, R., Gutterman, J. & Talpaz, M. (1988) *N. Engl. J. Med.* **319,** 990–998.
30. Rosenberg, N. & Witte, O. N. (1988) *Adv. Virus Res.* **39,** 39–81.
31. Konopka, J. B. & Witte, O. N. (1985) *Mol. Cell. Biol.* **5,** 3116–3123.
32. Lugo, T. & Witte, O. N. (1989) *Mol. Cell. Biol.* **9,** 1263–1270.
33. McLaughlin, J., Chianese, E. & Witte, O. N. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6558–6562.
34. Kelliher, M. A., McLaughlin, J., Witte, O. N. & Rosenberg, N. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6649–6653.
35. Scott, M. L., Van Etten, R. A., Daley, G. Q. & Baltimore, D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6506–6510.
36. Gishizky, M. L., Johnson-White, J. & Witte, O. N. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3755–3759.
37. Chen, Y.-Y. & Rosenberg, N. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6683–6687.
38. Afar, D. E. H., Goga, A., McLaughlin, J., Witte, O. & Sawyers, C. L. (1994) *Science* **264,** 424–426.
39. Hinds, P. W., Mittnacht, S., Dulic, V., Arnold, A., Reed, S. I. & Weinberg, R. A. (1992) *Cell* **70,** 993–1006.
40. Muller, A. J., Young, J. C., Pendergast, A.-M., Pondel, M., Landau, N. R., Littman, D. R. & Witte, O. N. (1991) *Mol. Cell Biol.* **11,** 1785–1792.
41. Sawyers, C. L., McLaughlin, J. & Witte, O. N. (1995) *J. Exp. Med.* **181,** 307–313.
42. Pear, W. S., Nolan, G. P., Scott, M. L. & Baltimore, D. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8392–8396.
43. Goga, A., McLaughlin, J., Afar, D. E. H., Saffran, D. C. & Witte, O. N. (1995) *Cell* **82,** in press.
44. McLaughlin, J., Chianese, E. & Witte, O. N. (1989) *Mol. Cell. Biol.* **9,** 1866–1874.
45. Sawyers, C. L., Callahan, W. & Witte, O. N. (1992) *Cell* **70,** 901–910.
46. Daksis, J. I., Lu, R. Y., Facchini, L. M., Marhin, W. W. & Penn, L. J. Z. (1994) *Oncogene* **9,** 3635–3645.
47. Jansen-Dürr, P., Meichle, A., Steiner, P., Pagano, M., Finke, K., Botz, J., Wessbecher, J., Draetta, G. & Eilers, M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3685–3689.
48. Oswald, F., Lovec, H., Moroy, T. & Lipp, M. (1994) *Oncogene* **9,** 2029–2036.
49. Bodrug, S. E., Warner, B. J., Bath, M. L., Lindeman, G. J., Harris, A. W. & Adams, J. M. (1994) *EMBO J.* **13,** 2124–2130.
50. Lovec, H., Grzeschiczek, A., Kowalski, M.-B. & Möröy, T. (1994) *EMBO J.* **13,** 3487–3495.
51. Palmero, I., Holder, A., Sinclair, A. J., Dickson, C. & Peters, G. (1993) *Oncogene* **8,** 1049–1054.
52. Kantarijan, H. M., Deisseroth, A., Kurzrock, R., Estrov, Z. & Talpaz, M. (1993) *Blood* **82,** 691–703.
53. Towatari, M., Adachi, K., Kato, H. & Saito, H. (1991) *Blood* **78,** 2178–2181.
54. Otterson, G. A., Kratzke, R. A., Coxon, A., Kim, Y. W. & Kaye, F. J. (1994) *Oncogene* **9,** 3375–3378.
55. Parry, D., Bates, S., Mann, D. J. & Peters, G. (1995) *EMBO J.* **14,** 503–511.
56. Sill, H., Goldman, J. M. & Cross, N. C. P. (1995) *Blood* **85,** 2013–2016.
57. Koh, J., Enders, G. H., Dynlacht, B. D. & Harlow, E. (1995) *Nature (London)* **375,** 506–510.