Physical and Functional Interactions between Pim-1 Kinase and Cdc25A Phosphatase

IMPLICATIONS FOR THE Pim-1-MEDIATED ACTIVATION OF THE c-Myc SIGNALING PATHWAY*

(Received for publication, November 23, 1998, and in revised form, April 6, 1999)

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The pim-1 oncogene encodes a serine/threonine kinase (Pim-1) involved in the transduction of cytokine-triggered mitogenic signals. Pim-1 is unique in that it closely cooperates with c-Myc not only in oncogenesis, but also in apoptosis induction. However, the molecular basis of Pim-1 function remains poorly understood, largely because the downstream effector molecule(s) for Pim-1 kinase has not been identified. Here we provide several lines of evidence that Cdc25A cell cycle phosphatase, a direct transcriptional target for c-Myc, is a substrate for Pim-1 kinase and functions as an effector for Pim-1. We found that Pim-1 physically interacts with Cdc25A both in vitro and in vivo and phosphorylates Cdc25A. We also observed that Pim-1-mediated phosphorylation of Cdc25A increases its phosphatase activity. In addition, wild-type Pim-1, but not kinase-inactive Pim-1, enhanced Cdc25A-mediated cellular transformation and apoptosis. Our results indicate that Cdc25A might be a key molecule that links Pim-1 and c-Myc and that also ties Pim-1-mediated mitogenic signals to cell cycle machinery.

The pim-1 oncogene was originally identified as a genetic locus frequently activated by the proviral insertion of Moloney murine leukemia virus into mouse T cell lymphomas (1–3). The pim-1 oncogene has also been implicated in human hematopoietic malignancies, with its overexpression frequently detected in human hematopoietic cell lines as well as in fresh tumor cells from patients with leukemia (4–6). Under physiological conditions, the pim-1 gene is expressed mainly in developing fetal hematopoietic tissues (6). The pim-1 gene product (Pim-1), identified as a serine/threonine kinase (7–9), has been thought to play a critical role in the transduction of mitogenic signals from cytokines since Pim-1 expression is rapidly induced after cytokine stimulation, and the proliferative response to cytokines is impaired in cells from pim-1-deficient mice (10–19). However, at present, the downstream signaling pathway for Pim-1 kinase leading to cell proliferation remains unknown.

One of the unique properties of pim-1 as an oncogene is its close cooperation with c-myc in oncogenesis (20–22). Although transgenic mice expressing pim-1 under the control of the immunoglobulin Eμ enhancer have been shown to develop lymphomas spontaneously, the appearance of monclonal lymphomas at a low incidence and with a long latency period suggests the requirement of additional genetic changes for lymphomagenesis (20). Indeed, treatment with chemical carcinogens or infection with Moloney murine leukemia virus significantly facilitated lymphomagenesis in Eμ-pim-1 mice, and it was also revealed that most of the lymphomas developing in Eμ-pim-1 transgenic mice have a concomitant overexpression of the c-myc oncogene, suggesting the strong dependence of pim-1 on the deregulated expression of c-myc (20). In support of this observation, Verbeek et al. (22) demonstrated that the coexpression of pim-1 and c-myc by crosses of Eμ-pim-1 and Eμ-myc mice causes the accelerated development of leukemias in utero. Although the mechanism underlying this strong synergistic oncogenesis between pim-1 and c-myc remains unclear, it has been proposed that Pim-1 may contribute to transformation by inhibiting apoptosis, similarly to antiapoptotic Bcl-2 oncoprotein, because pim-1 expression rescues both lymph node cells from rapid apoptosis in vitro and CD4+CD8+ double-positive thymocytes from dexamethasone-induced apoptosis in vivo in Eμ-pim-1 lpr/lpr mice (23).

c-Myc oncoprotein has been shown to trigger intracellular signals leading to both transformation and apoptosis (24), and the selective inhibition of the apoptotic signaling pathway by Bcl-2 has been suggested as a basis for the observed synergy between c-myc and bcl-2 in lymphomagenesis in transgenic mice (25–27). We therefore examined in a previous study whether pim-1 cooperates with c-myc in oncogenesis through the inhibition of c-Myc-mediated apoptosis (28) using a well established system for c-Myc-mediated apoptosis in which deregulated c-Myc expression causes the apoptosis of rat fibroblasts deprived of serum growth factors (29). However, quite unexpectedly, the expression of Pim-1 kinase enhanced the c-Myc-mediated apoptosis in serum-deprived Rat-1 fibroblasts (28), which was in sharp contrast to the inhibitory activity of Bcl-2 against c-Myc-mediated apoptosis (26, 27). Furthermore, Pim-1 expression alone did not cause apoptosis even in the absence of serum growth factors, indicating that Pim-1 kinase requires deregulated c-Myc expression for its apoptosis-promoting activity (28).
Activation of Cdc25A Phosphatase by Pim-1 Kinase

Thus, accumulating evidence now suggests that, in both cellular transformation and apoptosis induction, Pim-1 is dependent on and cooperates with deregulated c-Myc expression, and this gives rise to an intriguing possibility that Pim-1 kinase may function through the modulation of c-Myc-triggered intracellular signaling common to transformation and apoptosis. Therefore, in this study, we examined this possibility by exploring the target molecules for Pim-1 kinase, and we found that Cdc25A cell cycle phosphatase can function as a substrate and effector molecule for Pim-1 kinase. Based on the results, we suggest that Cdc25A, a direct transcriptional target of c-Myc implicated in both transformation and apoptosis induction, may act as a key molecule on which intracellular signals from c-Myc and Pim-1 converge.

EXPERIMENTAL PROCEDURES

Plasmids—pDNA3cCdc25A was created by subcloning rat cdc25A cDNA (30) containing the entire coding region into the mammalian expression vector pCDNA (Invitrogen). pFLAG-Pim-1 and pFLAG-Pim-1K67M were created by subcloning the entire coding region of pim-1 cDNA from pcDNA3pim-1 and pcDNA3pim-1K67M (28), respectively, into the pFLAG-CMV-2 expression vector (Eastman Kodak Co.). GST-Pim-1 and GST-Pim-1K67M were created by subcloning the entire coding region from pcDNA3pim-1 and pcDNA3pim-1K67M, respectively, in frame to the GST coding sequence of the pGEX2T vector (Amersham Pharmacia Biotech). GST-Pim-1 deletion mutants (amino acids 1–228, 1–96, 1–40, and 230–315) were similarly created by subcloning the EcoRI-BamHI, EcoRI-SacI, EcoRI-ApaI, and BamHI-EcoRI fragments of pcDNA3pim-1, respectively, into pGEX2T. GST-Cdc25A and GST-c-Myc(1–262) were created by subcloning the entire coding region of rat cdc25A cDNA and the human c-myc cDNA fragment from the initiator ATG codon to the Cld site, respectively, in-frame to GST of the pGEx2T vector. The affinity purification of the GST fusion proteins expressed in Escherichia coli (JM109) was done essentially as described previously (31).

Cell Culture, Apoptosis Assay, and Soft Agar Colony Formation Assay—Rat-1 fibroblasts were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (DMEM/FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin. To obtain Rat-1 cells constitutively overexpressing Cdc25A, Rat-1 cells were transfected with pcDNA3cCdc25A and selected with Genetecin (Life Technologies, Inc.). Stable Rat-1 transfectants expressing transfected Cdc25A (Rat-1/Cdc25A) were identified by a Northern blot analysis using rat cdc25A cDNA as a probe essentially as described previously (32). Rat-1/Cdc25A cells were further transfected with pFLAG-Pim-1 together with pCEP4 (carrying the hygromycin resistance gene) and pCEP4-Pim-1 (Carbomet). Stable transfectants thus obtained were examined for FLAG-tagged Pim-1 (FLAG-Pim-1) expression by immunoblot analysis using an anti-FLAG antibody (M5, Kodak), and clones expressing and not expressing FLAG-Pim-1 were designated Rat-1/Cdc25A/FLAG-Pim-1(+) and Rat-1/Cdc25A/FLAG-Pim-1(−), respectively. Rat-1/Cdc25A/FLAG-Pim-1K67M cells expressing FLAG-tagged, kinase-inactive Pim-1 were similarly obtained by transfecting Rat-1/Cdc25A cells with pFLAG-Pim-1K67M and pCEP4, followed by hygromycin B selection and immunoblot analysis.

Severed deprivation and the counting of apoptotic cells were done as described previously (28). To induce apoptosis in Rat-1 transfectants, cells were seeded 24 h before serum deprivation and then transferred to medium containing 0.1% FBS. Apoptotic cells were identified by their loss of adherence and typical nuclear changes (defined chromatin condensation and/or nuclear fragmentation). For detection of apoptotic nuclear changes, non-adherent cells were incubated in the presence of 0.5 μg/ml acridine orange (Sigma) and observed under a fluorescence microscope. The percentage of apoptotic cells was determined as 100 × (number of apoptotic cells/total cell count).

For colony formation assay, a total of 1 × 105 cells suspended in DMEM/FBS were mixed with an equal volume of DMEM/FBS containing 0.8% agarose (final 0.4% agarose) and poured onto a bed of DMEM/FBS containing 0.5% agarose in a 60-mm dish. After 1 week, photomicrographs were taken, and colonies were counted in three randomly selected visual fields (magnification × 100). In both the apoptosis and soft agar colony formation assays, multiple clones for each type of transfected cells were examined, with essentially similar results. Therefore, the results of representative transfected clones are presented.

RESULTS

Our previous results suggested that Pim-1 kinase stimulates c-Myc-mediated apoptosis in serum-deprived Rat-1 fibroblasts through the phosphorylation of its target molecule(s) (28). As one of the approaches to identify such a target(s) for Pim-1 kinase that might be involved in the intracellular signaling triggered by c-Myc, we searched for cellular proteins that are associated with Pim-1. A GST fusion protein containing full-length Pim-1 (GST-Pim-1) was bacterially expressed, purified, and then used to screen binding proteins in the Rat-1 cell lysate. When GST and GST-Pim-1 were incubated with a cell lysate and subeluting metabolically labeled Rat-1 cells, GST-Pim-1 bound several cellular proteins that did not bind to the control GST protein, suggesting the presence of cellular proteins that specifically bind to Pim-1 (Fig. 1A). Among these proteins, we were particularly interested in the one with a molecular mass of ~65 kDa (Fig. 1A, arrow) since a previous study also demonstrated the presence of an ~68-kDa phosphorylated protein complexed with Pim-1 in a Pim-1 immunocomplex kinase assay (8).

The ability of Pim-1 to cooperate with c-Myc in both cellular
transformation and apoptosis induction suggested the possibility that Pim-1 interacts with a molecule(s) regulating the common proximal signaling pathway required for both c-Myc-mediated cellular transformation and apoptosis induction (28). We therefore reasoned that the ~65-kDa protein might be c-Myc itself or its direct transcriptional target Cdc25A cell cycle phosphatase (34), both of which have a similar molecular mass of ~65 kDa. To test this possibility, we immunoblotted, with specific antibodies against c-Myc and Cdc25A, the proteins bound to GST-Pim-1 after incubation with the Rat-1 cell lysate. Although c-Myc was not found in the proteins bound to GST-Pim-1 in this in vitro binding assay (data not shown), a specific band was reproducibly detected around 65 kDa when immunoblotting was done using the anti-Cdc25A antibody (Fig. 1B). These results demonstrated that Pim-1 binds Cdc25A cell cycle phosphatase in this assay system.

Using this in vitro binding assay, we then determined which region of Pim-1 is responsible for the specific interaction with Cdc25A. We constructed deletion mutants of Pim-1 fused to GST (Fig. 2, A and B), and after incubating these GST-Pim-1 mutants with the Rat-1 cell lysate, we immunoblotted the bound proteins with the anti-Cdc25A antibody. As shown in Fig. 2C, Cdc25A preferentially bound to the N-terminal region of Pim-1 (GST-Pim-1-1–228; lane 3), but not to its C-terminal region (GST-Pim-1-230–313; lane 6). Although GST-Pim-1-(1–96) still bound Cdc25A efficiently (Fig. 2C, lane 4), GST-Pim-1-(1–40) failed to do so (lane 5). These results indicated that the N-terminal 96 residues of Pim-1 are necessary and sufficient for the interaction with Cdc25A.

We next examined whether the association of Pim-1 and Cdc25A observed in the in vitro binding assay is also relevant in vivo. Cell lysates were prepared from COS-7 cells transiently transfected with either a plasmid vector expressing FLAG-Pim-1 or a control empty vector (FLAG), and co-immunoprecipitation assays were performed using these lysates. As shown in Fig. 3A, the anti-FLAG antibody coprecipitated endogenous Cdc25A together with FLAG-Pim-1, but not with the FLAG tag alone. Conversely, the anti-Cdc25A antibody, but not control rabbit IgG, coprecipitated FLAG-Pim-1 together with Cdc25A (Fig. 3B). Thus, Pim-1 and Cdc25A form a stable complex in vivo as well as in vitro.

Given the physical interaction between Pim-1 and Cdc25A, we next investigated whether Cdc25A serves as a substrate for Pim-1 kinase. As shown in Fig. 4A, GST-Pim-1 phosphorylated GST-Cdc25A (lane 4) as efficiently as it phosphorylated histone H1 (lane 1), one of the best substrates for Pim-1 kinase known to date (9, 33). In contrast, GST-Pim-1 phosphorylated neither the control GST protein (Fig. 4A, lane 3) nor an irrelevant protein, the N-terminal 262 residues of human c-Myc fused to GST (lane 6) under these assay conditions, indicating that the phosphorylation of Cdc25A by Pim-1 is not a nonspecific event. The phosphorylation of Cdc25A was strictly dependent on the intrinsic kinase activity of Pim-1 because the kinase-inactive mutant of Pim-1 (Pim-1K67M), in which Lys-67 (essential for ATP binding) is mutated to Met (8, 33), failed to phosphorylate Cdc25A (Fig. 4A, lane 5) as well as histone H1 (lane 2).

Cdc25A phosphatase is thought to regulate cell cycle progression through the removal of phosphate groups from the conserved Thr-14 and Tyr-15 residues of cyclin-dependent kinases (30, 35), and it has been demonstrated that the phosphatase activity of Cdc25A can be enhanced through phosphorylation by serine/threonine kinases such as Cdk2 and Raf-1 (35, 36). To determine the effect of the Pim-1-mediated phosphorylation of Cdc25A on its phosphatase activity, we performed phosphatase assays using p-nitrophenyl phosphate, a chromogenic molecule that is structurally related to phosphorysine (37, 38), as a substrate. As shown in Fig. 4B, GST-Cdc25A, which was bacterially expressed and purified, had a basal phosphatase activity, dephosphorylating p-nitrophenyl phosphate in a dose-dependent manner, whereas the control GST protein had no such activity. We then analyzed the phosphatase activity of GST-Cdc25A after preincubation with GST fusion proteins including GST-Pim-1 under the kinase reaction conditions. The phosphatase activity of GST-Cdc25A increased from the basal level (Fig. 4C, bar 1) after preincubation with GST-Pim-1 (bar 5), but not with GST alone (bar 3), suggesting that Pim-1 activates Cdc25A. The activation of Cdc25A by Pim-1 was apparently dependent on phosphorylation by Pim-1 kinase since Pim-1 failed to activate Cdc25A significantly in the absence of ATP (Fig. 4C, bar 6), and the kinase-inactive Pim-1K67M mutant also failed to activate Cdc25A even in the presence of ATP (lane 7). Taken together, these results indicate that Pim-1 kinase activates the phosphatase activity of Cdc25A through its phosphorylation.

The finding that Pim-1 kinase is capable of phosphorylating and activating Cdc25A phosphatase in vitro, together with the observation that Pim-1 forms a stable complex with Cdc25A in vivo, strongly suggested the possibility that Pim-1 kinase modulates the biological functions of Cdc25A in living cells. To test this possibility, we first examined the effect of Pim-1 overexpression on the Cdc25A-mediated apoptosis of serum-deprived fibroblasts. Consistent with a previous report (34), the overexpression of exogenously introduced Cdc25A (Rat-1/Cdc25A; Fig. 5A, lane 2) efficiently induced apoptotic cell death in Rat-1 fibroblasts deprived of serum survival factors (Fig. 5, B and C). The concomitant overexpression of Pim-1 together with Cdc25A (Rat-1/Cdc25A/FLAG-Pim-1(+); Fig. 5A, lane 3) further stimulated the serum deprivation-induced apoptosis compared with the overexpression of Cdc25A alone (Rat-1/Cdc25A; Fig. 5, B and C), suggesting that Pim-1 is capable of enhancing Cdc25A-mediated apoptosis. The enhancement of Cdc25A-mediated apoptosis was actually dependent on the expression of Pim-1 and its intrinsic kinase activity since both Rat-1/Cdc25A/FLAG-Pim-1(−) cells, which do not overexpress Pim-1 despite transfection of the Pim-1 expression vector (Fig. 5A, lane 4), and Rat-1/Cdc25A/FLAG-Pim-1K67M cells, which overexpress kinase-inactive Pim-1 together with Cdc25A (lane 5), underwent apoptosis no more efficiently than did Rat-1/Cdc25A cells deprived of serum survival factors (B and C). Together, these results demonstrate that Pim-1 kinase enhances the ability of Cdc25A to induce apoptosis.
In addition to its role in apoptosis regulation, the perturbation of cell cycle control as a result of deregulated Cdc25A expression has been implicated in oncogenesis. For instance, Cdc25A is frequently overexpressed in human breast and gastric cancers and has been shown to cooperate with either oncopogenic Ha-Ras or a loss of RB1 in oncogenic transformation (39–41). We therefore investigated whether Pim-1 enhances the oncogenic potential of Cdc25A. The stable Rat-1 transfec-

![Region of Pim-1 responsible for the specific binding with Cdc25A.](image1)

FIG. 2. Region of Pim-1 responsible for the specific binding with Cdc25A. Pim-1 deletion mutants fused to GST (schematically presented in A) were expressed in E. coli, affinity-purified, and either separated by SDS-PAGE followed by Coomassie Blue staining (B) or subjected to binding analysis (C). In C, unlabeled Rat-1 cell lysate was incubated with the GST fusion products, and the bound proteins were separated by SDS-PAGE, followed by immunoblot analysis using the anti-Cdc25A antibody.

![Binding of Pim-1 and Cdc25A in vivo.](image2)

FIG. 3. Binding of Pim-1 and Cdc25A in vivo. COS-7 cells were transiently transfected with pFLAG-CMV-2 empty vector (FLAG) or pFLAG-Pim-1 (FLAG-Pim-1), and cell lysates were prepared 48 h after transfection. A, the cell lysates were immunoprecipitated (IP) with the anti-FLAG antibody, and the immunoprecipitates were immunoblotted with the anti-Cdc25A or anti-FLAG antibody. In the bottom panel, aliquots of total cell lysates were immunoblotted with the anti-Cdc25A antibody. B, the cell lysates were immunoprecipitated with the anti-Cdc25A antibody or with control IgG, and the immunoprecipitates were immunoblotted with the anti-FLAG or anti-Cdc25A antibody. In the bottom panel, aliquots of total cell lysates were immunoblotted with the anti-FLAG antibody.

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transfected phenotype of anchorage-independent growth in the presence of serum survival factors. As shown in Fig. 6 (A and B), the overexpression of either Cdc25A (Rat-1/Cdc25A) or Pim-1 (Rat-1/Pim-1) alone only marginally stimulated the colony formation of Rat-1 fibroblasts in soft agar. However, colony formation was substantially facilitated by the concomitant overexpression of Cdc25A and Pim-1 (Rat-1/Cdc25A/FLAG-Pim-1); Fig. 6, A and B). As was the case with the apoptosis assay, the enhanced colony formation required both the overexpression of Pim-1 and its kinase activity (Rat-1/Cdc25A/FLAG-Pim-1(-) and Rat-1/Cdc25A/FLAG-Pim-1K67M; Fig. 6, A and B). Notably, although the Rat-1/Cdc25A/FLAG-Pim-1(+) cells that overexpress Cdc25A and Pim-1 were capable of forming colonies in soft agar, they did not form foci under normal culture conditions. This may indicate that higher expression levels of Pim-1 and Cdc25A or additional oncogenes or loss of tumor suppressor genes is required for a fully transformed phenotype. Nevertheless, the results clearly indicate that Pim-1 kinase enhances the transforming potential as well as the apoptosis-inducing ability of Cdc25A.

**DISCUSSION**

In this study, we showed that Pim-1 kinase physically interacts with Cdc25A and increases its phosphatase activity through phosphorylation. We also showed that Pim-1 kinase enhances the biological activities of Cdc25A, including apoptosis induction. Cdc25A has been identified as a direct transcriptional target for the c-Myc transcription factor, and it has also been demonstrated that the deregulated expression of Cdc25A is required for the induction of c-Myc-mediated apoptosis in rodent fibroblasts (34). In addition to these findings, we previously reported that Pim-1 expression can stimulate the apoptotic signaling pathway elicited by c-Myc in Rat-1 fibroblasts depending on its kinase activity (28). Taken together, these lines of evidence suggest that Pim-1 kinase activates the c-Myc-mediated apoptotic signaling through the direct activation of Cdc25A up-regulated by c-Myc.

Our present results suggested that Pim-1 behaves as a pro-apoptotic molecule in the c-Myc- and Cdc25A-mediated apo-
ptosis of serum-deprived Rat-1 cells. However, it seems that the effect of Pim-1 on apoptosis depends on the cell type. For instance, Pim-1 has been implicated in the apoptosis induction of a murine myeloma cell line (42), whereas Pim-1 has been shown to function as an antiapoptotic factor in lymphocytes from lpr/lpr mice (23) and in a murine myeloid precursor cell line (43). Similarly, the role of c-Myc-mediated signaling in apoptosis regulation appears to be different depending on the cell type. Indeed, in the interleukin (IL)-3-dependent pro-B cell line Ba/F3, c-Myc overexpression rescues cells from the IL-3 deprivation-induced apoptosis associated with a decrease in c-Myc expression, demonstrating that c-Myc functions as a survival factor rather than as an apoptosis inducer under this condition (44). On the other hand, c-Myc overexpression facilitates the IL-3 deprivation-induced apoptosis of the myeloid cell line 32D (45). From these observations, we propose that Pim-1 may be simply stimulating the c-Myc-mediated signaling pathway in every case, and the net effect of Pim-1 expression on apoptosis may be dependent on whether c-Myc acts proapoptotically or antiapoptotically in a given cell type. However, it is also possible that Pim-1 kinase has multiple target molecules, including Cdc25A, some of which are implicated in apoptosis induction and others in apoptosis inhibition. If so, the further identification of the target molecules of Pim-1 should provide...
more insights into the versatile apoptosis regulation by Pim-1.

At present, the precise mechanism underlying Pim-1-mediated cellular transformation remains unknown. Although it has been proposed based on the antiapoptotic activity of Pim-1 kinase that Pim-1 contributes to transformation by inhibiting apoptosis (23), it is unlikely that the inhibition of apoptosis is the sole mechanism. First, pim-1 has been shown to effectively cooperate with bcl-2, a representative antiapoptotic oncogene, in lymphomagenesis (46, 47). Second, gfi-1, identified through the proviral tagging approach as a gene whose overexpression can further facilitate lymphomagenesis in myc/pim-1 bitransgenic mice, has been shown to function as an apoptosis inhibitor through the transcriptional repression of proapoptotic members of the bcl-2 gene family such as bax and bak (48, 49). If Pim-1 is functioning solely as an apoptosis inhibitor, these combinations should be redundant rather than cooperative. In this regard, our present results give rise to an attractive hypothetical model in which Pim-1 contributes to cellular transformation through the activation of Cdc25A cell cycle phosphatase, which has recently been reported to be overexpressed in human aggressive lymphomas overexpressing c-myc (50). Although the role of Cdc25A in lymphomagenesis is yet to be demonstrated, deregulated Cdc25A expression may well be oncogenic to hematopoietic cells, given its critical role in cell cycle progression (30, 35) and its well documented oncogenic potential in fibroblasts (39). More important, this model can

Fig. 6. Pim-1 and Cdc25A synergistically induce the anchorage-independent growth of Rat-1 cells in the presence of serum survival factors. A, the indicated Rat-1 cells were plated and cultured in soft agar containing 10% fetal bovine serum as described under "Experimental Procedures." Rat-1/Pim-1 cells, which overexpress transfected Pim-1, have been previously described (28). Photomicrographs were taken under a phase-contrast microscope 1 week after plating (original magnification × 100). B, shown is a quantitative summary for A. The numbers of colonies were counted after 1 week of culture in soft agar. The results are the means ± S.D. of two separate experiments.
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provide a clear explanation for the close cooperation between pim-1 and c-myc in lymphomagenesis, one of the most characteristic features of the pim-1 oncogene. It would therefore be of interest to investigate whether Cdc25A has a role and can cooperate with Pim-1 in lymphomagenesis.

Aside from its function as an oncoprotein, Pim-1 kinase also plays a crucial role in mediating the mitogenic signals from cytokines such as IL-3 and IL-7 in hematopoietic cells (10, 12–14). However, how the mitogenic signals lead to stimulated cell proliferation remains unknown. Cdc25A cell cycle phosphatase has been shown to act as a critical regulator of cell cycle transition from G1 to S (30, 35), and its phosphatase activity is regulated by phosphorylation, as shown in this study as well as in others (35, 36). It is therefore expected that mitogen-induced Pim-1 expression leads to the increased phosphorylation and activation of Cdc25A, which in turn stimulates cell proliferation by facilitating cell cycle progression. Thus, our identification of Cdc25A as an effector molecule for Pim-1 kinase provides a possible link between Pim-1-mediated mitogenic signaling and cell cycle progression.

Acknowledgments—We thank Drs. Shigeaki Jinno, Akihisa Nagata, and Hirota Okayama for the generous gift of Cdc25A cDNA.

REFERENCES

1. Cuyppers, H. T., Selten, G., Quint, W., Zijlstra, M., Maandag, E. R., Boelens, W., van Wezenbeek, P., Melief, C., and Berns, A. (1984) Cell 37, 141–150
2. Selten, G., Cuyppers, H. T., and Berns, A. (1985) EMBO J. 4, 1793–1798
3. Selten, G., Cuyppers, H. T., Boelens, W., Robanus-Maandag, E., Verbeek, J., Domen, J., van Beveren, C., and Berns, A. (1986) Cell 46, 603–611
4. Nagarajan, L., Louie, K., Tsujimoto, Y., Ar-Rushdi, A., Heubner, K., and Creece, C. M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 2556–2560
5. Meeker, T. C., Nagarajan, L., Ar-Rushdi, A., Rovera, G., Heubner, K., and Creece, C. M. (1987) Oncogene Res. 1, 87–91
6. Amson, R., Sigaux, F., Prezdelborski, S., Flandrin, G., Givol, D., and Telerman, A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8857–8861
7. Sarias, C. J. M., Domen, J., and Berns, A. (1991) EMBO J. 10, 655–664
8. Padma, R., and Nagarajan, L. (1991) Cancer Res. 51, 2484–2489
9. Hooyer, D., Friedmann, M., Reeves, R., and Magnuson, N. S. (1991) J. Biol. Chem. 266, 14018–14023
10. Dautry, P., Weil, D., Yu, J., and Dautry-Varsat, A. (1988) J. Biol. Chem. 263, 17615–17620
11. Wingett, D., Reeves, R., and Magnuson, N. S. (1991) J. Immunol. 147, 3653–3659
12. Lilly, M., Le, T., Holland, P., and Hendrickson, S. L. (1992) Oncogene 7, 727–732
13. Domen, J., van der Lught, N. M. T., Acton, D., Laird, P. W., Linders, K., and Berns, A. (1993) J. Exp. Med. 178, 165–176
14. Domen, J., van der Lught, N. M. T., Laird, P. W., Sarias, C. J. M., Clarke, A. R., Hooper, M. L., and Berns, A. (1993) Blood 82, 1445–1452
15. Miura, O., Miura, Y., Nakamura, N., Quelle, F. W., Witholthu, B. A., Ible, J. N., and Aoki, N. (1994) Blood 84, 4135–4141
16. Buckley, A. R., Buckley, D. J., Leff, M. A., Hooyer, D. S., and Magnuson, N. S. (1995) Endocrinology 136, 5252–5259
17. Nagata, Y., and Todokoro, K. (1995) FEBS Lett. 377, 497–501