# Tcl1 enhances Akt kinase activity and mediates its nuclear translocation

**Yuri Pekarsky*, Anatoliy Koval*, Cora Hallas*, Roberta Bichi*, Maria Tresini*, Scott Malstrom*, Giandomenico Russo†, Philip Tsichlis*, and Carlo M. Croce*‡**

*Kimmel Cancer Center, Jefferson Medical College, Philadelphia, PA 19107; and †Istituto Dermopatico dell'Immacolata, Laboratory of Vascular Pathology, 00167 Rome, Italy

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**The *TCL1* oncogene at 14q32.1 is involved in the development of human mature T-cell leukemia. The mechanism of action of Tcl1 is unknown. Because the virus containing the *v-akt* oncogene causes T-cell lymphoma in mice and Akt is a key player in transduction of antiapoptotic and proliferative signals in T-cells, we investigated whether Akt and Tcl1 function in the same pathway. Coimmunoprecipitation experiments showed that endogenous Akt1 and Tcl1 physically interact in the T-cell leukemia cell line SupT11; both proteins also interact when cotransfected into 293 cells. Using several *AKT1* constructs in cotransfection experiments, we determined that this interaction occurs through the pleckstrin homology domain of the Akt1 protein. We further demonstrated that, in 293 cells transfected with *TCL1*, the endogenous Akt1 bound to Tcl1 is 5–10 times more active compared with Akt1 not bound to Tcl1. The intracellular localization of Tcl1 and Akt1 in mouse fibroblasts was investigated by immunofluorescence. When transfected alone, Akt1 was found only in cytoplasm whereas Tcl1 was localized in the cytoplasm and in the nucleus. Interestingly, Akt1 was also found in the nucleus when *AKT1* was cotransfected with *TCL1*, suggesting that Tcl1 promotes the transport of Akt1 to the nucleus. These findings were supported by the intracellular localization of Akt1 or Tcl1 when Tcl1 or Akt1, respectively, were confined to the specific cellular compartments. Thus, we demonstrate that Tcl1 is a cofactor of Akt1 that enhances Akt1 kinase activity and promotes its nuclear transport.**

The *TCL1* gene at chromosome 14q32.1 is often activated in human T-cell malignancies by chromosomal inversions and translocations such as inv(14)(q11;q32) and t(14;14)(q11;q32) or t(7;14)(q35;q32) (1). Normally, *TCL1* expression is observed in early T-cell progenitors ($CD4^-$, $CD8^-$, $CD3^-$), in pre B-cells and immature IgM-expressing B-cells (1). Introduction of a *TCL1* transgene in mice under the control of the proximal *lck* promoter resulted in mature T-cell leukemia in mice at the age of 15–20 months (2). The second member of the *TCL1* gene family, *MTCP1*, is located at Xq28 and is activated in rare cases of mature T-cell leukemia showing rearrangements at Xq28 (3). Recently, we identified the third member of this family, *TCL1b,* also located at 14q32.1 and activated by chromosomal rearrangements involving the *TCL1* locus (4). In the mouse, *Tcl1b* is represented by five homologues (5). Although the crystal structure of Tcl1 suggests that it may play a role in the transport of small molecules such as retinoids, nucleotides, and fatty acids (6), the function of the 14-kDa Tcl1 protein is still not known. Cell fractionation experiments in lymphoid cells have shown that Tcl1 is localized in both the nucleus and the cytoplasm (7).

The protein kinase Akt/PKB is the homologue of *v-akt*, isolated from the retrovirus AKT8, which causes T-cell lymphomas in mice (8). The Akt protein contains a pleckstrin homology (PH) domain and kinase domain (9). Activation of Akt by insulin and various growth and survival factors involves a phosphatidylinositol 3-kinase (PI-3K)-dependent membrane translocation step that is attributable to the binding of the PH domain to D3 phosphoinositides and a PDK1-mediated phosphorylation step at Thr308 and Ser473 (reviewed in ref. 9). Treatment with wortmannin, a (PI-3K)-kinase inhibitor, completely inhibits the activation of Akt (reviewed in ref. 9). Recent studies showed that Akt is a key player in the transduction of antiapoptotic and proliferative signals in T-cells (refs. 10 and 11; reviewed in ref. 9). Activated Akt enhances both cell cycle progression and IL2 production through the inhibition by phosphorylation of the proapoptotic factor Bad (11). Introduction of a constitutively activated *AKT1* transgene under the control of the proximal *lck* promoter causes T-cell lymphomas in mice (S.M. and P.T., unpublished data). In cultured cells, Akt1 can be localized in both the nucleus and cytoplasm (12). In addition, it has been claimed that Akt1 translocates to the nucleus in insulin-stimulated 293 cells (13). The mechanism of the nuclear translocation of Akt1 is not known. In this report, we present evidence that Tcl1 and Akt1, the protein products of two oncogenes involved in T-cell leukemogenesis, interact with each other. The interaction mediated by the PH domain of Akt1 enhances the kinase activity and promotes the nuclear translocation of the Akt1 kinase.

## Materials and Methods

**Cells Lines.** 293 and NIH 3T3 cells were purchased from the American Type Culture Collection. MEF cells were obtained from CLONTECH. SupT11 T-cell leukemia cells were described previously (1).

**Constructs and Transfection.** HA-*AKT1*, (Δ11-60)-HA-*AKT1*, (Thr308/Ala, Ser473/Ala)-HA-*AKT1*, (Lys179/Met)-HA-*AKT1*, and HA-*AKT2* constructs were previously described (14). All HA-AKT constructs contain murine *Akt1* or *Akt2* ORF and the HA tag on the N terminus of an encoded protein. The myristoylated Myc-*AKT1* construct and Akt1 PH domain glutathione *S*-transferase (GST) fusion protein were purchased from Upstate Biotechnology (Lake Placid, NY). Full length *TCL1* cDNA was amplified by PCR from SupT11 mRNA and was cloned into pcDNA3, pCMV/myc/nuc vectors (Invitrogen), and into pEGFPN1 and pEGFPC1 vectors (CLONTECH). Transfections were carried out by using Fugene 6 reagent (Roche Molecular Biochemicals) according to the manufacturer's instructions.

**Protein lysates, Immunoprecipitation, and Western Blotting.** Cells were grown in RPMI 1640 or MEM medium with 10% FCS and were lysed by using Nonidet P-40 lysis buffer containing 50 mM

Abbreviations: PH domain, pleckstrin homology domain; GST, glutathione *S*-transferase; PDGF, platelet-derived growth factor; PI-3K, phosphatidylinositol 3-kinase.

‡To whom reprint requests should be addressed at: 233 South 10th Street, Room 1050, Bluemle Life Sciences Building, Philadelphia, PA 19107-5541. E-mail: croce@calvin.jci.tju.edu.

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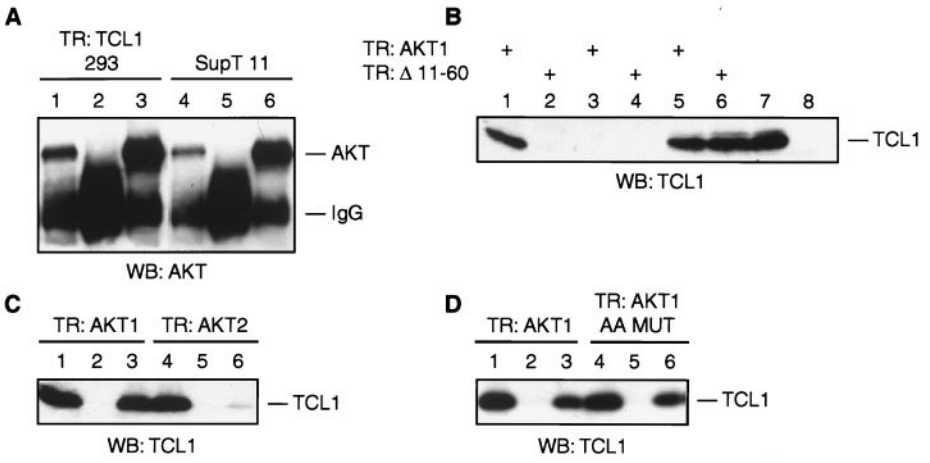


**Fig. 1.** Tcl1 interacts with Akt. (*A*) Immunoprecipitation of Akt1 with anti-Tcl1 antibody. Detection of Akt1 in the immunoprecipitates was carried out by Western blotting using a mouse monoclonal anti-Akt1 antibody. Lysates used were 293 cells transfected with *TCL1* (lanes 1–3) and SupT11 cells (lanes 4–6). Antibodies used for immunoprecipitation were anti-Tcl1 (lanes 1 and 4), mouse IgG (lanes 2 and 5), and mouse monoclonal anti-Akt1 (lanes 3 and 6). (*B*) Akt1 interacts with Tcl1 through PH domain. 293 cells were cotransfected with *TCL1* and HA-*AKT1* or HA-(Δ11-60) *AKT1* mutant as indicated. Immunoprecipitations were carried out with an anti-HA antibody (lanes 1 and 2), mouse IgG (lanes 3 and 4), or anti-Tcl1 antibody (lanes 5 and 6) and were detected by Western blotting with anti-Tcl1 antibody. Lanes 7 and 8: The lysate was coprecipitated with 5 $\mu$g of Akt1 PH domain-GST fusion protein (lane 7) or GST alone (lane 8). (*C*) Akt1 but not Akt2 strongly interacts with Tcl1. 293 cells were cotransfected with *TCL1* and HA-*AKT1* (lanes 1–3) or HA-*AKT2* (lanes 4–6). Immunoprecipitations were carried out with anti-Tcl1 antibody (lanes 1 and 4), mouse IgG (lanes 2 and 5), and anti-HA antibody (lanes 3 and 6) and were detected with anti-Tcl1 antibody. (*D*) Interaction with Tcl1 is independent of Akt1 phosphorylation. 293 cells were cotransfected with *TCL1* and HA-*AKT1* (lanes 1–3) or HA-*AKT1* AA mutant (Thr308/Ala Ser473/Ala). Immunoprecipitations and Western blot detection were performed as in *C*. Expression levels of exogenous and endogenous Tcl1 and Akt were checked in each experiment (where applicable) and were found similar.

Tris (pH 7.5), 150 mM NaCl, 10% glycerol, 0.5% NP40, and protease inhibitors. Immunoprecipitations were carried out overnight in the same buffer using 0.5 mg of protein, 5 $\mu$g of antibody, and 40 $\mu$l of protein A/G PLUS agarose (Santa Cruz Biotechnology) and were washed four times with the same buffer containing 0.1% NP40. Antibodies used were Anti-HA.11 (Babco, Richmond, CA), anti-PKB$\alpha$/Akt clone 7 (Transduction Laboratories, San Diego), or anti-Akt/PKB rabbit polyclonal antibody (New England Biolabs), anti-phospho-Akt(Ser 473) rabbit polyclonal antibody (New England Biolabs), and anti-Tcl1 clone 27D6 mouse monoclonal antibody (G.R., unpublished work). Western blotting was performed under standard conditions (7).

**Kinase Assay.** These experiments were carried out by using the Akt kinase assay kit from New England Biolabs according to the manufacturer's recommendations; in some experiments, anti-Tcl1 or anti-HA antibodies were used for immunoprecipitations.

**Immunofluorescence.** Cells were seeded on fibronectin-covered cell culture slides (Becton Dickinson), fixed for 10 min in 3.7% PBS-buffered formaldehyde and were permeabilized with 0.05% Triton X-100 in PBS for 5 min. Cells were then blocked for 1 hour in 100% goat serum (Sigma) and were incubated with a primary antibody for 1 hour in 10% goat serum in PBS and with a secondary antibody under the same conditions. Antibodies used were anti-Tcl1 clone 27D6 mouse monoclonal antibody, anti-PKB$\alpha$/Akt1 clone 7, rabbit anti-Akt antibody, anti-Myc rabbit polyclonal antibody (Upstate Biotechnology), anti-mouse Texas Red-conjugated antibody (Oncogene Research, Cambridge, MA), and anti-rabbit FITS conjugated antibody (Amersham Pharmacia). Cells were examined by using a confocal microscope (Bio-Rad) under 63× magnification.

## Results

**Tcl1 Interacts with Akt1.** To explore the possibility that Tcl1 and Akt1 function in the same pathway, we first investigated whether Tcl1 and Akt1 physically interact. Immunoprecipitation with anti-Tcl1 antibodies followed by Western blotting with the monoclonal anti-Akt1 antibody revealed that Tcl1 interacts with endogenous Akt1 when transfected into 293 embryonic kidney cells (Fig. 1*A*, lanes 1–3). Endogenous Tcl1 and Akt1 also interact in SupT11 T-cell leukemia cells carrying a t(14;14)(q11;q32.1) translocation (Fig. 1*A*, lanes 4–6). Because the Akt PH domain functions both as a phosphoinositide and as a protein binding module (reviewed in ref. 9), we examined whether Akt1/Tcl1 interaction is mediated by the Akt1 PH domain. For this purpose, we cotransfected 293 cells with a *TCL1* construct and HA-tagged *AKT1* constructs expressing the wild-type Akt1 protein or an Akt1 mutant protein (Akt1 Δ11-60), carrying a 50-amino acid PH domain deletion. Subsequently, we immunoprecipitated Akt1 with the anti-HA antibody. Western blots of the immunoprecipitates were probed with the anti-Tcl1 antibody. Fig. 1*B* shows that Tcl1 interacts with wild-type Akt1, but not with Akt1 (Δ11-60) (lanes 1 and 2). To prove that the PH domain is indeed responsible for this interaction, we used an Akt1 PH domain GST fusion protein in pulldown experiments. Fig. 1*B* (lanes 7 and 8) shows that Tcl1 binds to the PH domain GST fusion protein but not to GST alone. Because the anti-Akt1 antibody used in Fig. 1*A* recognizes both Akt1 and Akt2 (not shown), we proceeded to determine which isoform(s) of Akt actually interacts with Tcl1. 293 cells were transfected with HA-tagged constructs of *AKT1* or *AKT2* in combination with the *TCL1* construct. Lysates of the transfected cells were subjected to immunoprecipitation with an anti-HA antibody. Fig. 1*C* shows that Tcl1 strongly interacts with Akt1. Almost as much Tcl1 was precipitated with the anti-HA antibody as with the anti-Tcl1 antibody. In contrast, only a faint band of Tcl1 was observed in the Akt2 immunoprecipitates, even after prolonged exposures, suggesting that Tcl1 has a much stronger affinity for Akt1 than Akt2. Immunoprecipitation of Tcl1 also led to the coimmunoprecipitation of Akt1 (Fig. 1*A*), but not Akt2 (not shown). Human Tcl1b did not coimmunoprecipitate with Akt1 or Akt2 (not shown).

**Tcl1 Enhances the Akt1 Kinase Activity.** To determine whether Tcl1 affects the kinase activity of Akt1, we transfected 293 cells with a *TCL1* expression construct or vector only. Endogenous Akt1 was immunoprecipitated 48 hours later from lysates of the transfected cells by using anti-Tcl1 or anti-Akt1 antibodies. The kinase activity associated with these immune complexes was measured by using a GST-GSK3-$\beta$ fusion protein as a specific substrate. Fig. 2*A* shows that the specific activity of Tcl1-bound Akt1 immunoprecipitated from *TCL1* transfected cells is 5–10 times higher than the specific activity of Akt1 immunoprecipitated from vector-transfected cells (Fig. 2*A*). The specific ac-

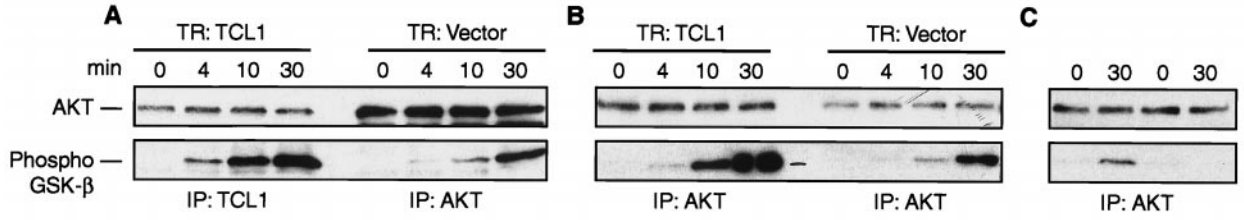


**Fig. 2.** Tcl1 enhances Akt1 kinase activity. Endogenous Akt1 was immunoprecipitated from 293 cells transfected with the indicated constructs. Kinase activity was determined by using GSK3-$\beta$-GST fusion protein as a substrate. Each reaction was terminated after 0, 4, 10, and 30 min. Amounts of Akt (upper panels) and phospho-GSK3-$\beta$ (lower panels) were determined by Western blotting with rabbit anti-Akt antibody and anti-phospho-GSK3-$\beta$ antibody, respectively. (*A*) Akt1 was immunoprecipitated from *TCL1*-transfected cells with a anti-Tcl1 antibody (*Left*) or vector-transfected cells with anti-Akt antibody (*Right*). (*B*) Same lysates as in *A*, but immunoprecipitations were carried out with an anti-Akt antibody only. (*C*) Lysates of thymus from a *TCL1*-transgenic mouse (2) (left two lanes) or a wild-type mouse (right two lanes) were immunoprecipitated with anti-Akt antibody. For immunoprecipitation of Akt, we used anti-PKB$\alpha$/Akt1 clone 7 antibody, anti-Akt/PKB rabbit polyclonal antibody, or anti-Akt antibody included with Akt kinase assay kit, with consistent results.

tivity of Akt1 immunoprecipitated with the anti-Akt1 antibody is also higher in Tcl1 transfected cells versus vector transfected cells (Fig. 2*B*). The more moderate increase of the kinase activity of Akt1 immunoprecipitated from Tcl1 transfected cells versus vector transfected cells in Fig. 2*B* versus Fig. 2*A* may be attributable to the fact that only a fraction of Akt1 immunoprecipitated with the anti-Akt1 antibody is bound to Tcl1. Nevertheless, in both panels, the activity of Akt1 is higher in Tcl1 transfected cells at 10 min of incubation (Fig. 2 *A* and *B*). To verify that the kinase activity in the Akt1 immunoprecipitates is attributable to Akt1 and not another associated kinase, we checked the activity of a kinase-dead mutant of Akt1 (Lys179/Met) expressed under similar conditions. As expected, immunoprecipitates of this mutant did not show any kinase activity (not shown). Our findings were further confirmed by experiments showing that Akt1 is constitutively active in the thymus of transgenic mice expressing Tcl1 under the control of the proximal *lck* promoter (2) but not in the thymus of wild-type mice (Fig. 2*C*).

The increased activity of Akt1 bound to Tcl1 may be attributable to the fact that Tcl1 binds only to active (phosphorylated at Thr308 and Ser473) Akt1. Alternatively, Tcl1 may be a cofactor that facilitates the activation of Akt1. To address this question, we examined the binding of Tcl1 to kinase-inactive Akt1 mutants. Fig. 1*D* shows that Tcl1 interacts equally well with wild-type Akt1 and the Akt1 Thr308/Ala;Ser473/Ala mutant (AA mutant), a mutant that cannot be activated by phosphorylation. In addition, Tcl1 immunoprecipitates equally well with wild-type and the kinase-dead Akt1 mutant Lys179/Met (not shown). This indicates that binding of Tcl1 to Akt1 is independent of Akt1 phosphorylation or activation status. Activation of Akt1 by platelet-derived growth factor (PDGF) is caused by D3 phosphoinositides-dependent phosphorylation by PDK1 (reviewed in ref. 9). Treatment of PDGF-stimulated NIH 3T3 cells with wortmannin, a PI-3K inhibitor, prevents Akt1 phosphorylation and activation (14, 15). Fig. 3*B* shows that wortmannin inhibits the phosphorylation of Akt1 in both untransfected and Tcl1 transfected NIH 3T3 cells. This suggests that the stimulatory effect of Tcl1 on the activity of Akt1 is PI-3K-dependent and that the binding of Tcl1 to the Akt1 PH domain cannot substitute phosphoinositide binding. Because the functional outcome of Akt1 phosphorylation is the activation of the Akt1 kinase, we examined whether overexpression of Tcl1 enhances the phosphorylation of Akt1 at Ser473 by PDGF stimulation. To our surprise, we observed that this is not the case (Fig. 3*A*). We conclude, therefore, that the effect of Tcl1 on Akt1 activation is PI-3 K-dependent but independent of phosphorylation at Ser473. This suggests that phosphorylation by PDK1 and binding to Tcl1 may synergize for Akt1 activation.

**Tcl1 Promotes Akt1 Nuclear Translocation.** Akt1 is primarily localized in the cytoplasm (12), although in some cells Akt is localized in the nucleus (12), and it was reported that, in insulin-stimulated 293 cells, activated Akt1 translocates into the nucleus (13). Tcl1, on the other hand, is localized in both the cytoplasm and in the nucleus (7). Thus, we examined whether coexpression of Tcl1 and Akt1 affects the subcellular localization of both proteins. The results of these experiments are shown in Fig. 4. MEF cells were transiently transfected with *TCL1* and/or *AKT1*, and the intracellular localization of both proteins was determined by immunofluorescence. Under normal growth conditions (10% serum), Akt1 was localized in the cytoplasm in >90% of cells transfected with *AKT1* alone (Fig. 4*A Left*). Under the same growth conditions, Tcl1 was localized in both the cytoplasm and the nucleus in >90% of cells transfected with *TCL1* alone or *TCL1*-GFP (Fig. 4*A Center* and *Right*). However, when Tcl1 or a GFP-Tcl1 fusion protein (with GFP attached to the N terminus of Tcl1) were coexpressed with Akt1 in the same cells, both proteins were

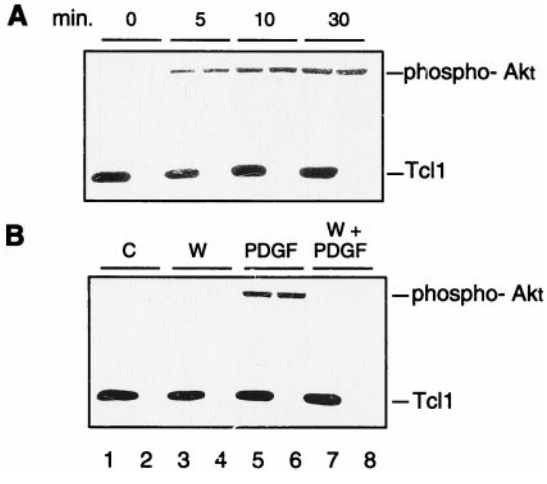


**Fig. 3.** The expression of Tcl1 does not increase Akt1 phosphorylation or interfere with effect of wortmannin. NIH 3T3 cells were transfected with *TCL1* (lanes 1, 3, 5, and 7) or vector (lanes 2, 4, 6, and 8) and were starved with media without FCS overnight. (*A*) Cells were treated with 100 ng/ml PDGF for the indicated period of time and were lysed. Western blotting was performed by using anti-phospho-Akt and anti-Tcl1 antibody. Each lane contains the same amount of protein (not shown). (*B*) NIH 3T3 were transfected and starved as in *A*. Cells were not treated (lanes 1 and 2); were treated with 200 nM wortmannin for 1.5 hours (lanes 3 and 4); were treated with 100 ng/ml PDGF for 30 min (lanes 5 and 6); and were treated with 200 nM wortmannin for 1 hour followed by PDGF for 30 min (lanes 7 and 8). Western blotting was performed as in *A*.

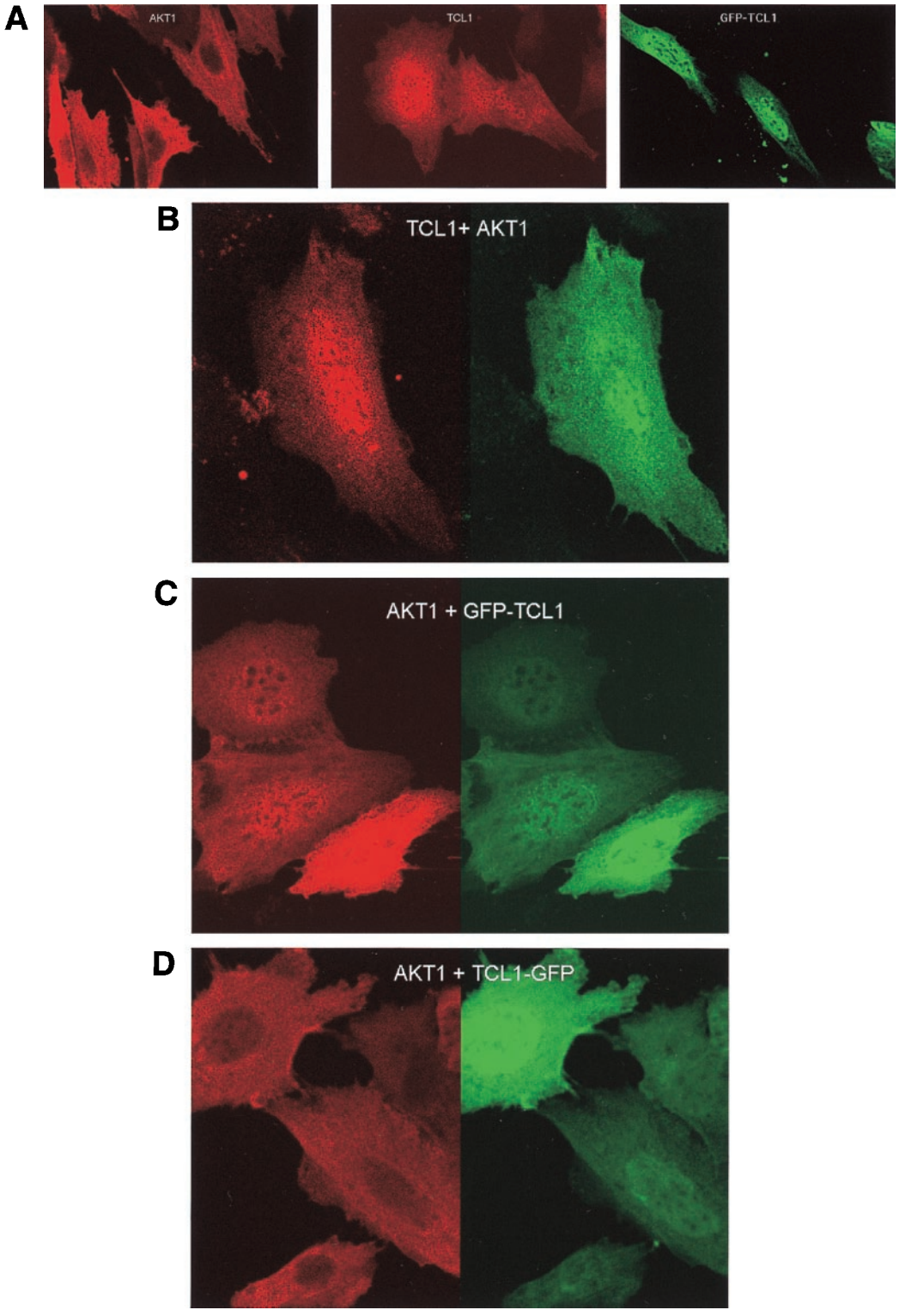


**Fig. 4.** Tcl1 promotes nuclear translocation of Akt1. MEF cells were transfected or cotransfected with indicated constructs. (*A*) Intracellular localization of Akt1 (*Left*), Tcl1 (*Center*), and GFP-Tcl1 (*Right*). (*B*) Colocalization of Akt1 (green) and Tcl1 (red). (*C*) Colocalization of Akt1 (red) and GFP-Tcl1 (green). (*D*) Intracellular localization of Akt1 (red) and Tcl1-GFP (green).

colocalized in the cytoplasm as well as in the nucleus in >90% of the cells (Fig. *B* and *C*). Thus, Tcl1 promotes the nuclear translocation of Akt1.

In contrast, coexpression of Tcl1-GFP (with GFP attached to the C terminus of Tcl1) and Akt1 resulted in localization of Akt1 in the nucleus in ≈30% of the cells (not shown) whereas in ≈60% of the cells Akt1 was detected mostly in the cytoplasm; Tcl1-GFP remained in its location in the nucleus and in the cytoplasm (Fig. 4*D*). This suggests that the addition of GFP at the C terminus of Tcl1 to a certain extent inhibits the transport of the Tcl1-Akt1

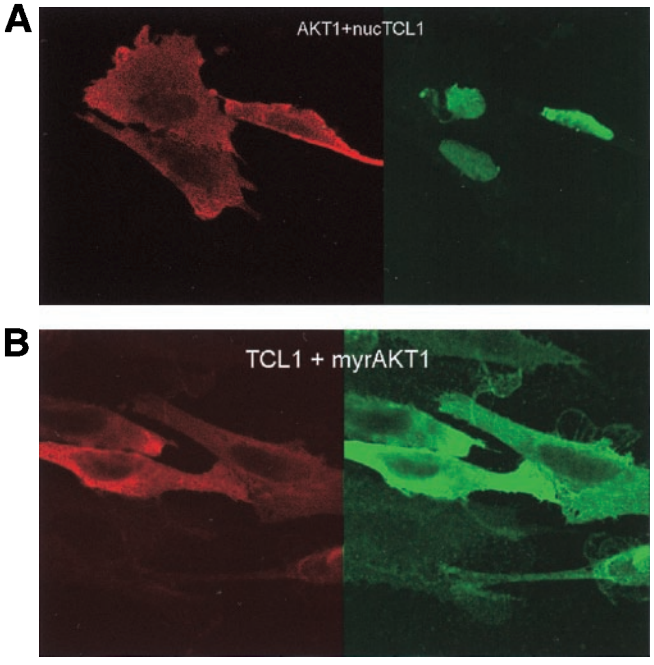


**Fig. 5.** Nuclear translocation of Akt1 by Tcl1 require their interaction in the cytoplasm. MEF cells were transfected or cotransfected with indicated constructs. (*A*) Intracellular localization of Akt1 (red) and nuc-Tcl1 (green) in the same cells. (*B*) Intracellular localization of myristoylated Akt1 (green) and Tcl1 (red) in the same cells.

complexes to the nucleus, possibly because of the partial interference with the interaction of Akt1 and Tcl1.

Because both Tcl1 and Akt1 are also localized in the cytoplasm, we speculate that the interaction of Tcl1 and Akt1 takes place in the cytoplasm followed by the translocation of this complex into the nucleus. To test this hypothesis, we used a *TCL1* construct containing a nuclear localization signal leading to the expression of Tcl1 only in the nucleus (nucTcl1). Fig. 5*A* shows that, in cells expressing nuclear Tcl1, Akt1 was located exclusively in the cytoplasm. This may suggest that Akt1 needs to interact with Tcl1 in the cytoplasm to be transported to the nucleus. Although interaction of Tcl1 with wild-type Akt1 led to the nuclear translocation of Akt1, interaction of Tcl1 with membrane-associated myrAkt1 led to the cytoplasmic localization of Tcl1 (Fig. 5*B*). These results indicate that, indeed, the binding between the two proteins affects the subcellular localization of both. Biologically relevant appears to be the nuclear translocation of wild-type Akt1 in cells coexpressing both proteins.

The biological consequences of the enhancement of the Akt1 activity have not been determined to date. However, our data indicate that expression of Tcl1 does not increase the Akt1-mediated phosphorylation of Bad, p70 S6 kinase, or IκB (11, 16, 17) (not shown).

## Discussion

In this report, we showed that Akt1 and Tcl1 physically interact and that this interaction results in the enhancement of the Akt1 kinase activity and in the translocation of the Akt1 kinase into the nucleus. Although Akt1 and Akt2 are closely related proteins, our data indicate that Tcl1 interacts specifically with Akt1. Furthermore, both Akt1 and Akt2 did not interact with the Tcl1-related protein, Tcl1b.

The process of Akt activation consists of three distinct steps: a PH domain-dependent, growth factor-independent step, marked by constitutive phosphorylation of Thr450; a growth factor-induced PI-3K-dependent membrane translocation step; and a PI-3K-dependent step characterized by phosphorylation at Thr308 and Ser473 (14). Both PI-3K-dependent steps are inhibited by wortmannin, a PI-3K inhibitor (15). Our data demonstrate that Tcl1 does not activate Akt1 in wortmannin-treated cells, and, therefore, binding of Tcl1 to the Akt1 PH domain cannot substitute for D3-phosphoinositide binding. Moreover, Tcl1 does not enhance Akt1 phosphorylation, suggesting that binding to Tcl1 may synergize with phosphorylation to induce activation of Akt1. Alternatively, the Tcl1-Akt1 complex may recruit additional proteins, which enhance the activity of Akt1.

Recent studies showed that Akt1 can be found in the nucleus (12), and, in insulin-stimulated 293 cells, its nuclear translocation may take place after its membrane translocation and activation (13). Our data provide clues regarding the mechanism of the nuclear translocation of Akt1, specifically in MEF cells grown under normal conditions. In cells coexpressing Akt1 and Tcl1, Akt1 was constitutively localized in the nucleus. The change in the subcellular localization of Akt1 appears to depend on the interaction between the two proteins. This is suggested by findings showing that membrane-associated myrAkt1 forces Tcl1 into the cytoplasm. The interaction between Akt1 and Tcl1 responsible for the nuclear translocation of Akt1 appears to occur in the cytoplasm. These data suggest that Tcl1 does not only facilitate the activation of Akt1 but also promotes its nuclear translocation. The latter may be attributable to the fact that Tcl1 functions as a direct transporter of Akt1 or may contribute to the assembly of a complex that promotes the nuclear transport of Akt1. Because Tcl1 is expressed only in certain lymphoid cells (1), and the nuclear translocation of Akt1 was reported in cells not expressing Tcl1 (13), additional molecules, perhaps related to Tcl1, responsible for Akt1 nuclear translocation may exist.

The biological outcome of the Tcl1-induced enhancement of Akt1 activity is expected to occur through the phosphorylation of Akt1 specific targets. Because the Tcl1-activated Akt1 translocates into the nucleus, the most likely targets of the Tcl1-Akt1 complex are nuclear. To address these questions, we examined the phosphorylation of previously reported cytoplasmic proteins directly or indirectly phosphorylated by Akt1. The results to date suggest that Tcl1 does not enhance the Akt1-mediated phosphorylation of p70 S6 kinase, Bad, and IκB. Future studies will investigate the phosphorylation of nuclear targets.

Because both Tcl1 and Akt1 cause T-cell malignancies in transgenic mice, it will be of considerable interest to determine whether *TCL1* and *AKT1* double transgenic mice develop leukemia faster or show more severe phenotype. Thus, it seems likely that Tcl1 participates in PI-3 K-dependent Akt1 signaling pathway by enhancing Akt1 kinase activity and mediating Akt1 nuclear translocation.

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