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A Proline-Rich Motif in the C Terminus of Akt Contributes to Its Localization in the Immunological Synapse

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The serine/threonine kinases of the Akt/protein kinase B family are regulated in part by recruitment to the plasma membrane, which is accomplished by the binding of an N-terminal PH domain to the phosphatidylinositol 3-kinase products phosphatidinositol 3,4,5-trisphosphate and phosphatidinositol 3,4-bisphosphate. We have examined Akt localization in a murine T cell clone (D10) before and after stimulation by APC/Ag, and we found that whereas the pleckstrin homology domain is required for plasma membrane recruitment of Akt upon T cell activation, the C terminus of the kinase restricts its cellular localization to the immunologic synapse formed at the site of T cell/APC contact. A recently described proline-rich motif in this region appears to be important for proper localization of full-length Akt. Moreover, a form of Akt in which this motif was mutated acts as a potent dominant negative construct to block T cell activation. Therefore, multiple mechanisms are involved in the proper targeting of Akt during the early events of T cell activation. The Journal of Immunology, 2004, 172: 5441–5449.

The Akt (or protein kinase B) kinase is a member of the AGC family of serine/threonine kinases, a family that also includes the ribosomal S6 kinases and protein kinase C (PKC)4-related kinases, among others (1, 2). These kinases are characterized in part by a regulatory serine or threonine in the activation loop of the kinase domain, phosphorylation of which is required to achieve maximal kinase activity. This phosphorylation is conducted by 3-phosphoinositide-dependent kinase-1 (PDK-1) in the case of Akt and several other AGC kinases (2). The AGC kinases are also positively regulated by serine or threonine phosphorylation at a C-terminal hydrophobic motif. However, the identity of the kinase(s) responsible for this phosphorylation is still controversial. Recent structural studies have elegantly demonstrated that this C-terminal motif positively regulates kinase activity by interacting with and causing an allosteric change in the kinase domain (3, 4).

All three mammalian Akt isoforms (as well as homologues in lower species) contain an N-terminal pleckstrin homology (PH) domain. This modular domain in Akt can independently bind to the 3'-phosphorylated inositol phospholipids phosphatidinositol 3,4,5-trisphosphate (PI(3,4,5)P3) and phosphatidinositol 3,4-bisphosphate (PI(3,4)P2) that are generated by the activation of phosphatidylinositol 3-kinase (PI-3-kinase). As these lipids are generated at the plasma membrane by PI-3-kinase, increases in their concentration contribute to the recruitment of PH domain-containing proteins to the plasma membrane. Indeed, previous studies have shown that the isolated PH domain of Akt (5–9) is sufficient to mediate plasma membrane recruitment of green fluorescent protein (GFP) in T cells and other cell types. Likewise, membrane recruitment of full-length Akt after growth factor or chemotactic stimulation is dependent upon an intact PH domain (1, 10, 11). Other proteins containing PH domains, such as the tyrosine kinases Tec and Itk, are also recruited to the plasma membrane after T cell activation (12, 13).

Enzymatic activation of Akt occurs in a stepwise fashion (14), beginning with the activation of PI-3-kinase, whose phosphorylation products PI(3,4,5)P3 and PI(3,4)P2 recruit Akt to the plasma membrane. It is here that Akt can be phosphorylated by the kinase PDK-1 at threonine 308 (in the case of Akt1) and by an activity termed PDK-2 at serine 473, resulting in the fully active form of Akt. Once activated, Akt promotes various physiological outcomes, including cell survival, growth, and glucose metabolism. It does so through effects on both transcription and translation, as well as by post-translational modification of specific substrates (1, 15–17). We previously reported that Akt can replace the T cell costimulatory function that is normally provided by CD28 for the production of IL-2 and IFN-γ (18). This is most likely due to the ability of Akt to target the NF-κB transcription factor pathway in T cells (19).

Efficient T cell activation is thought to depend upon the formation of a complex macromolecular structure at the interface between the T cell and an APC known variously as the supramolecular activation cluster (SMAC) or immunological synapse (20, 21). However, the precise role of this structure in T cell activation is still controversial (21–23). The hallmarks of SMAC formation include concentration of the TCR, the costimulatory molecule CD28, and the serine/threonine kinase PKCθ in a tight cluster known as the central SMAC (c-SMAC) (20). In fact, the importance of PKCθ for T cell activation was first suggested by its selective localization, among all PKC isoforms, to the c-SMAC (24). A central role for PKCθ in T cell activation was later confirmed by the analysis of mice lacking its expression (25, 26). We and others (18, 27) previously showed that PKCθ can functionally

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cooperate and physically associate with Akt for the activation of NF-κB in T cells.

Our observations concerning the function of Akt in T cells (discussed above) raised a paradox, i.e., they did not explain why, in the absence of CD28, the well-established ability of the TCR to activate PI-3-kinase and Akt (28) was not sufficient for complete T cell activation. Another intriguing aspect of Akt function is its functional and physical connection to PKCθ (18, 27), the localization of the latter having been instructive in uncovering its function. For these reasons, we sought to better understand the requirements for localization of Akt during the early stages of T cell activation. In this study we report that the localization of Akt in T cell/APC conjugates is influenced not only by the PH domain, but also by the C terminus of the protein. A proline-rich motif in this region appears to contribute to this effect, and a form of Akt in which the prolines are mutated acts to block T cell activation. Thus, the hydrophobic motif as well as the proline-rich motif in the C terminus of Akt are both important regulatory sites in that region of the kinase.

Materials and Methods

DNA constructs/abs

Murine Akt, either full-length or the indicated truncations, was amplified by PCR from cDNA with high fidelity PWO polymerase and cloned into the pN1- or pCl1-enhanced GFP vector (BD Clontech Laboratories, Palo Alto, CA). Point mutants of Akt-GFP were generated with the QuickChange system (Stratagene, San Diego, CA). The fidelity of all constructs was verified by automated sequencing. A vector encoding PKCθ-GFP was obtained from BD Clontech Laboratories.

Rabbit anti-PKCθ was obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and used at a dilution of 1/50. Anti-hermaggutinin (anti-HA) Ab was obtained from Covance (Berkeley, CA) and revealed by secondary staining with anti-mouse Ab conjugated to Alexa-555 (Molecular Probes, Eugene, OR). Phosphospecific rabbit Abs to Akt were obtained from Cell Signaling (Beverly, MA) and used at a dilution of 1/50. Rabbit Ab to GFP was obtained from BD Clontech Laboratories and used for Western blotting, which were performed as previously described (18).

Cell lines/peptides

A rapidly dividing variant of the D10 T cell clone was obtained from M. Krummel (University of California, San Francisco, CA). Cells were re-stimulated every 3–4 wk with chicken conalbumin (Sigma-Aldrich, St. Louis, MO) and irradiated or mitomycin C-treated APCs (RBC-depleted splenocytes from B10.BR mice). During the intervening periods, cells were maintained in complete D10 medium (RPMI 1640 supplemented with 10% heat-inactivated FCS, 50 μg/ml 1-glutamine, 0.1 mM nonessential amino acids, 50 μM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM/ml HLF-2 (Roche, Indianapolis, IN)). CH27 murine B cells were maintained in RPMI 1640 supplemented with 5% FCS, 1-glutamine, and antibiotics. Before conjugate formation, CH27 cells were loaded overnight with whole chicken conalbumin (100 μg/ml) or 3–4 h with cognate peptide, or the E8A variant thereof (29), at 8 μM. HPLC-purified conalbumin peptides were obtained from the Bionucler Research Center at University of California, San Francisco.

Transfections

D10 cells at least 1 wk poststimulation were resuspended at 35 × 10⁶ cells/ml in RPMI 1640 without supplements; 0.4 ml of cells in a 0.4-cm cuvette were electroporated in a Gene-Pulser (Bio-Rad, Hercules, CA) at 250 V at 960 μF, then cultured overnight in 10 ml of complete D10 medium, including IL-2. The next day, live cells were isolated on Lymphoprep (Cedarslane Laboratories, Hornby, Canada) and recultured for 3–4 h in complete D10 medium, excluding IL-2.

Conjugate formation/staining

D10 cells were mixed with equal numbers of Ag-loaded CH27 B cells (in both RPMI 1640 and 5% FCS) and spun at 4000 rpm for 1 min in a microcentrifuge. Conjugates were allowed to form for 20 min at 37°C. After aspiration of medium, cell pellets were gently resuspended in PBS and 1% BSA by pipetting up and down three times with a 1-ml micropipette. Approximately 100,000 cells were then applied to a poly-L-lysine-coated slide and allowed to settle for 10 min. Paraformaldehyde was added to a final concentration of 4%, and fixation was allowed to proceed for 20 min, after which cells were washed once in PBS. Cells were permeabilized with 0.2% Triton X-100 for 4 min and blocked for at least 10 min with 1% BSA/4% normal goat serum. Staining was performed for 30 min at room temperature, with Ab diluted in blocking buffer. After each staining step, cells were washed three times for 5 min each time with PBS and 0.2% BSA. After the last washing step, coverslips were mounted with polyvinyl alcohol mounting medium (Sigma-Aldrich).

Wide-field fluorescence microscopy/data analysis

Images were collected on a Marnian system (III; Intelligent Imaging Innovations, Denver, CO), consisting of an Axiovert microscope (Zeiss, Deerfield, IL) fitted with a Zeiss Plan-Apochromat ×63/1.4 NA oil objective, and a Sensicam cooled CCD camera ( Cooke, Auburn Hills, MI). Collection times were calibrated to fall within the linear range of the camera. For any given construct, cells expressing very dim or bright levels were ignored; in general, average fluorescence intensity fell between 200 and 400 arbitrary fluorescence units. Fifteen to 20 fluorescence and differential interference contrast (DIC) images were collected per stack.

Data stacks (z-axis) of fluorescence images were subjected to constrained iterative deconvolution with Slidebook software (Intelligent Imaging Innovations, Denver, CO). Unless otherwise noted, figures contain mid-plane images from deconvolved stacks. Quantitation was performed on mid-plane images by drawing masks across the interface between the T cell and the APC or around the remainder of the T cell plasma membrane and obtaining the mean fluorescence intensity for the highlighted area. Quantitative data were obtained from multiple experiments.

SDS-PAGE/Western blotting

Appropriate expression of GFP constructs was confirmed by SDS-PAGE and Western blotting, which were performed as previously described (18).

Luciferase assays

Jurkat T cells were transfected using the same conditions as those described above for D10 cells. Luciferase activity was determined as described previously (19).

Results

System used to study Akt localization during T cell activation

The localization of Akt during T cell activation was investigated with the well-characterized D10 T cell clone, which recognizes a peptide fragment of chicken conalbumin presented by I-A^d (30). We formed conjugates of D10 cells with CH27 B cells that had been loaded with the cognate Ag. Images were collected using wide-field immunofluorescence microscopy and were processed by deconvolution (20). We took advantage of the fact that a particular subline of D10 was observed to grow for several weeks in culture without Ag stimulation, although IL-2 was still required for proliferation (L. P. Kane, unpublished observations). This allowed us to easily introduce various constructs to the D10 cells by transient transfection via electroporation. The efficiency of transfection (30–40%; data not shown) was sufficiently high for the microscopy studies described below. This approach also eliminated possible confounding effects of selecting stable clones that may not be representative of the original starting population of cells. Finally, we were interested in the disposition of Akt in stable, mature conjugates, so we tracked the formation of such conjugates by staining cells with an Ab to the immunological synapse marker PKCθ. Conjugates displaying distinct, tight PKCθ clustering at the contact site (i.e., the c-SMAC) were studied further for Akt localization.

In most experiments the localization of Akt was followed by a GFP fusion protein containing enhanced GFP at the C terminus of full-length murine Akt1, separated by a linker of ~20 aa. The full-length Akt-GFP fusion was expressed at the predicted size and still possessed biological activity, as measured in an NF-κB reporter assay (data not shown), consistent with our earlier studies that showed synergistic activation of NF-κB by PMA and overexpressed wild-type Akt (19). D10 T cells transiently expressing...
Akt-GFP were left unstimulated or were mixed with Ag-loaded CH27 B cells to form conjugates. As shown in Fig. 1A, Akt-GFP in unconjugated D10 cells displayed a diffuse cytoplasmic localization. However, in D10 cells that were found in mature conjugates with APCs (i.e., those displaying tight contact site PKCθ staining), there was an efficient recruitment of Akt-GFP to the plasma membrane, especially at the contact site (Fig. 1B, left panel). The distribution of Akt-GFP was almost always broader than that of PKCθ in the conjugates with mature synapses (Fig. 1 and data not shown).

Many previous studies have demonstrated that D3 phosphoinositide generation is required for membrane recruitment and activation of Akt (11). We confirmed this with Akt-GFP by pretreating D10 cells with LY294002, a specific inhibitor of PI-3-kinase (31). As shown in Fig. 1C, LY294002 severely impacts the ability of Akt-GFP to be recruited to the plasma membrane of D10 cells in conjugates. However, the c-SMAC/immunologic synapse marker PKCθ is recruited to the T cell/APC contact site, so immunologic synapse formation still occurs in the presence of a potent PI-3-kinase inhibitor. Similar results are seen when a novel PI analog inhibitor of Akt is used (Fig. 1D). Like LY294002, this compound (SH-6) inhibits Akt plasma membrane recruitment and activation (32–34) (data not shown). Thus, although plasma membrane localization of Akt-GFP was observed in some conjugates, it did not result in tight contact site concentration of the molecule. As seen with LY294002 (Fig. 1C), localization of PKCθ appeared to be unaffected by SH-6, despite its effects on Akt. Neither LY294002 nor SH-6 had any detectable impact on conjugate formation between D10 and CH27 cells (data not shown). The results obtained with these inhibitors suggest that proper localization of at least the majority of Akt is not required for c-SMAC localization of PKCθ despite the fact that these two kinases can functionally and physically interact (18, 27, 35). Also, we noted that Akt-GFP adopted relatively restricted localization to the site of T cell/APC contact in stable conjugates. We next sought to determine which intrinsic elements of Akt are responsible for this pattern of localization.

**FIGURE 1.** Effects of PI-3-kinase and PI analog inhibitors on Akt and PKCθ localization. A, Localization of Akt-GFP in an unconjugated D10 cell. B, Representative control conjugate, showing GFP localization (left panel), PKCθ (middle panel), and DIC (right panel). C, Representative conjugate using D10 cells pretreated with the PI-3-kinase inhibitor, LY294002. D, Representative images of conjugates containing D10 cells pretreated with the PI analog, Akt inhibitor, SH-6. Two representative images are shown in this study to illustrate the fact that treatment with this drug led to two distinct patterns of Akt-GFP localization that were observed in approximately equal proportions.
Localization of full-length Akt vs its isolated PH domain

Previous studies have analyzed the localization of the PH domain of human Akt, fused to the enhanced GFP, as a marker for the generation of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) in T cells (8, 9, 36). These studies found that the Akt PH domain can be rapidly recruited from the cytoplasm to the plasma membrane of activated T cells. We investigated the recruitment of this construct and compared it with the activity of full-length Akt fused to GFP. D10 cells not found in conjugates generally displayed diffuse, mainly cytoplasmic, localization of both Akt-GFP and PH-GFP (Fig. 2, left-most panels). We observed that full-length Akt, as before, was recruited to the plasma membrane of T cells in conjugates with APC and adopted a relatively restricted distribution at the contact site compared with the rest of the membrane (Fig. 2A). In agreement with previous reports (8, 9, 36), we saw very efficient membrane recruitment of the Akt PH domain, which was somewhat more concentrated at the site of T cell/APC contact (Fig. 2B).

In general, the isolated PH domain was recruited to the membrane more efficiently than the full-length molecule (Fig. 2 and data not shown). However, there was also a significant difference in the scope of plasma membrane recruitment between the two constructs. Thus, full-length Akt in conjugated T cells was more restricted to the part of the plasma membrane in closest contact with the APC; in contrast, the PH-GFP construct was more diffusely located around the entire plasma membrane. The differences in plasma membrane recruitment are quantified in Fig. 2C. Thus, using mid-plane images, we compared the intensity of GFP fluorescence at the T cell/APC contact site to that of the remainder of the plasma membrane to obtain a ratio. This ratio was ~3-fold higher for full-length Akt-GFP compared with the PH-GFP construct. These results suggest that full-length Akt is recruited more efficiently to the T cell/APC contact site than the PH domain alone.

**FIGURE 2.** Comparison of localization of Akt PH-GFP and full-length Akt-GFP. Conjugates of D10 T cells and CH27 B cells were formed, fixed, and analyzed as described in Materials and Methods. The day before conjugate formation, D10 cells were electroporated with plasmids encoding either full-length Akt-GFP (A) or just the PH domain fused to GFP (B). These constructs are represented schematically above their respective fluorescence images. Several representative mid-plane fluorescence (top row of each set) and DIC (bottom row of each set) images are shown for each construct, in addition to one non-conjugated T cell example (left-most images). The graph in C represents the average ratio (±SD) of contact site fluorescence to that of the rest of the membrane, as determined from 15–20 mid-plane images of conjugates with D10 expressing each construct.
We also saw a similar concentration of Akt-GFP at the contact site when fixed cell images of Akt were displayed in false color mode, either as two-dimensional projection images (Fig. 3A), or mid-plane fluorescence images superimposed on gray-scale DIC images (Fig. 3B). The middle panel of Fig. 3B confirms that an intact PH domain is required for proper recruitment of Akt-GFP, as this construct is lacking critical residues for PH domain function (37). Finally, a similar enrichment of Akt-GFP at the T cell/APC contact site was observed when live cells were imaged (Fig. 3C), demonstrating that it is not only a feature of fixed cell conjugates.

This pattern of localization of full-length Akt could also be seen when a HA-tagged form of the molecule was imaged, as shown in Fig. 4A. Furthermore, although it was difficult to image endogenous Akt with several commercially available Abs (data not shown), the activated form of the endogenous kinase could be tracked with phosphospecific Abs directed against either the C-terminal tail (Fig. 4B, left two panels) or the kinase domain itself (right two panels). The phosphorylation of both of these residues correlates well with catalytic activity (38). Both these Abs produced significant staining above background compared with a control stain (bottom panels). Fluorescence images for the secondary Ab alone control were collected with the same exposure time as the above images.

Role of the C terminus and kinase activity in Akt localization
As our results demonstrated that the PH domain of Akt does not fully account for its localization, we investigated what other regions of the protein might be involved. We began by constructing C-terminal truncated forms of Akt, removing either the last 16 or 56 amino acids, fused to GFP. Full-length Akt-GFP and PH-GFP are shown again for comparison (Fig. 5A). Removing aa 421–476, i.e., most of the protein after the kinase domain, has a significant impact on the efficiency of contact-site localization of Akt (Fig. 5B), compared with the full-length form. This construct nearly recapitulates the distribution of the Akt PH domain-GFP, as quantified in Fig. 5D. We confirmed by anti-GFP western blotting that this construct is expressed at the appropriate size (data not shown).
As shown in Fig. 5C-D, by contrast, removal of only aa 461–476 has little if any impact on Akt localization. These results suggest that the C terminus of Akt is important for the correct localization of the protein during Ag recognition by T cells.

The above results prompted us to determine whether the C terminus of Akt by itself might mediate specific subcellular localization of a heterologous protein, namely GFP. We therefore fused the last 76 aa of Akt to the C terminus of GFP (Fig. 6B). Full-length Akt is shown for comparison (Fig. 6A). As shown in Fig. 6B, the distribution of C-terminal Akt-GFP does not appear to change significantly upon contact with an Ag-loaded APC (lower four panels) compared with cells not in conjugates (Fig. 6B, upper panels). This is also apparent when GFP fluorescence is displayed in false color mode, overlaid onto DIC images (Fig. 6C). Thus, the C terminus of Akt apparently plays a role in limiting the localization of Akt after recruitment to the membrane via its PH domain.

Recent studies have demonstrated a functional role for a putative Src homology 3-binding, proline-rich motif in the C-terminal portion of Akt (39). This motif is conserved among Akt isoforms and across species. As the motif lies within the part of Akt that appeared to have an effect on localization, as shown above in Fig. 5, we mutated the two prolines in the full-length Akt-GFP construct and assessed its localization. As shown in Fig. 7, mutation of...
prolines 421 and 424 to alanine resulted in a pattern of localization reminiscent of that seen with the PH-only construct. Thus, although this construct was still apparently recruited to the plasma membrane, it did not concentrate as tightly as the wild-type construct at the T cell/APC contact site.

In an effort to begin to understand the relevance of this proline-rich motif for Akt function in T cells, we tested the activity of the GFP construct containing it in an NF-κB reporter assay, because we had shown previously that Akt could contribute to NF-κB activation in T cells (19). As shown in Fig. 8A, the proline mutant of Akt-GFP inhibited CD28-mediated induction of an NF-κB luciferase reporter in a dose-dependent manner. This effect was not attributable to a general effect on cell viability, because the same construct did not affect expression of a cotransfected reporter construct driven by a constitutive promoter (Fig. 8B). Thus, an allele of Akt lacking the conserved proline-rich sequence downstream of its kinase domain acts as a potent dominant negative to block T cell activation pathways.

Discussion

In this study we have presented evidence that localization of the Akt kinase during T cell activation is more complex than previously appreciated. Specifically, we show that the full-length kinase adopts a more restricted localization to the immunologic synapse than does the isolated PH domain. This pattern of Akt localization has been demonstrated in three ways: first, with a GFP fusion to full-length Akt; second, with an epitope-tagged form of Akt; and third, with phosphospecific Abs that detect activated Akt. Consistent with previous results, inhibition of PI-3-kinase prevents efficient plasma membrane recruitment of Akt, as does a novel PI analog that appears to be a more specific inhibitor of Akt, i.e., further downstream of PI-3-kinase. We also show with Akt-GFP that removal of the C-terminal region, after the kinase domain, nearly recapitulates the localization of the isolated PH domain. Despite an apparent role for the C terminus, however, this region of Akt is insufficient by itself to mediate plasma membrane or contact site localization of a heterologous protein. Finally, the restriction of Akt localization by its C terminus requires an intact proline-rich motif, mutation of which creates a molecule with a potent ability to inhibit T cell activation.

Differences between full-length Akt and PH-only GFP fusions were also noted in a B cell line by Cantrell and colleagues (5), who found that upon ligation of surface Ig, the PH-only construct was recruited more efficiently to the membrane than the full-length Akt-GFP. Furthermore, they found that a detectable fraction of full-length Akt translocated to the nucleus after activation, consistent with findings made in other cell types (40, 41). We also observe less efficient total recruitment of full-length Akt compared with the PH domain alone (Figs. 2 and 3), although significant nuclear localization has not been obvious in our experiments (L. P. Kane and A. Weiss, unpublished observations). Detection of a nuclear pool of Akt in T cells may require biochemical, rather than microscopic, analysis. Taken together, our findings and those of Cantrell suggest that the contribution of the C-terminal portion of Akt to its localization is not a T cell-specific phenomenon.
There are now several examples of protein-protein interactions mediated by the C terminus of Akt. The hydrophobic motif within the C terminus folds back to interact with the kinase domain of Akt itself, greatly increasing its kinase activity (42). A novel negative regulator of Akt, termed C-terminal modulatory protein, also appears to bind in the vicinity of the hydrophobic motif (43). Finally, a recent report has demonstrated that the proline-rich motif studied in this report can mediate an interaction with the Src tyrosine kinase through its Src homology 3 domain (39). Given this finding, we have investigated whether Akt may bind the Src family kinase Lck, which is expressed in T cells. To date, we have been unable to demonstrate such an interaction, and we are currently undertaking a broader search for proteins that interact with Akt in a proline-motif-dependent manner. In any case, it seems unlikely that binding to Lck will completely account for the distribution of full-length Akt that we have observed, because the pattern of Lck redistribution (transient localization to the synapse) after T cell activation is distinct from that of Akt (44).

It is still unclear precisely how the proline mutant of Akt acts at the biochemical or cell biological level to inhibit T cell activation. It may more than likely does not merely act as a sink for PIP3, as overexpression of the PH domain alone does not inhibit NF-κB induction (data not shown) or T cell proliferation (36). Consistent with our findings (Fig. 7), a recent study noted (as data not shown) that mutation of the proline-rich motif in the C terminus of Akt did not prevent plasma membrane recruitment of Akt after growth factor stimulation (39). Thus, the mutation may have a more subtle effect on localization to a particular region of the plasma membrane, e.g., lipid rafts. In preliminary experiments we have observed a strong correlation between Akt-GFP localization and a lipid raft marker (unpublished observations), so it may be instructive to examine the effects of this proline mutation on Akt-GFP localization in T cell lipid rafts. Such mislocalization could result in inefficient Akt activation and/or an inability to interact with substrates. Regardless of the mechanism by which the prolines in the C terminus of Akt restrict its localization, mutation of these residues creates a molecule with potent dominant negative activity in T cells. In fact, this construct appears to be a more potent inhibitor than other kinase-de

References