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Pak1 protein kinase of Schizosaccharomyces pombe, a member of the p21-GTPase-activated protein kinase (PAK) family, participates in signaling pathways including sexual differentiation and morphogenesis. The regulatory domain of PAK proteins is thought to inhibit the kinase catalytic domain, as truncation of this region renders kinases more active. Here we report the detection in the two-hybrid system of the interaction between Pak1 regulatory domain and the kinase catalytic domain. Pak1 catalytic domain binds to the same highly conserved region on the regulatory domain that binds Cdc42, a GTPase protein capable of activating Pak1. Two-hybrid, mutant, and genetic analyses indicated that this intramolecular interaction rendered the kinase in a closed and inactive configuration. We show that Cdc42 can induce an open configuration of Pak1. We propose that Cdc42 interaction disrupts the intramolecular interactions of Pak1, thereby releasing the kinase from autoinhibition.

The p21-GTPase-activated protein kinase (PAK) family is present in all eukaryotes. Genetic evidence suggests that STE20, one of three Saccharomyces cerevisiae homologs of PAK, mediates signaling of pheromone response from receptor-coupled heterotrimeric G proteins to the mitogen-activated protein kinase (MAPK) cascade, which includes STE11, STE7, and the pair FUS3 and KSS1 (13, 14, 28). STE20 can phosphorylate STE11 in vitro (25, 36). Another homolog, CLA4, appears to regulate normal localization of cell growth and cytokinesis (7), and a third, SKM1, has broad functions in morphogenesis and growth (20). In the fission yeast, Schizosaccharomyces pombe, Pak1 (also known as Shk1) seems to be involved in both sexual differentiation and morphogenesis (17) and has a structural and functional homolog, Shk2 (26, 37). Pak1 has been shown to release the intramolecular and, presumably, autoinhibitory interactions of Byr2, the S. pombe homolog of STE11 (31). Mammalian PAK proteins have three major isoforms, and they appear to be mediators of signaling from members of the p21-GTPase family such as Rac1 and Cdc42 to the MAPKs including Jun kinase and p38 MAPKs (1, 3, 6, 11, 23, 27, 38).

All PAKs have an N-terminal regulatory domain and a conserved C-terminal kinase catalytic domain. The regulatory domains are poorly conserved except for a 70-amino-acid stretch, named CRIB (Cdc42-Rac interactive binding) domain, which is known to bind the small Rho-family GTPases (4). Cdc42 can activate PAK proteins in vitro, inducing a PAK autophosphorylation event (16). Two mechanistic models are consistent with the in vitro biochemical data: Cdc42-Rac directly induces an active conformation of the catalytic region, or the GTPases antagonize an autoinhibitory mechanism.

We have been utilizing genetic analysis and the two-hybrid system of Fields and Song (8) to probe the regulatory mechanisms of kinases in the RAS signaling pathways of yeast and mammalian systems (2, 5, 17, 18, 31, 32, 35). Byr2, one of the S. pombe Ras1 effectors that is required for sexual differenti-

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MATERIALS AND METHODS

Yeast, media, and genetic manipulations. S. cerevisiae L40, a lacZ-based two-hybrid reporter strain with both HIS3 and lacZ as reporter genes (33), was used to study two-hybrid interactions. AN43-5A has a FUS1-lacZ reporter system and was used to measure the activity of the X. cerevisiae mating signaling pathway (17). S. cerevisiae cultures were grown in YPD (2% peptone, 1% yeast extract, 2% glucose) or in dropout (DO) synthetic minimal medium (0.67% yeast nitro-
gen base without amino acids, 2% glucose) with appropriate autotrophic supplements. The lithium acetate protocol was used for yeast transformation (12). Generating Pak1 and Cdc42 clones. PCR (24) was used to generate all constructs. Cdc42-Reg and the catalytic domain of Pak1 that encodes the C-terminal 385 amino acids, was made previously (31). Pak1-Reg, which encodes the N-terminal 284 amino acids, was made with the following pair of oligonucleotides: CCCCCCA AACCTTCTCCATTAGTTTCCAGC to 375 amino acids of Pak1, which was generated by PCR with the following pair of pLBD vectors. Full-length Pak1 was made by fusing Pak1-Reg to the C-terminal -galactoside (ONPG) was used as the substrate in the nyl- The PCR product was digested with BamHI and SalI and cloned into pGAD and pLBD vectors. Full-length Pak1 was made by fusing Pak1-Reg to the C-terminal -galactoside (ONPG) was used as the substrate in the nyl- The PCR product was digested with BamHI and SalI and cloned into pGAD and pLBD vectors. Full-length Pak1 was made by fusing Pak1-Reg to the C-terminal -galactoside (ONPG) was used as the substrate in the nyl- The PCR product was digested with BamHI and SalI and cloned into pGAD and pLBD vectors. Full-length Pak1 was made by fusing Pak1-Reg to the C-terminal -galactoside (ONPG) was used as the substrate in the nyl- The PCR product was digested with BamHI and SalI and cloned into pGAD and pLBD vectors. Full-length Pak1 was made by fusing Pak1-Reg to the C-terminal -galactoside (ONPG) was used as the substrate in the nyl- The PCR product was digested with BamHI and SalI and cloned into pGAD and pLBD vectors. Full-length Pak1 was made by fusing Pak1-Reg to the C-terminal -galactoside (ONPG) was used as the substrate in the nyl- The PCR product was digested with BamHI and SalI and cloned into pGAD and pLBD vectors. Full-length Pak1 was made by fusing Pak1-Reg to the C-terminal -galactoside (ONPG) was used as the substrate in the nyl- The PCR product was digested with BamHI and SalI and cloned into pGAD and pLBD vectors. 

RESULTS


domains block truncated and activated Pak1 in vivo. The standard autoinhibition model for protein kinases predicts that the regulatory domain inhibits the catalytic activity, and for Pak1, this is supported by the truncation experiments that have been performed and reported by others (17, 28). If our two-hybrid data correctly identifies the region of the regulatory molecule that binds to the catalytic domain, and the truncated Pak1 is activated because of the loss of the
inhibitory influence of the regulatory domain, then expression of that domain should inhibit the activity of the truncated Pak1 when it is expressed in trans. To test this prediction, we exploited the observation that expression of the truncated Pak1 is somewhat toxic to \textit{S. cerevisiae}. We thus performed an expression toxicity assay. L40 was transformed with either GAD-Pak1-Reg, GAD-Pak1149–210, GAD-Pak1157–210, or GAD alone, all carrying the \textit{LEU2} marker, and either pLS104-Pak1-Cat or pLS104 vector alone, each carrying \textit{ADE2}. Cells were plated on medium lacking leucine and adenine (DO-LA), and transformants were patched out on fresh DO-LA plates. The patches were then replica plated and grown for several days on the nonselective medium, YPD, before being replica plated back on the selective medium. Cells expressing toxic \textit{ADE2} plasmids will tend to lose the same, which we can assay in two ways: by failure to thrive on the selective plates and by the red color characteristic of cells lacking \textit{ADE2}. Cells with Pak1-Cat and GAD alone failed to grow effectively on the selective medium, and the patches displayed a red color. However, those with Pak1-Cat with either GAD-Pak1-Reg, GAD-Pak1149–210, or GAD-Pak1157–210 grew more effectively, and the patches displayed a pink to white color (Fig. 3). These studies confirm that the region we have identified not only binds to the catalytic domain but also inhibits it, even when expressed in trans. If we correctly surmise that wild-type Pak1 failed to bind Pak1-Reg because of intramolecular interactions, we should be able to readily isolate Pak1 mutants that gain the ability to bind Pak1-Reg, and such mutants should have regulatory and catalytic domains that are no longer able to interact.

Regulatory and catalytic interaction keeps wild-type, full-length Pak1 in a closed configuration. Since Pak1, like Byr2, contains a regulatory domain capable of interacting with its catalytic domain, we suspected that full-length Pak1, like full-length Byr2, would exist in a closed configuration in which the catalytic domain is occupied by the regulatory domain. In support of this hypothesis, we found that although we could readily detect binding between Pak1-Reg and Pak1, we could not detect the binding of Pak1-Reg to full-length Pak1, even though the latter was perfectly capable of binding Cdc42\textit{V12,C189S} (Fig. 1). (Note: in these experiments, the Cdc42\textit{V12,C189S} protein, lacking the farnesylation site, was used because the combined expression of Pak1 and Cdc42\textit{V12} is toxic.) These results suggest that an intramolecular interaction exists between the regulatory and catalytic domains in full-length Pak1. This hypothesis is further strengthened by the experiments, described below, in which we searched for, found, and analyzed mutants of Pak1 that were in an open configuration.
Pak1-Reg was randomly mutagenized by PCR and fused to Pak1-Cat in to form a library of LBD fusions of full-length, mutagenized Pak1. The DNA of this mutant library was trans-formed into L40 together with GAD-Pak1-Reg, and cells were plated in the absence of histidine to select for mutant, full-length Pak1 capable of interacting with the isolated regulatory domain. Colonies that grew on the His\textsuperscript{+} plates were patched out and tested for the production of β-galactosidase. Twenty-five colonies that were both His\textsuperscript{+} and LacZ\textsuperscript{+} were isolated, and the LBD plasmids from these cells were recovered, amplified, and transformed back into L40 together with GAD-Pak1-Reg or GAD. Nineteen of the 25 LBD-Pak1 plasmids interacted with GAD-Pak1-Reg but not with GAD. Figure 4 shows the two-hybrid interactions of the 19 LBD-Pak1 mutants with GAD-Pak1-Reg and with the negative control. Since these Pak1 mutants can bind Pak1-Reg, we call them Pak1\textsuperscript{open} mutants hereafter.

The regulatory domains of the 19 Pak1\textsuperscript{open} mutants were sequenced. All of them contain a single mutation between residues 161 and 200, that is, within the CRIB domain, the highly conserved region that binds both Cdc42 and Pak1-Cat (Table 1). Several mutations were encountered more than once, and the mutants fell into 13 groups. All mutations except M200T and M200R were mapped to residues conserved among PAK proteins. Figure 5 shows the multiple alignments of this conserved region with representative homologs, with the sites of mutation indicated.

As the first step towards characterizing these Pak1\textsuperscript{open} mutants, we tested the binding of the Pak1-Reg of these mutants to Pak1-Cat and Cdc42. The Pak1-Reg plasmids were excised and fused to GAD, and the GAD fusions were tested with LBD-Pak1-Cat and LBD-Cdc42, individually. GAD-Pak1wt-Reg and GAD were tested alongside. The two-hybrid results are presented in Fig. 6. Significantly, but not surprisingly, the regulatory domains of all of the Pak1\textsuperscript{open} mutants failed to bind LBD-Pak1-Cat, while all still bound Cdc42 to varying degrees.

These results demonstrate that all 19 Pak1\textsuperscript{open} mutants contain mutations in the CRIB domain that abolish binding to the catalytic domain Pak1 and argue strongly that the loss of intramolecular interaction is the cause for the open configuration.

**Genetic characterizations of Pak1\textsuperscript{open} mutants.** If the disruption of the regulatory-catalytic interactions were sufficient to activate Pak1, we would expect the Pak1\textsuperscript{open} mutants to be...
activated. To test this, we examined the activity of Pak1open mutants in comparison with that of the wild-type Pak1. Three phenotypes associated with the dominant activated Pak1, Pak1-Cat, were assayed: toxicity (references 17 and 26 and as described above), activation of the FUS1-lacZ reporter system (17), and the induction of the open configuration of Byr2 (31). The format for expression toxicity assays was described above. Pak1, Pak1open mutants, Pak1-Cat, and Pak1K415,416R-Cat were cloned under an alcohol dehydrogenase promoter into an Ade2-based 2 μm plasmid, pLS104. L40 cells transformed with various pLS104 clones were plated on Ade− plates. Transformsants were then patched onto fresh Ade− plates and replica plated on nonselective medium and then again on Ade− plates. Cells that grow well while carrying the pLS104 derivatives grow robustly as white patches on Ade− plates, while cells with pLS104 derivatives that cause toxicity or growth defects, to avoid the potential complications due to the toxicity of the expressed gene. Cells containing the plasmids were patched on medium rich in glucose and then replica plated to medium depleted of glucose but rich in galactose (2%). The amount of β-galactosidase in these cells was monitored by the conversion of X-Gal, and the results are presented in Fig. 8. As expected, Pak1open mutants could activate the S. cerevisiae mating pathway and stimulate β-galactosidase production by the reporter system.

TABLE 1. Mutations in Pak1open mutants

<table>
<thead>
<tr>
<th>Mutant group</th>
<th>Mutation</th>
<th>Member(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>F161S</td>
<td>Pak1open-1</td>
</tr>
<tr>
<td>II</td>
<td>G166W</td>
<td>Pak1open-9</td>
</tr>
<tr>
<td>III</td>
<td>E167G</td>
<td>Pak1open-17</td>
</tr>
<tr>
<td>IV</td>
<td>F168S</td>
<td>Pak1open-11</td>
</tr>
<tr>
<td>V</td>
<td>W175R</td>
<td>Pak1open-5, Pak1open-10</td>
</tr>
<tr>
<td>VI</td>
<td>L179P</td>
<td>Pak1open-6, Pak1open-18</td>
</tr>
<tr>
<td>VII</td>
<td>I184T</td>
<td>Pak1open-3, Pak1open-12, Pak1open-13, Pak1open-15</td>
</tr>
<tr>
<td>VIII</td>
<td>P193S</td>
<td>Pak1open-2</td>
</tr>
<tr>
<td>IX</td>
<td>P193Q</td>
<td>Pak1open-19</td>
</tr>
<tr>
<td>X</td>
<td>A195V</td>
<td>Pak1open-4</td>
</tr>
<tr>
<td>XI</td>
<td>A195T</td>
<td>Pak1open-7, Pak1open-8</td>
</tr>
<tr>
<td>XII</td>
<td>M200T</td>
<td>Pak1open-14</td>
</tr>
<tr>
<td>XIII</td>
<td>M200R</td>
<td>Pak1open-16</td>
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The third assay for Pak1 activation was based on its ability to induce the open configuration of Byr2. We have previously shown that expression of the dominant activated Pak1, Pak1-Cat, but not the wild-type full-length kinase, induced the two-hybrid interaction between GAD-Byr2-CBD (the GAD fusion to the smallest subregion of the regulatory domain of the Byr2 kinase sufficient to bind to its catalytic domain) and LBD-Byr2 (31). We therefore tested if Pak1open mutants were more effective than wild-type Pak1 at inducing this interaction. L40 was transformed with pGAD-Byr2-CBD, pLBD-Byr2, and either pLS104-Pak1-Cat, pLS104-Pak1K415,416R-Cat, pLS104-Pak1, pLS104-Pak1L179P, pLS104-Pak1I184T, pLS104-Pak1A195T, pLS104-Pak1W175R, or Pak1P193S, Pak1P193Q, Pak1A195V, or Pak1M200R grew, but their patches were pink; cells with Pak1M200T were dark pink; and cells with Pak1L179P, Pak1I184T, or Pak1A195T grew poorly after being replica plated back to the Ade− plates, and the patches had a red color, much like those with dominant activated Pak1. From this, we conclude that the majority of Pak1open mutants have higher activity than wild-type Pak1, and the results support the model that Pak1 intramolecular interaction is responsible for autoinhibition.

The FUS1-lacZ reporter system provides an indicator for the activity of the S. cerevisiae mating signaling pathway (10). We have shown previously that dominant activated forms of STE20 and Pak1 can activate this pathway in an STE11-dependent manner (17). It was noted then that full-length wild-type Pak1 failed to activate the pathway. Therefore, we asked if any of the Pak1open mutants could activate the S. cerevisiae mating pathway and stimulate β-galactosidase production by the reporter system. Pak1open mutants were cloned under a galactose-inducible GAL1 promoter, as described in Materials and Methods, and the results support the model that Pak1 intramolecular interaction is autoinhibitory.

The patches of cells expressing Pak1-Cat, Pak1K415,416R-Cat, the kinase-defective Pak1-Cat. While cells containing wild-type Pak1 were unable to activate the reporter system detectably, several of the strains carrying Pak1open mutants were able. In fact, some produced β-galactosidase as well as did cells carrying Pak1-Cat. These results demonstrate that some Pak1open mutants are more active than wild-type Pak1 and further confirm that intramolecular interaction is autoinhibitory.
tants that were most active in the previous assays), or just pLS104 vector alone. Transformants were tested quantitatively for the production of β-galactosidase. The results are presented in Fig. 9. As expected, Pak1wt-Cat induced the interaction between GAD-Byr2-CBD and LBD-Byr2 to about six times above the background level, while kinase-defective Pak1K415,416R-Cat, failed to enhance this interaction. Wild-type full-length Pak1 also failed to increase this interaction, but both Pak1open mutants were able to induce levels twofold over the background level. These results once again confirm that the intramolecular interaction is autoinhibitory.

Cdc42 promotes the open configuration of Pak1. It has been shown in vitro, with a gel overlay assay, that purified Rac-Rho-Cdc42 can induce an autophosphorylation and activation event of Pak1 (16). Cdc42 is now known to be an upstream activator of Pak1 in vivo (17, 26), although the activation mechanism remains unknown. We have shown that Cdc42 and Pak1-Cat interact with a tightly overlapping region on Pak1-Reg. Moreover, we failed to find evidence for a trimeric complex among Cdc42V12, Pak1-Reg, and Pak1-Cat, detectable by the two-hybrid system, suggesting that the three-way interaction is sterically forbidden (data not shown). Therefore, we speculated that Cdc42 activates Pak1 by directly relieving Pak1 of...
the autoinhibition that results from the intramolecular binding of the regulatory and catalytic domains. This speculation led us to predict and test whether Cdc42 could induce the open configuration of Pak1.

We have successfully used the two-hybrid system to identify signaling components that can induce the open configuration of Byr2 (31), and we applied the same principles to Pak1. The Pak1 opening assay was performed in the following fashion: L40 was transformed with (i) either GAD-Pak1-Reg, GAD-Pak1149–210, GAD-Pak1157–210, or GAD alone; (ii) either LBD-Pak1 or LBD-Ras1 as a control; and (iii) either pLS104-Pak1-Cat, pLS104-Pak1K415,416R-Cat, pLS104-Pak1-K415,416R, or pLS104-Pak1, 13 pLS104-Pak1open mutants. Transformants were initially plated and then patched in groups of four on medium lacking adenine. The Ade+ cells were then replica plated on the nonselective medium, YPD, for several days, before being replica plated on medium lacking adenine (DO-adenine). Pictures were taken of the patches on DO-adenine, and the color of the patches was noted. The wild-type ADE2 allele was included for comparison.

The mutations, S148A and H155A, were each independently tested for intramolecular interaction within Pak1, the fission yeast homolog of PAK. Expression of segment fusions indicated that the highly conserved region of the regulatory domain of Pak1 (Pak1-Reg), to which Cdc42 also binds, was capable of binding to the catalytic domain. This mapping was later confirmed by point mutation analysis. The mutations, S148A and H155A, were each independently tested for intramolecular interaction within Pak1, the fission yeast homolog of PAK. Expression of segment fusions indicated that the highly conserved region of the regulatory domain of Pak1 (Pak1-Reg), to which Cdc42 also binds, was capable of binding to the catalytic domain. This mapping was later confirmed by point mutation analysis.

Previous studies showed that the intramolecular interaction between Byr2 regulatory and kinase catalytic domains keeps that kinase in a closed configuration and establishes autoinhibition (31). In this report, we first describe a similar potential for intramolecular interaction within Pak1, the fission yeast homolog of PAK. Expression of segment fusions indicated that the highly conserved region of the regulatory domain of Pak1 (Pak1-Reg), to which Cdc42 also binds, was capable of binding to the catalytic domain. This mapping was later confirmed by point mutation analysis.

DISCUSSION

Previous studies showed that the intramolecular interaction between Byr2 regulatory and kinase catalytic domains keeps that kinase in a closed configuration and establishes autoinhibition (31). In this report, we first describe a similar potential for intramolecular interaction within Pak1, the fission yeast homolog of PAK. Expression of segment fusions indicated that the highly conserved region of the regulatory domain of Pak1 (Pak1-Reg), to which Cdc42 also binds, was capable of binding to the catalytic domain. This mapping was later confirmed by point mutation analysis.

In the case of Byr2, the intramolecular interaction causes autoinhibition, and its release is associated with kinase activation. The same appears to be true for Pak1. First, the expression of the smallest regulatory region of Pak1 capable of binding the catalytic region, a region that does not bind to Cdc42, inhibits the toxicity resulting from expression of the free catalytic domain. Second, the majority of Pak1open mutants are more active than wild-type Pak1, and some of them behaved similarly to the activated Pak1 lacking its regulatory domain. Third, Cdc42, a known activator of Pak1, both in vivo and in vitro, induces the open configuration, as discussed below.

Our genetic results indicate that not all Pak1open mutants are equally activated, and none are as active as the construct which lacks the entire regulatory region. There are many possible explanations for this. First, these proteins may be expressed at different levels. Second, although we cannot detect intramolecular interaction in the mutants by two-hybrid analysis, the mutants may nevertheless have a closed configuration in vivo. Third, there may be other features of the regulatory domain that are inhibitory for full biological activity. Indeed, other proteins that bind to the regulatory domain of Pak1 have recently been identified (9). Our studies are not designed to resolve these questions.
In our previous studies, we found that activated Pak1 could induce the open configuration of Byr2. We suspected that Cdc42 might do the same to Pak1. First, it was known that Cdc42 was an activator. Second, Cdc42 and the catalytic domain bind to overlapping regions of the regulatory domain. Third, we could not observe a stable trimeric complex among Cdc42, Pak1-Reg, and Pak1-Cat. We thus tested if Cdc42 could open Pak1. We used three different molecular probes for the open configuration of Pak1: GAD-Pak1-Reg, GAD-Pak1149–210, and GAD-Pak1 157–210. None are able to bind full-length Pak1, all three are able to bind the isolated catalytic domain, and only the first is also able to bind Cdc42. Indeed, when Cdc42 was overexpressed, the release of the kinase catalytic domain of full-length Pak1 to bind GAD-Pak1149–210 and GAD-Pak1157–210 was clear. Moreover, opening by Cdc42 could not be observed on mutant Pak1 proteins that do not bind Cdc42.

Although Cdc42 does activate Pak1, binds to Pak1, and opens its conformation and the open-conformation mutants are more active than wild type, these experiments do not rule out additional functions for Cdc42 in the activation of Pak1. For example, Cdc42 may facilitate the localization of Pak1 or the binding of other activating proteins.

It may be useful to draw a parallel between the interactions of Cdc42 and those of Ras1 with their respective protein kinase targets. Many of the same relations are retained: Ras1 is an in vivo regulator of Byr2, it binds directly to Byr2, and its domain of interaction overlaps with the site where the catalytic subunit also binds (22, 31, 32). Yet we were unable to demonstrate the opening of Byr2 by Ras1. In fact, no direct in vitro activation of Byr2 by Ras1 (or of Raf by H-ras) has been observed, and we have observed a stable complex between Ras1 and the Byr2 catalytic domain bridged by a mutant regulatory domain of Byr2 with enhanced affinity for the catalytic domain (30a). Thus, the mechanisms of action of these two very similar GTPases on two similar protein kinases are likely to be very different.

FIG. 8. Activity of the Pak1<sup>open</sup> mutants in the FUS1-lacZ induction assay. AN43-5A was transformed with either pYX113 (the empty vector with the GAL1 promoter), pYX113-Pak1-Cat, pYX113-Pak1<sup>K415,416R</sup>-Cat, pYX113-Pak1, 13 of the pYX113-Pak1<sup>open</sup> mutants, or pYX113-lacZ. Transformants were subjected to a 20-h galactose induction before being assayed for β-galactosidase production from the FUS1 promoter. Overlay filters were incubated with X-Gal for 2 to 6 h, and results of quadruplicate transformants are shown. Cultures were also harvested for β-galactosidase liquid assays, performed on four independent transformants. The assay results, ± standard deviations, are shown at right.

FIG. 9. Induction of the open configuration of Byr2 by the overexpression of Pak1<sup>open</sup> mutants. L40 was transformed with either pGAD-Byr2-CBD or pGAD; either pLBD-Byr2 or pLBD-Lamin; and either pLS104-Pak1-Cat, pLS104-Pak1<sup>K415,416R</sup>-Cat, pLS104-Pak1<sup>137P</sup>, pLS104-Pak1<sup>179I</sup>, pLS104-Pak1<sup>184T</sup>, or just the pLS104 vector alone. Transformants were tested quantitatively for β-galactosidase production. Values shown are relative levels. Standard deviations from at least four independent transformants are shown by error bars.
The region of the Pak1 regulatory domain that can bind to both Cdc42 and the catalytic domain is highly conserved among all members of the PAK family. Hence, this intramolecular interaction is highly likely to be conserved among them as well. Indeed, during the preparation of this paper, Zhao et al. reported the identification of a conserved negative regulatory region in αPAK (39). The authors showed that mutations on residues 101 to 137 of αPAK render that kinase constitutively active. They further provided evidence that αPAK83–149, a 67-amino-acid peptide, can block PAK activation by Cdc42 in vitro and suppresses PAK functions in vivo. This conserved negative regulatory region on αPAK corresponds to the Pak1 autoinhibitory region reported here. Our results are exactly complementary.

It may be proper to think of four kinases comprising the prototypic MAPK module: MAPK, MEK, MEKK, and PAK. MAPKs and MEKs have limited regions outside of the kinase catalytic domain and need to be phosphorylated at conserved residues in the catalytic domain to gain maximum kinase activities (34) (reviewed in reference 19). Thus, MEKs and MAPKs are predominantly regulated by dynamic phosphorylation and dephosphorylation and perhaps do not display autoregulation. MEKKs, like MEKs, also utilize regulatory and catalytic interaction to exert kinase autoregulation. Both PAKs and MEKKs can be regulated by p21 GTPases. However, while PAK regulation by Rho-family GTPases may be caused in part by direct release from autoinhibition, the regulation of MEKKs by GTPases may be more indirect (15, 30).

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