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The SH3 domain of the tight junction protein ZO-1 binds to a serine protein kinase that phosphorylates a region C-terminal to this domain

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Abstract ZO-1 is a tight junction phosphoprotein partially homologous to a tumor suppressor in *Drosophila*. The homologous region contains an SH3 domain with an unidentified function. Using fusion proteins containing the SH3 domain and various N- and C-terminal sequences, we tested for association of a kinase with this protein domain in extracts of MDCK cells. We show that the SH3 domain of ZO-1 binds a serine protein kinase that phosphorylates a region immediately C-terminal to the SH3 domain. This kinase associates specifically with the SH3 domain of ZO-1 and appears to be also associated with junctional complexes extracted from MDCK cells.

Key words: Tight junction; ZO-1; SH3 domain; Serine protein kinase

1. Introduction

To understand the development of multicellular tissues and the pathology of cancer, it is necessary to know more about the regulation of cell-cell adhesion and the assembly and disassembly of intercellular junctions. The epithelial junctional complex is formed by gap junctions, desmosomes, adherens junctions and tight junctions [1]. Some proteins that localize to these structures have been identified as oncogenes or tumor suppressors suggesting that intercellular junctions form a specialized signal transduction complex that regulates cell growth and differentiation [2–4]. For this reason, the identification and characterization of new junctional components with enzymatic properties enabling them to either transmit signals or regulate the interplay of structural components of intercellular junctions is of primary cell biological importance and is likely to improve our understanding of the regulation of cell growth and differentiation.

Tight junctions are the most apical structure of the junctional complex between epithelial cells. They regulate the diffusion of molecules through the paracellular pathway and participate in the maintenance of the differential distribution of lipids and, perhaps, some proteins between the apical and the basolateral cell surface domain [5]. Several peripheral membrane proteins that localize at the tight junction have been identified but only two have been sequenced: ZO-1 [6–8] and ZO-2 [9,10]. Both proteins show homology to a tumor suppressor in *Drosophila*, disc large A (dlgA). This growing family of proteins includes also lin-2, a component of the ras pathway that induces vulval differentiation in *C. elegans*, SAP90/PSD95, a protein associated with synapses, and p55, a cytoskeletal protein of the erythrocyte membrane [11–15]. These proteins possess a domain homologous to yeast guanylate kinase, an SH3 domain, and, three repeated regions called PDZ (for PSD95, Dlg A and ZO-1) domain. Interestingly, PDZ repeats seem to be important for the clustering of signal transduction molecules at specific membrane domains (reviewed in [16]). In contrast, nothing is known about molecules interacting with the SH3 domain of any member of this protein family.

SH3 domains were originally found in non-receptor protein tyrosine kinases of the Src family [17–19]. Since then, however, SH3 domains have been found in various proteins involved in different enzymatic as well as structural functions. Furthermore, since SH3 domains interact with specific PXXP motifs of other proteins, this protein domain appears to be a rather general mediator of protein-protein interactions [20–24]. Interestingly, the SH3 domains of Src, Nck, and Grb2 have been shown to interact with kinases, suggesting that this type of protein domain can also provide a link to other regulatory components [25–27].

Protein kinases appear to play a central role in the regulation of tight junction sealing [28–37]. Pharmacological studies suggested that the assembly of tight junctions in a Ca-switch model is directly or indirectly regulated by protein kinase C as well as other signal transducers like G-proteins, phospholipase C, and calmodulin [38,39]. Conversely, the disassembly of the junctional complex is also regulated by protein kinases [40].

In an effort to identify proteins interacting with the tight junction protein ZO-1 we used fusion proteins to identify associated proteins. Because almost all proteins associated with tight junctions are phophoproteins, we investigated whether fusion proteins containing parts of ZO-1 associate with a kinase that also phosphorylates the fusion protein. We show here that the SH3 domain of ZO-1 binds a serine kinase that phosphorylates a region C-terminal to this domain. This kinase can utilize GTP as a phosphoryl donor instead of ATP, and does not associate with other SH3 domains like those of Abl, Src, Crk, Grb2 or Nck. Moreover, immunoprecipitates of ZO-1 extracted from MDCK cells were also found to contain a kinase activity that phosphorylated the same fusion protein.

2. Materials and methods

2.1. Construction of fusion proteins

Fusion proteins consisting of glutathione *S*-transferase (GST) spliced to different parts of ZO-1 were generated using the pGEX-3X vector (Pharmacia, Uppsala, Sweden). For the extended PDZ3-SH3 fusion protein, a fragment obtained by digestion of the human

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Abbreviations: ZO-1, zonula occludens-1; ZO-2, zonula occludens-2; SH3, Src homology domain 3; GST, glutathione *S*-transferase; PDZ domain, PSD95-Dlg A-ZO-1 homology domain; ZAK, ZO-1 Associated Kinase

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ZO-1 cDNA [7] with *StuI* was ligated into the *SmaI* site of pGEX-3X. All other fusion proteins containing the SH3 domain and various neighboring regions were generated by PCR using a 5'-primer containing a *Bam*HI site and a 3'-primer containing an in-frame stop codon followed by an *Eco*RI site. PCR products were generated with Pfu polymerase (Strategene, USA) and cloned into the *Bam*HI and *Eco*RI sites of pGEX-3X (see Fig. 2). Fusion proteins encoding the SH3 domains of Abl, Src, nSrc, Crk and full-length Grb2 and Nck were kindly provided by Bruce Mayer (Department of Microbiology and Molecular Genetics, Harvard Medical School) [41,42]. GST fusion proteins were prepared and purified as described [43].

2.2. Cell culture and preparation of cell lysates

MDCK cells were cultured in DMEM supplemented with 10% of fetal calf serum and antibiotic as described [44]. Cells from confluent dishes were washed twice in ice-cold phosphate-buffered saline containing 1 mM PMSF, scraped off, and pelleted. The cell pellets were lysed in PBS containing 1% Triton X-100 and 1 mM phenylmethylsulphonyl fluoride. Lysates were spun for 10 min at 13000 rpm in a microcentrifuge and the supernatants were either directly used or stored at −80°C.

2.3. Kinase assays, phosphoamino acid analysis, and immunoprecipitation

For GST-fusion protein pull-down assays, approx. 1–3 μg of fusion protein and 40 μl of glutathione-Sepharose beads (1:2 slurry) were used per precipitation with 500 μl of MDCK cell extract containing 100–200 μg of total soluble proteins. Incubation of the fusion protein with the cell extract was carried out overnight at 4°C on an end-over-end rotator. Then, samples were spun and washed three times with PBS containing 1% Triton X-100 and twice with 50 mM HEPES, pH 7.4. Kinase reactions were carried out in 40 μl of kinase buffer (50 mM HEPES pH 7.4, 10 mM $MgCl_2$, 2 mM $MnCl_2$) containing 2.5 μCi of [γ-^{32}P]ATP (Hartman Analytic, Germany, 11 Ci/mmol). Reactions were performed at 30°C for 60 min and terminated by adding SDS-PAGE sample buffer followed by heating for 10 min at 75°C. Samples were fractionated on 8–20% gradient mini SDS-PAGE gels and phosphorylated bands were visualized by autoradiography.

Phosphoamino acid analysis was conducted as previously described using 2-dimensional thin-layer electrophoresis [45]. Immunoprecipitations where performed as described [39] using the monoclonal antibody R40.76 specific for ZO-1 [46] coupled to CNBr-activated Sepharose. Control incubations were performed with inactivated Sepharose. The same buffer as used for the pull-down assay described above was employed and the in vitro kinase assay was performed in the presence of 1 mM Na_3VO_4 and 50 ng/μl of okadeic acid.

3. Results

3.1. The SH3 domain of ZO-1 binds a serine kinase

The homologous region between the tight junction protein ZO-1 and the *Drosophila* tumor suppressor protein dlgA contains an SH3 domain [7]. SH3 domains are often found in signal transduction proteins and generally bind to proline-rich sequences of specific proteins [17,24,19]. To identify the signal transduction pathway that regulates tight junction assembly and sealing, we sought to identify the protein(s) which interact(s) with the SH3 domain of ZO-1. To do this, we generated a fusion protein consisting of GST and amino acids 295–633 of ZO-1, which include the third PDZ domain as well as the SH3 domain. Because tight junction assembly and sealing has been shown to be regulated by kinases, we investigated first whether this fusion protein binds to a kinase that also phosphorylates it.

Fig. 1A shows that when the fusion protein containing the third PDZ domain and the SH3 domain of ZO-1 (GST-PDZ3-SH3ex) was incubated with MDCK cell extract, extensively washed and then tested in vitro for kinase activity, very strong phosphorylation of the fusion protein was observed (Fig. 1A, lane 1). The specificity of this interaction was assessed by incubating GST with cell extract and, after extensive washing, 2 μg of the GST-PDZ3-SH3ex fusion protein was added prior to the in vitro kinase assay. No phosphorylation of the fusion protein was observed in such samples (Fig. 1A, lane 2). This experiment shows that the fusion protein containing the third PDZ repeat and the SH3 domain of ZO-1 binds to a protein kinase that phosphorylates the fusion protein itself.

Kinases can be classified by the amino acid that they phosphorylate [47]. For this reason, we performed a phosphoami-

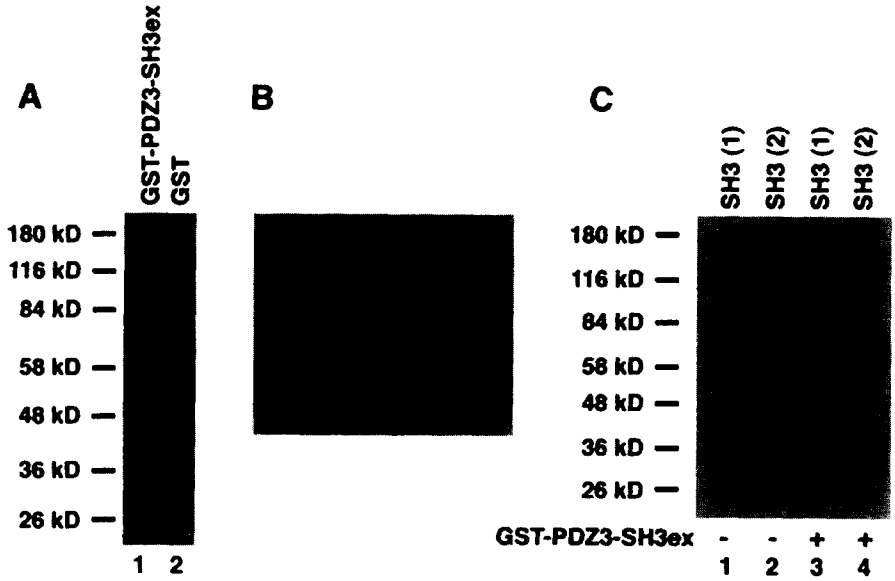

Fig. 1. The SH3 domain of ZO-1 binds to a serine kinase. (A) A fusion protein containing the third PDZ and the SH3 domain of ZO-1 (GST-PDZ-SH3ex) or GST only was incubated with MDCK cell extract, extensively washed and associated proteins were tested for kinase activity in vitro. 2 μg of GST-PDZ3-SH3ex was added to the GST bound proteins. (B) The band of the phosphorylated ZO-1 fusion protein was excised from the gel and subjected to phosphoamino acid analysis. Indicated are the positions of phosphoserine, phosphothreonine and phosphotyrosine. (C) Two different GST fusion proteins containing only the SH3 domain (apparent molecular mass of approx. 35 kDa) were generated and tested for binding and phosphorylation in the absence (lanes 1,2) or presence of GST-PDZ3-SH3ex fusion protein (lanes 3,4).

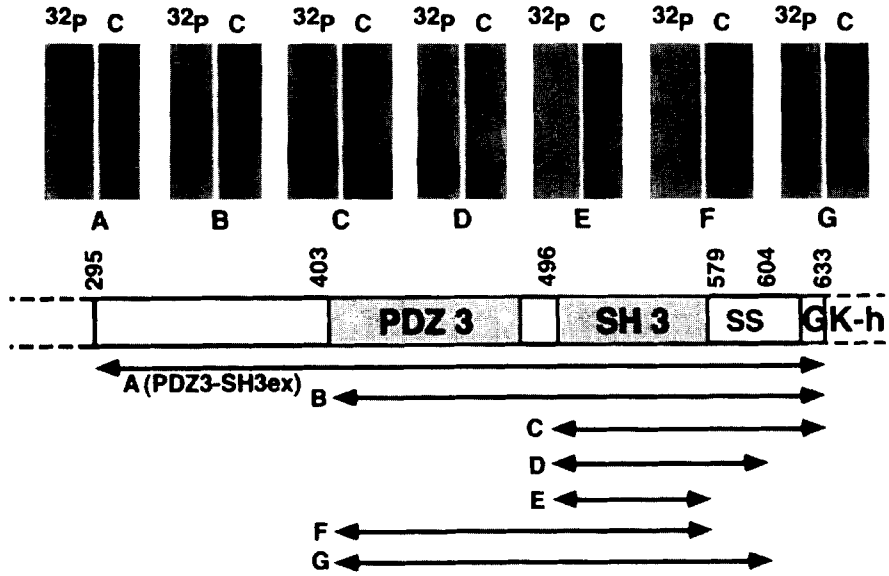

Fig. 2. The kinase phosphorylates serine residue(s) C-terminal to the SH3 domain. Several constructs containing the SH3 domain and different C- and N-terminal sequences were generated according to the schematic description in the bottom part of the figure and labeled from A to G. These fusion proteins were analyzed for phosphorylation in vitro. Autoradiographs are indicated as ^{32}P and Coomassie stains as C. The letter underneath each set of gels corresponds to the letter indicated in the schematic representation of the constructs.

no acid analysis of the GST-PDZ3-SH3ex fusion protein phosphorylated in vitro. Fig. 1B shows that the only phosphorylated amino acid we could detect was phosphoserine. Phosphorylation on tyrosine residues could also not be detected when the in vitro phosphorylated fusion protein was probed with anti-phosphotyrosine antibodies on immunoblots (not shown). Therefore, the precipitated kinase appears to phosphorylate the fusion protein only on serine residues. Interestingly, ZO-1 also contains phosphoserine (as well as phosphothreonine) in vivo [46], suggesting that ZO-1 could indeed be a physiological substrate for the precipitated kinase.

To determine whether the kinase binding site is indeed the SH3 domain of ZO-1, we generated two different GST fusion proteins containing the SH3 domain (SH3(1), amino acids 496–579; SH3(2), amino acids 506–579, apparent molecular masses approx. 35 kDa). These fusion proteins were subjected to the same assay as that described in Fig. 1A, however, no phosphorylation of the fusion proteins could be detected (Fig. 1C, lanes 1,2). This negative result could have been due either to failure of the kinase to bind to these shorter fusion proteins or, alternatively, to the fact that they do not contain the phosphorylation site(s). Therefore, we added the original fusion protein as a substrate to the kinase assays performed with the precipitates of the SH3-fusion proteins. Indeed, this experiment demonstrated that the SH3-fusion proteins precipitated the kinase since phosphorylation of the GST-PDZ3-SH3ex fusion protein could be detected (Fig. 1C, lanes 3,4). Thus, the SH3 domain of ZO-1 appears to be sufficient to bind the kinase but does not contain the phosphorylation site(s).

To localize the phosphorylation site(s), we generated different fusion proteins containing the SH3 domain, to ensure binding of the kinase, and different upstream and downstream sequences as shown in Fig. 2. Fusion proteins lacking either only the additional sequences N-terminal to the third PDZ domain (Fig. 2B) or also the PDZ domain and some additional amino acids linking the latter domain to the SH3 domain (Fig. 2C) were phosphorylated to a degree similar to that of the original fusion protein (Fig. 2A: GST-PDZ3-SH3ex). Furthermore, an additional short C-terminal deletion also did not affect the observed phosphorylation (Fig. 2D). As described in Fig. 1C, a fusion protein containing only the SH3 domain did not become phosphorylated (Fig. 2E). However, the Coomassie staining shows that this fusion protein was not very stable and became degraded. Even though the experiment in Fig. 1C showed that this small amount of fusion protein was still capable of binding the kinase, the absence of phosphorylation could have been due to the low amount of protein. Therefore, we assessed whether fusion proteins analogous to D and E but containing in addition the third PDZ domain would exhibit a similar phosphorylation behavior. Indeed, the fusion protein containing no amino acids C-terminal to the SH3 domain did not become phosphorylated (Fig. 2F) while the one which included amino acids 579–604 was phosphorylated (Fig. 2G). This result shows that the major, if not the only, phosphorylation site is within residues 579–604. Interestingly, the phosphorylated region contains only two serine residues that are immediately adjacent to each other ($S^{597}S^{598}$). These two serine residues are within a basic environment that is similar to the consensus site for protein kinase C [47]. Interestingly, H7 and staurosporine, inhibitors of protein kinase C as well as other protein kinases, inhibit tight junction assembly and disassembly upon addition or removal, respectively, of calcium [38–40,48,49]. Furthermore, protein kinase C purified from brain phosphorylates immunoprecipitated ZO-1 in vitro, and protein kinase C ζ localizes at the lateral membrane of MDCK cells [50]. Therefore, we determined next whether the kinase activity was inhibited by staurosporine. Since we did not observe inhibition by staurosporine at low concentrations (10 nM or less), it is not likely that the precipitated kinase is protein kinase C (not shown). In contrast, 100 nM staurosporine inhibited phosphorylation of the GST-PDZ3-SH3ex fusion protein by approx. 50% (not shown). Since such high concentrations of staurosporine inhibit many kinases including protein kinase C, protein kinase A, tyrosine kinases and myosin light chain

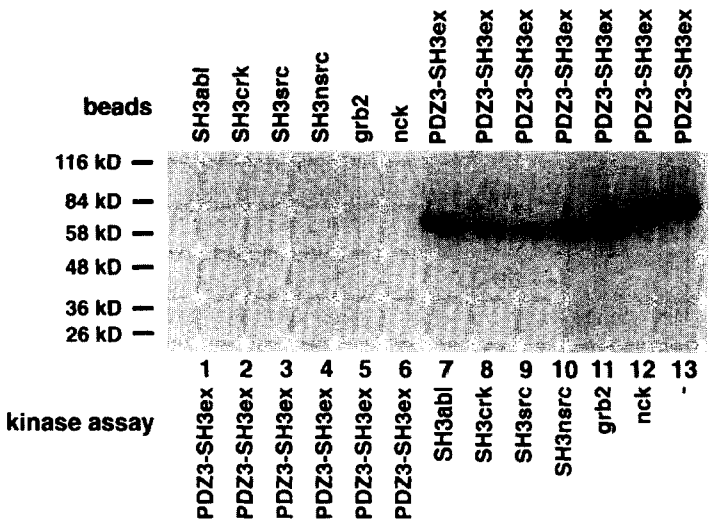

Fig. 3. The serine kinase binds specifically to the SH3 domain of ZO-1. Fusion proteins containing SH3 domains of Abl, Crk, Src, nSrc, or the entire coding sequences of Grb2 and Nck were bound to glutathione beads, incubated with MDCK cell extract, and then assayed for kinase activity by adding GST-PDZ3-SH3ex fusion protein as substrate (lanes 1–6). Alternatively, the kinase precipitated with the GST-PDZ3-SH3ex fusion protein was tested by adding the above-described fusion proteins as additional substrates (lanes 7–13).

kinase [51], we analyzed whether antibodies specific for those kinases (anti-protein kinase C ζ from Boehringer Mannheim; anti-protein kinase A R-I and R-II kindly provided by B.A. Hemmings, Friedrich Miescher Institute, Basel, Switzerland [52]; and anti-myosin light chain kinase from Sigma) would also precipitate the kinase that phosphorylates the GST-PDZ3-SH3ex fusion protein. However, we did not observe phosphorylation of the fusion protein in kinase assays with these immunoprecipitates. The kinase assay of the immunoprecipitated kinases showed phosphorylated proteins of molecular weight different from that of the fusion protein which can be taken as a control that the immunoprecipitated kinases were indeed active but did not phosphorylate the fusion protein. Furthermore, chelerythrine, a kinase inhibitor more specific for protein kinase C than staurosporine, [53] did not inhibit kinase activity (data not shown).

Next, we characterized some of the biochemical properties of the ZO-1 SH3-binding kinase. The original assay conditions tested included 10 mM magnesium and 2 mM manganese, therefore, we analyzed whether both cations are required for optimal activity. We obtained the same activity using 12 mM of either magnesium or manganese. Replacement by calcium, cadmium or zinc did not give full activity (data not show). Furthermore, the precipitated kinase was capable of using GTP instead of ATP as phosphoryl donor (not shown). A known kinase that is capable of using GTP instead of ATP is casein kinase II [54]. However, we could not detect this kinase in fusion protein precipitates using specific antibodies (Boehringer Mannheim, not shown) for immunoblot and the immunoprecipitated casein kinase II did not significantly phosphorylate the fusion protein (data not shown). Furthermore, the phosphorylated serine residue(s) are not within a consensus site for casein kinase II phosphorylation.

3.2. *The kinase associated with the SH3 domain of ZO-1 does not associate with the SH3 domains of Abl, Src, Crk, Grb2 or Nck*

To characterize the specificity of the kinase associated with the SH3 domain of ZO-1 we analyzed other SH3 domains such as those of Abl, Src, nSrc, Crk, and full length Grb2 and Nck (GST fusion proteins were kindly provided by Bruce Mayer). First, we incubated the different fusion proteins with MDCK cell extracts and, after washing, the PDZ3-SH3ex fusion protein was added and an in vitro kinase assay was performed and SDS-PAGE analysis was carried out.

Fig. 3 shows that none of these fusion proteins precipitated a kinase that was capable of phosphorylating the GST-PDZ3-SH3ex fusion protein. Thus, the SH3 domain of ZO-1 binds to a kinase that does not associate with the SH3 domains of Abl, Src, nSrc, Crk, and full-length Grb2 and Nck. This not only suggests that binding to the SH3 domain of ZO-1 is specific but also rules out that the kinase that binds to the SH3 domain of ZO-1 is identical to one of the protein kinases that bind to the SH3 domain of Src and Nck [25,26].

To examine also the specificity of the phosphorylation, we added the above-mentioned purified fusion proteins as substrates to kinase assays of precipitates generated with the GST-PDZ3-SH3ex fusion protein. This experiment showed that only the GST-PDZ3-SH3ex fusion protein became phosphorylated (Fig. 3). This suggests not only that binding to the SH3 domain of ZO-1 is specific but also that not just any SH3 domain-containing protein can serve as a substrate for this kinase.

3.3. *A kinase that phosphorylates the GST-PDZ3-SH3ex fusion protein co-immunoprecipitates with ZO-1*

In vivo ZO-1 is phosphorylated on Ser and Thr [46]. To evaluate whether ZO-1 is associated to a kinase in vivo that may phosphorylate the PDZ3SH3 fusion protein, we immunoprecipitated ZO-1 with a specific monoclonal antibody (R40.76) [46] and then analyzed the immunoprecipitate in an in vitro kinase assay in the absence or presence of the GST-PDZ3-SH3ex fusion protein.

Fig. 4 shows that the GST-PDZ3-SH3ex fusion protein only became phosphorylated when it was added to a kinase assay with precipitates generated with beads carrying the anti-ZO-1 antibody (lane 2) but not if it was added to a assay with negative beads (lane 1). Furthermore, a phosphorylated band

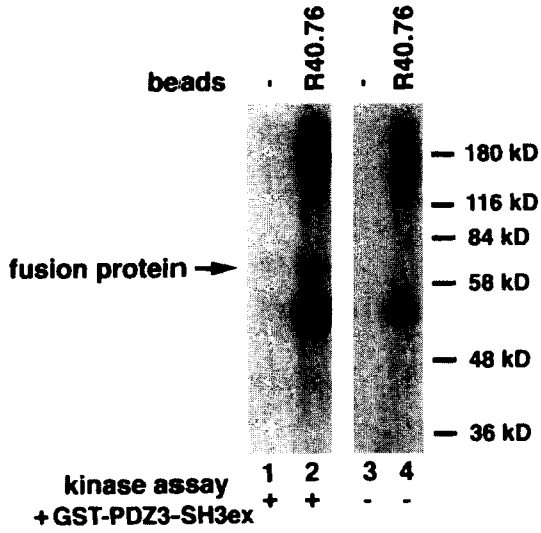

Fig. 4. ZO-1 co-precipitates with a kinase that phosphorylates the PDZ3-SH3 ex fusion protein. Immunoprecitpitated ZO-1 or inactivated Sepharose associated proteins were analyzed in an in vitro kinase assay in the presence (lanes 1,2) or in the absence (lanes 3,4) of GST-PDZ3-SH3ex fusion protein. The position of the GST-PDZ3-SH3ex fusion protein is indicated with an arrow.

with the molecular weight of the fusion protein was only observed in its presence (compare lanes 2 and 4) indicating that this phosphorylated band indeed corresponded to the fusion protein. Interestingly, in the absence as well as presence of the fusion protein, four other phosphorylated bands could be observed. While the high molecular mass bands are likely to correspond to ZO-1 (210 kDa), ZO-2 (160 kDa) and p130, all three are known to be phosphoproteins [39]. The identity of the band migrating at approx. 52 kDa is not clear but could be either immunoglobulin heavy chain or an uncharacterized protein.

This result indicates that a kinase capable of phosphorylating the GST-PDZ3-SH3ex fusion protein is also associated with ZO-1 immunoprecipitated from MDCK cell extracts. This suggests that a kinase that associates with and phosphorylates the fusion protein containing the third PDZ domain and the SH3 domain of ZO-1 also associates with ZO-1 in vivo.

4. Discussion

We found that fusion proteins containing the SH3 domain of ZO-1 bind to a protein kinase that phosphorylates serine residue(s) C-terminal to the binding domain. A similar kinase activity co-immunoprecipitated with ZO-1 extracted from MDCK cells. Since ZO-1 is phosphorylated in vivo in Ser and this kinase appears to bind specifically to the SH3 domain of ZO-1 and not to other SH3 domains tested, we propose to call this serine protein kinase ZAK (ZO-1 Associated Kinase) until further characterization.

Our results are significant in several aspects. First, even though there are many publications on the involvement of kinases in the assembly and disassembly of tight junctions and ZO-1 is a phosphoprotein, this is the first time a kinase has been shown to interact directly with one of the components of this intercellular junction. Second, while it is known that ZO-1 directly or indirectly interacts with several other proteins, this is the first time that such an interaction has been localized to a particular domain of this protein. Third, since ZO-1 is a putative tumor suppressor, this interacting kinase is not only potentially involved in the regulation of tight junctions but might also be involved in the regulation of cell growth and differentiation. Fourth, ZAK not only extends the short list of kinases specifically interacting with a particular SH3 domain but is also the first one that in addition phosphorylates a region C-terminal to the SH3 domain.

Many pharmacological experiments have been reported that suggest that protein kinases are involved in the regulation of the sealing of tight junctions (see Section 1). Furthermore, antibodies against E-cadherin, an epithelial calcium-dependent cell-cell adhesion molecule, perturb cell-cell contact [55] and inhibit the assembly of the tight junction triggered by the addition of calcium [56]. Interestingly, the cell-cell contact perturbation can be prevented by activation of protein kinase A [57] or by the addition of diC8, suggesting an involvement of protein kinase C [39]. Other experiments confirmed the possible participation of protein kinase C in the assembly of tight junctions [49,50]. Moreover, protein kinases are also involved in the disassembly of tight junctions [40]. While the drugs generally used suggest an involvement of protein kinases C, A and tyrosine kinases, it is still not clear whether the observed alterations were due to direct or indirect effects. Furthermore, no kinase has so far been shown to interact with any of the known components of tight junctions. Therefore, the serine protein kinase activity described in this paper is an attractive candidate for a kinase directly involved in the assembly and sealing of tight junctions. The total phosphate content of ZO-1 is different in two strains of MDCK cells which differ in transepithelial resistance [30], however, it is the same [39] or just slightly different [58,50] in low versus high calcium cells. This apparent contradiction could mean that the total phosphocontent does not significantly change but that the phosphocontent at specific sites might change [58]. ZAK appears to phosphorylate ZO-1 itself, it will be of interest to analyze further whether this protein kinase is involved in the assembly of the junction. Interestingly, essentially all of the protein kinase inhibitors tested affect not only assembly but also disassembly, which could mean that the same kinase(s) are regulating both processes. Thus, transient phosphorylations might be involved in the mobilization of junctional components or the dissociation of existing interactions. Other factors like cell-cell interactions or cytoskeletal architecture then determine whether upon phosphorylation or dephosphorylation ZO-1 will be used for the assembly of tight junctions or associates with other components, for instance, β-catenin, an interaction that takes place in the absence of calcium and assembled tight junctions [59].

Since a serine kinase activity was also found to co-immunoprecipitate with ZO-1 from cell extracts derived from MDCK cell monolayers, it could also be that ZAK regulates an interaction occurring within assembled tight junctions. ZO-1 was found to interact directly in vitro with the C-terminal domain of occludin, the only known transmembrane protein of tight junctions [60]. Furthermore, experiments using stably transfected MDCK cells expressing wild-type or mutant chicken occludin suggested that the C-terminal domain of occludin is important for the regulation of epithelial paracellular permeability [61]. Hence, one could also imagine that ZAK regulates the interaction between the C-terminal cyto-

plasmic domain of occludin and ZO-1 and, thereby, paracellular permeability.

Another interesting feature of ZO-1 is that it belongs to a family of proteins homologous to disc large A, a tumor suppressor protein originally identified in *Drosophila* that localizes at the septate junction [2]. Besides the SH3 domain, all members of this family possess three PDZ domains and a guanylate kinase domain. While no associated protein for the domain homologous to guanylate kinase has been reported, PDZ domains of a neuronal member of this protein family, PSD95/SAP90, have been described to mediate interactions with NMDA-receptor, ion channels as wells as nitric oxide synthase (reviewed in [16]). In the case of ZO-1, however, no proteins are known to interact specifically with any of its domains. We have now shown that its SH3 domain interacts with a serine protein kinase. Because of the tumor suppressor activity of disc large A and, the role of lin-2 in differentiation [3], it is tempting to speculate that other members of this family might have a similar activity and, therefore, ZAK could also be part of a signal transduction mechanism of tight junctions involved in the regulation of cell growth and differentiation. Interestingly, mutations in the SH3 and guanylate kinase domain of dlgA cause loss of normal cell proliferation control [62].

As mentioned in Section 1, binding of protein kinases to SH3 domains is a new type of interaction recently identified for the SH3 domains of Src and Nck [26,25]. The fact that the SH3 domain of ZO-1 associates with a protein kinase suggests that this type of binding partner for SH3 domains might be a more general phenomena. More importantly, in the case that we describe here, the protein kinase not only associated with the SH3 domain but also phosphorylated sequences specific C-terminal to the SH3 domain. This suggests that kinase binding to the SH3 domain of a protein can have direct structural and, most likely, functional consequences for the protein.

The approach and observations described here open up new possibilities to look at how tight junction assembly and function are regulated by kinases. Once the chemical identity of this kinase has been determined, it will possible to express it in a wild-type or mutant configuration and study the physiological consequences.

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