Association of the Ste20-like Kinase (SLK) with the Microtubule

ROLE IN Rac1-MEDIATED REGULATION OF ACTIN DYNAMICS DURING CELL ADHESION AND SPREADING*

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Cytoskeletal remodeling events are tightly regulated by signal transduction systems that impinge on adhesion components and modulators of cellular architecture. We have previously shown that the Ste20-like kinase (SLK) can induce apoptosis through the induction of actin disassembly and cellular retraction (Sabourin, L. A., Tamai, K., Seale, P., Wagner, J., and Rudnicki, M. A. (2000) Mol. Cell. Biol. 20, 684–696). Using immunofluorescence studies, we report that SLK is redistributed with adhesion components at large podosome-like adhesion sites in fibronectin-stimulated fibroblasts. However, in vitro kinase assays demonstrate that its activity is not modulated following fibronectin stimulation. Double immunofluorescence studies in exponentially growing or spreading fibroblasts show that SLK is associated with the microtubule network and can be coprecipitated with α-tubulin. Furthermore, the stimulation of adhesion site formation by microtubule-disrupting agents induces the relocation of SLK with unpolymerized α-tubulin to large vinculin-containing adhesion complexes. These results suggest that SLK is part of a microtubule-associated complex that is targeted to adhesion sites and implicated in the regulation of cytoskeletal dynamics.

Cells reside in a protein network called the extracellular matrix, which they secrete and mold into the intercellular space. The extracellular matrix exerts profound control over the cells. The effects of the matrix are primarily mediated by integrins, a family of cell surface receptors that attach cells to the matrix and mediate mechanical and chemical signals from it. These signals regulate the activities of cytoplasmic kinases, growth factor receptors, and ion channels and control the organization of the intracellular actin cytoskeleton (1).

Integrin receptor binding to extracellular matrix proteins generates intracellular signals through tyrosine phosphorylation events that are important for cell growth, survival, and migration (2). In various cell types, integrin clustering triggers tyrosine phosphorylation of signaling proteins through the activation of a large number of non-receptor protein tyrosine kinases such as focal adhesion kinase (FAK)1 and the Src-family of kinases. Increased tyrosine phosphorylation of intracellular proteins is one of the earliest responses stimulated by integrin receptor activation when cells contact matrix protein such as fibronectin (FN). Upon replating of cells on fibronectin, FAK becomes activated either through conformational changes or as a result of aggregation mediated by integrin clustering. FAK autophosphorylation at Tyr-397 under these conditions promotes the transient association of Src-family protein tyrosine kinases with FAK and the formation of a signaling complex. In addition, FAK associates with several different signaling proteins such as p130Cas, Shc, Grb2, phosphatidylinositol 3-kinase, and paxillin. This association enables FAK to function within a network of integrin-stimulated signaling pathways, leading to the activation of targets such as the ERK and JNK/mitogen activated protein kinase pathways (1–5).

For many cellular processes, microtubules and the actin cytoskeleton dynamics must be coordinated. Recent studies suggest that there may be signal transduction systems that integrate the responses of the two systems (6–15). Previous data have suggested the existence of a factor associated with the microtubules that can be released and affect assembly of the actin meshwork (16). Interestingly, microtubule breakdown stimulates stress fiber formation in fibroblasts (17), requires active Rho, and involves elevated myosin light chain phosphorylation (14, 18, 19), suggesting a coordinate regulation of the actin cytoskeleton and the microtubule network. This is accompanied by an assembly of adhesion plaques and tyrosine phosphorylation of the adhesion components FAK and paxillin (10). Tension exerted by microtubules on the actin cytoskeleton has been hypothesized to counteract stress fiber formation (10). Recently, it has been shown that there is direct physical interaction between the microtubules and adhesion sites, suggesting that relaxation signals are delivered via the microtubules to focal contacts to modulate the development of adhesions in a region-specific manner (11, 15, 20, 21).

We and others (22–25) have previously identified a novel Ste20-related kinase termed SLK that is involved in cytoskeletal reorganization and apoptosis (24, 25). Overexpression of SLK in various cell lines was shown to induce the rapid disassembly of actin stress fibers and cell death (25). Relatively high

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1 The abbreviations used are: FAK, focal adhesion kinase; FN, fibronectin; ERK, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; MEF, mouse embryonic fibroblast; DME medium, Dulbecco’s modified Eagle’s medium; PBS, fetal bovine serum; PBS, phosphate-buffered saline; TRITC, tetramethylrhodamine isothiocyanate; RIPA, radioimmune precipitation assay; HA, hemagglutinin; SLK, Ste20-like kinase.
levels of SLK protein and activity have been observed in all cell types analyzed, suggesting a functional role for SLK in physiological processes other than apoptosis. Because of its ability to induce cytoskeletal remodeling when overexpressed in a variety of cultured cell lines, we have analyzed the levels, distribution, and activity of SLK protein during cell spreading on FN, a process that requires extensive cellular remodeling (2, 26).

Using indirect immunofluorescence microscopy and biochemical techniques, we have determined that SLK is redistributed to large adhesion complexes during cell spreading on FN and that it is associated with the microtubule network. Using microinjection studies, we show that ectopic expression of activated SLK induces the disassembly of actin stress fibers, a process that can be inhibited by dominant negative Rac1. Finally, the overexpression of SLK by adenoviral infection inhibits cell spreading on FN. These results suggest that SLK may be delivered to adhesion sites via the microtubule network where it regulates actin dynamics.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture**—The mouse fibroblast cell lines MEF-3T3 (MEF Tet-On, C3018, Clontech) and Swiss 3T3 mouse embryonic fibroblast (ATCC catalog no. 4925) were used in all experiments. MEF-3T3 and Swiss 3T3 cell lines were maintained at 37°C, 5% CO2 in Dulbecco’s modified Eagle’s medium (DME medium, Bio-Whitaker) supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 50 μg/ml penicillin, and 50 μg/ml streptomycin. For FN replating assays, sub-confluent MEF-3T3 cultures were serum-starved in 0.25% FBS-DME medium for 24 h and harvested by trypsin-EDTA treatment as described previously (27). The trypsin was inactivated using soybean trypsin inhibitor (0.5 mg/ml), and the cells were collected by centrifugation and resuspended in 0.1% bovine serum albumin-DME medium. After 1 h at 37°C in suspension, the cells were plated onto fibronectin-coated plates or coverslips. The culture dishes (or coverslips) were precoated with fibronectin (10 μg/ml) or poly-l-lysine (100 μg/ml, Sigma) in PBS overnight at 4°C, rinsed with PBS, and warmed to 37°C for 1 h prior to replating. For cytoskeletal disruption experiments, MEF-3T3 cells were plated on FN-coated (10 mg/ml) plates and then serum-starved overnight on 0.25% FBS-DME medium. The plates were then treated with colchicine (10 μg/ml) or nocodazole (10 μM) for 30 min prior to lysis. An analysis of SLK protein expression and activity was performed by Western blot and in vitro kinase assay as described below.

To disrupt the microtubule network prior to FN stimulation, colchicine was added to the cells in suspension in the final 30 min of incubation.

For immunofluorescence studies, the coverslips were fixed in 4% paraformaldehyde for 10 min at room temperature, washed in PBS, and blocked with 0.1% bovine serum albumin and 200 μg/ml ChromaPure Goat IgG (Jackson ImmunoResearch Laboratories). SLK was detected using an anti-SLK rabbit polyclonal antibody (24) in conjunction with fluorescein isothiocyanate-labeled second antibodies. The anti-Rac1 monoclonal antibody was obtained from Transduction Laboratories. Actin was detected by incubation with fluorescein isothiocyanate-labeled or tetramethyl rhodamine isothiocyanate (TRITC)-labeled secondary antibodies. Vinculin protein was detected with a mouse anti-vinculin monoclonal (clone VIN11-5, Sigma) in conjunction with fluorescein isothiocyanate-labeled secondary antibodies. Tubulin protein was detected using an anti-α-tubulin monoclonal antibody (clone DM1, Sigma) and TRITC-labeled secondary antibodies. The anti-Rac1 monoclonal antibody was obtained from Transduction Laboratories. Actin was detected by incubation with TRITC-conjugated phalloidin. Samples were visualized with a Zeiss Axioskop100 epifluorescence microscope equipped with appropriate filters and photographed with a digital camera (Sony Corporation). The images were analyzed with commercially available software, and the microscopy was digitized and processed for publication.

**Western Blot and In Vitro Kinase Assays**—At various times after replating, the attached cells were rinsed in PBS and protein extracts were made in modified RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1% Nonidet P-40, and protease inhibitors (Sigma inhibitor mixture). For SLK expression analysis, 20 μg of total cell lysate was subjected to Western blot with anti-SLK antibodies and TRITC-labeled secondary antibodies. The anti-Rac1 monoclonal antibody was obtained from Transduction Laboratories. Actin was detected by incubation with TRITC-conjugated phalloidin. Samples were visualized with a Zeiss Axioskop100 epifluorescence microscope equipped with appropriate filters and photographed with a digital camera (Sony Corporation). The images were analyzed with commercially available software, and the microscopy was digitized and processed for publication.

**RESULTS**

**Fig. 1. SLK localizes to podosome-like structures during cell adhesion and spreading.** Exponentially growing MEF cells immunostained for SLK (A) and P-actin (B) are shown. Endogenous SLK expression was found to be absent in actin fiber-rich regions (arrows). The asterisk shows a cell bearing stress fibers expressing low levels of SLK. Following replating of MEF cells on FN-coated coverslips (10 min), endogenous SLK was found to be predominantly perinuclear (C) and not associated with newly formed adhesion complexes detected by an anti-vinculin antibody (D). At later time points following replating on FN (20 min), a proportion of SLK (E) was found to be associated with vinculin (F) in diffuse podosome-like adhesion structures (arrows). This colocalization was observed in 60 ± 7% cells (n = 200) 20 min after replating. The cells were photographed at ×400 (A and B) and ×1000 (C–F).

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FIG. 2. SLK is not activated during cell adhesion and spreading on fibronectin. A, serum-starved MEF cells (C) were held in suspension (S), replated on FN-coated dishes or polylysine (PL), and sampled at timed intervals for SLK expression and activity. The activation of FAK (determined by Tyr-397 phosphorylation) and tyrosine phosphorylation of paxillin were analyzed for comparison (B). Normalization to total immunoprecipitated SLK (data not shown) shows that SLK is not activated following FN stimulation. In contrast, FAK and paxillin were activated at 10 min following replating.

C-terminal truncation, which results in SLK activation (HA-SLK1-373). Plasmid HA-SLK1-373(C63) is the kinase inactive version of HA-SLK1-373 (25). For coinjections, FLAG-tagged RacN17 and HA-SLK1-373 were mixed (each at 100 ng/ml) and injected in MEF-3T3 cells. The injected cells were incubated for 3 h or 8 h in coinjections) at 37 °C, fixed in 4% paraformaldehyde, and processed for indirect immunofluorescence staining. Coverslips were double-stained for HA using anti-HA rabbit polyclonal antibody (sc-805, Santa Cruz Biotechnology, Inc.) and anti-vinculin or actin as described above. To quantitatively measure the phenotypic changes induced by SLK ectopic expression, cells that stained positive for the HA epitope and displayed an absence of cytoplasmic stress fibers were scored. At least 50 injected cells were counted in three separate experiments, and the average is shown. In spreading assays, MEF-3T3 cells were infected with adenovirus expressing either of the two HA-tagged truncations of SLK. The cells were serum-starved in 0.25% FBS-DME medium overnight and infected for 90 min at a MOI of 100 in serum-free medium and allowed to express the tagged proteins for 5 h. The cells were then collected by centrifugation, held in suspension for 1 h, and plated onto fibronectin-coated coverslips and allowed to spread for 10–20 min prior to fixation and immunofluorescence staining. To quantitatively assess the effect of SLK overexpression during cell spreading, HA-positive cells that displayed a rounded “non-spread” morphology (HA-SLK1-373) or increased density of stress fibers (HA-SLK1-373(C63)) were enumerated. At least 100 HA-positive cells were counted in three separate experiments.

RESULTS

We have previously demonstrated that the overexpression of the SLK induces actin stress fiber disassembly and cell death (24, 25). Because of its ability to induce cytoskeletal remodeling when overexpressed in a variety of cultured cell lines, we analyzed the levels, distribution, and activity of SLK protein during cell spreading on FN, a process that requires extensive cellular remodeling (2, 26).

To gain insight into the role of SLK during normal cell growth, we first investigated the cellular distribution of endogenous SLK in exponentially growing MEF-3T3 cells. Supporting the observation that SLK can induce stress fiber disassembly, endogenous SLK distribution was found to correlate with an absence of actin stress fibers (see Fig. 1), suggesting a role for SLK in the regulation of actin dynamics. Interestingly, the levels of SLK protein were also found to be variable, suggesting that its expression may be regulated throughout the cell cycle.

The replating of suspended cells on FN-coated substrates has been shown to trigger the recruitment of adhesion complexes and subsequent actin polymerization from adhesion sites (2, 28). To investigate the function of SLK in cytoskeletal remodeling events, MEF-3T3 cells were immunostained for SLK during adhesion and spreading shortly after FN stimulation. When MEF cells held in suspension were replated onto FN-coated substrates, SLK was found to be redistributed to the cell periphery and to colocalize with the adhesion protein vinculin (Fig. 1, E and F) and FAK (data not shown). Surprisingly, SLK was observed to colocalize with vinculin at large structures resembling lamellipodia that formed abundantly 20 min post-replating (Fig. 1, E and F). A poor association was observed at discrete adhesion complexes that formed in the initial 10 min of spreading (Fig. 1, C and D), suggesting that SLK may be associated with adhesion complex disassembly or turnover. To investigate potential changes in SLK kinase activity during cell spreading on FN, extracts from replated cells were prepared and subjected to Western blot analysis and in vitro kinase assays. Following replating and Western blot analysis, total SLK levels remained unchanged during cell spreading (Fig. 2A). Interestingly, following in vitro kinase assay and normal-
Microtubule-disrupting agents have been shown to impair cell spreading and focal adhesion turnover (11). Therefore, we next examined whether SLK was also associated with the mi-

FIG. 4. SLK is redistributed to adhesion complexes induced by microtubule disruption. Serum-starved cells were immunostained for SLK (A), vinculin (B), α-tubulin (C) prior to the addition of nocoda-

zole. The addition of nocodazole to the cultures for 30 min induced the formation of large adhesion complexes (E) and the redistribution of SLK to these sites (D). Similarly, SLK was also localized to these sites (F) along with depolymerized α-tubulin (G). Photomicrographs are shown at ×630.

FIG. 5. SLK colocalizes with the microtubule network during adhesion and spreading. Cells suspended in serum-free medium were replated onto FN-coated coverslips for 20 min in the absence (A and B) or in the presence (C–F) of nocodazole and immunostained for SLK, α-tubulin, and vinculin. SLK (A) was observed to colocalize with the microtubule network (B) during cell spreading on FN. Similarly, in the few cells that spread in the presence of colchicine, SLK (C) was also found to colocalize with depolymerized α-tubulin (D, arrows). As for untreated cells, SLK (E) was also colocalized with vinculin (F) at diffuse adhesion sites in colchicine-treated cells (arrows). Photomicrographs are shown at ×630.

SLK activity remained unchanged following replating onto FN-coated plates (Fig. 2A). Similarly, no changes were observed during cell adhesion (20 min) to poly-L-lysine-coated substrates. Over the same time period, the phosphorylation of FAK at tyrosine 397 (28) and increased paxillin tyrosine phosphorylation (2, 28) did occur, indicating that the adhesion components were activated (Fig. 2B). These results suggest that SLK distribution is modulated by adhesion without any detectable changes in its kinase activity. One possibility is that specific redistribution of SLK leads to localized changes in substrate phosphorylation and downstream signaling events.

SLK Is Associated with the Microtubules and Adhesion Components during Cell Spreading—The observation that SLK colocalizes with adhesion components during late spreading events raises the possibility that it is implicated in adhesion complex disassembly or turnover. Recently, adhesion site contact by the microtubule network has been shown to promote their dissociation (11, 15). In addition, the disruption of microtubules has been shown to induce the assembly of focal adhesions and to stimulate the formation of actin stress fibers (10). Therefore, we investigated the distribution of SLK and α-tubulin in double immunostaining experiments. As shown in Fig. 3, a very strong colocalization was observed between the microtubule network and endogenous SLK protein in exponentially growing MEF cells (>95% of exponentially growing cells). In some areas at the cell periphery, SLK staining presented as a “filament-like” pattern (see Fig. 3, C and D) that colocalized with the microtubule network, suggesting that SLK is a microtubule-associated kinase. Interestingly, this association of SLK with the microtubules appeared to be preferentially non-centrosomal. Similarly, non-centrosomal microtubule nucleation has been observed at focal contacts in 3T3 cells during recovery from nocodazole or taxol treatment (15). To test whether SLK forms part of a complex with the microtubule, whole cell extracts obtained under different lysis conditions were subjected to immunoprecipitations using anti-SLK followed by Western blot analysis. Our results show that under all of the conditions tested, α-tubulin could be coprecipitated with SLK (Fig. 3E). However, in glutathione S-transferase pull-down assays using glutathione S-transferase-tubulin, SLK did not interact directly with α-tubulin (data not shown), suggesting that SLK is indirectly associated with the microtubule and that they are part of a higher order protein complex.

The treatment of serum-starved fibroblasts with microtubule disrupting agents has been previously shown to stimulate the formation of large adhesion complexes containing tyrosine-phosphorylated FAK and paxillin at the cell periphery (10, 14). To test whether SLK can redistribute to newly formed adhesion sites following microtubule depolymerization, serum-starved MEF cells grown on FN were treated with nocodazole for 30 min and subjected to double immunostaining for SLK and vinculin or α-tubulin. Induction of focal adhesion assembly by nocodazole resulted in the redistribution of SLK to the cell periphery with vinculin and α-tubulin, suggesting that SLK can be targeted to newly formed adhesion sites (Fig. 4, D and E). Interestingly, this can be achieved in the absence of an intact microtubule network (Fig. 4, F and G).

Microtubule-disrupting agents have been shown to impair cell spreading and focal adhesion turnover (11). Therefore, we next examined whether SLK was also associated with the mi-
crotubule network during cell adhesion and spreading on FN. The replating of suspended cells on FN-coated substrates resulted in SLK localization to the cell periphery with polymerized microtubules (Fig. 5, A and B). Interestingly, in the presence of colchicine, SLK was also colocalized with α-HA (A, C, E, and G) and TRITC-phalloidin (B and D) or anti-vinculin (F and H). Coinjection of dominant negative Rac1, RacN17, inhibited stress fiber disassembly and cell retraction for up to 8 h post-injection (I and J). Prolonged expression (8 h) of SLK1-373 induced retraction and extensive blebbing (I and J, insets). Cells expressing active SLK1-373 displayed altered morphology with actin redistribution to the periphery (B, arrow) and much smaller fibrillar adhesions (F, double arrow). Peripheral adhesions did not appear to be affected (F, arrow). No effect on stress fibers or adhesion sites was observed in SLK1-373K63R-expressing cells. Photomicrographs are shown at ×630. K, cells that stained positive for the HA epitope and displayed an absence of cytoplasmic stress fibers were scored. At least 50 injected cells were counted in three separate experiments, and the average is shown. Stress fiber disassembly induced by expression of SLK1-373 (87 ± 7% cells) could be inhibited by coinjection with RacN17 (loss of stress fibers in 32 ± 7% of the injected cells following RacN17 coinjection).

Regulation of Cell Adhesion and Spreading by SLK

Overexpression of activated SLK promotes cellular retraction and inhibits cell spreading—To gain insight into the role of SLK during cell adhesion and spreading, MEF cells grown on FN-coated substrates were microinjected with epitope-tagged SLK expression vectors encoding the activated truncation SLK1-373 (or the kinase inactive mutant SLK1-373K63R) and analyzed for the presence of actin stress fibers and focal adhesions. Cells overexpressing activated SLK1-373 displayed a retracted morphology (rounded appearance and loosely adherent), a marked reduction in actin stress fibers and extensive actin reorganization at the periphery (Fig. 6, A and B). Quantitative analysis showed that >85% (86.6 ± 7%) of cells expressing SLK1-373 displayed that phenotype. In contrast, no change in stress fiber density was observed in cells injected with the kinase-inactive SLK1-373K63R (97 ± 2% retained stress fibers, see Fig. 6, C and D). When SLK1-373-overexpressing cells were surveyed for focal adhesions using anti-vinculin antibodies, peripheral adhesion sites were smaller and fibrillar adhesions were greatly reduced or absent, consistent with the disassembly of actin stress fibers (Fig. 6, E and H). Overexpression of SLK1-373K63R had no effect on the density, size, and distribution of vinculin-containing adhesion sites in all injected cells (Fig. 6, G and H). No obvious differences were observed in the organization of the microtubule network of microinjected cells (data not shown). These results suggest a functional role for SLK in the
process of actin stress fiber disassembly rather than a direct destabilization of adhesion structures.

The small GTPase Rac1 has been previously shown to induce stress fiber disassembly as well as lamellipodia formation (29). Interestingly, Rac1 also binds directly to the microtubule in its GTP-bound form (30) and becomes activated by microtubule growth in fibroblasts (21). Therefore, we tested the possible involvement of Rac1 in SLK-mediated stress fiber disassembly. As shown in Fig. 6, the coinjection of both HA-SLK1-373 and a dominant negative form of Rac1, RacN17, resulted in a marked inhibition of stress fiber disassembly (86% compared with 32% in the presence of RacN17) and subsequent cellular retraction for up to 8 h post-injection. Over the same period, cells expressing HA-SLK1-373 alone had completely retracted and displayed extensive blebbing, a hallmark of apoptotic cells.

Furthermore, as for vinculin, in 60% (60/110065%) of the cells, SLK was found to colocalize with Rac1 following replating of MEF-3T3 cells onto FN (Fig. 7). Together, these results suggest a functional interaction between these two proteins and that Rac1 may be a mediator of SLK-induced actin remodeling.

Whether SLK directly regulates Rac1 or an upstream guanine exchange pathway remains to be elucidated.

To test the effect of SLK overexpression on cell spreading, MEF cells were infected with adenoviral constructs encoding SLK1-373 (C and G) or inactive kinase K63R (A and E), held in suspension, and replated on FN-coated coverslips for 20 min. The cells were immunostained using anti-HA and TRITC-phalloidin (B and D) or anti-vinculin antibodies (F and H). >90% SLK1-373-expressing cells (92 ± 6%) failed to spread and did not assemble actin fibers (G and H, arrows). Cells expressing lower levels of SLK1-373 (~5% HA-positive cells) (panel C) were able to spread but displayed much smaller adhesion sites. In contrast, 95 ± 5% of cells expressing the kinase-inactive SLK1-373K63R displayed an increased density of stress fibers. For both expression plasmids, at least 100 HA-positive cells were counted in three separate experiments. Uninfected cells are marked with an asterisk.
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Efficient targeting of adhesion sites by the microtubule for further delivery of destabilizing signals (15).

Direct interaction between SLK and α-tubulin was not observed by in vitro binding assays, suggesting an indirect interaction. One possibility is that post-translational modification of one or both proteins is required for their interaction, a signal that could be transduced downstream of adhesion complex formation. Alternatively, an adapter protein is required to recruit SLK to the microtubule. Whether these interactions are tightly regulated through post-translational modifications downstream of adhesion complex formation remains to be investigated.

The Rho family of small GTPases has been implicated as a mediator of cytoskeletal remodeling events such as the formation of stress fibers, lamellipodia, and ruffling (29, 33–35). The small GTPase Rac1 has been shown to be activated following microtubule growth and to promote lamellipodial protrusion (21). Specifically, GTP-Rac1 can directly bind β-tubulin (30), suggesting that it plays a role in cytoskeletal remodeling events regulated by microtubule growth such as adhesion disassembly. In addition, RhoA and mDia have been demonstrated to stabilize the microtubule network by capping (14, 18, 19), presumably to stabilize adhesion sites and the actin network (36). Our results show that SLK and Rac1 localization overlap extensively during cell spreading on FN at membrane protrusions resembling lamellipodia (cellular structures that can be induced by activated Rac1), suggesting that Rac1 may be a downstream effector of SLK. Furthermore, dominant negative Rac1 inhibits the cytoskeletal remodeling events induced by activated SLK, suggesting a functional interaction between these proteins.

Recently, the overexpression of β-tubulin 1 in MDCK cells has been demonstrated to inhibit cell spreading and adhesion (8). In addition, delayed formation of adhesion sites was reported in these cells shortly after replating (8). Interestingly, microtubule-disrupting agents induce the formation of adhesion sites and actin stress fibers, supporting the notion that the growing microtubule carries actin- and adhesion-destabilizing signals. Therefore, we propose a model whereby specific adhesion components through direct interaction with tubulin can target the microtubule network to adhesion sites (see Fig. 9).

Targeting of SLK to adhesion sites by the microtubules can lead to actin network destabilization, a process required for further cytoskeletal remodeling and cell movement.

Overall, we have established a novel molecular link between the adhesion-destabilizing activity of the microtubule network and actin-based cytoskeletal remodeling events. Further studies will be necessary to identify upstream and downstream signals required for SLK-dependent remodeling events implicating Rac1 and the microtubule network.

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