Ste20-like protein kinase SLK (LOSK) regulates microtubule organization by targeting dynactin to the centrosome

Olga N. Zhapparova[a], Artem I. Fokin[a], Nadezhda E. Vorobyeva[b], Sofia A. Bryantseva[a], and Elena S. Nadezhdina[a,c]

[a]A. N. Belozersky Institute for Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia; [b]Institute of Gene Biology, Russian Academy of Sciences, Moscow 119334, Russia; [c]Institute of Protein Research, Russian Academy of Sciences, Moscow 117334, Russia

ABSTRACT **The microtubule- and centrosome-associated Ste20-like kinase (SLK; long Ste20-like kinase [LOSK]) regulates cytoskeleton organization and cell polarization and spreading. Its inhibition causes microtubule disorganization and release of centrosomal dynactin. The major function of dynactin is minus end–directed transport along microtubules in a complex with dynein motor. In addition, dynactin is required for maintenance of the microtubule radial array in interphase cells, and depletion of its centrosomal pool entails microtubule disorganization. Here we demonstrate that SLK (LOSK) phosphorylates the p150^{Glued} subunit of dynactin and thus targets it to the centrosome, where it maintains microtubule radial organization. We show that phosphorylation is required only for centrosomal localization of p150^{Glued} and does not affect its microtubule-organizing properties: artificial targeting of nonphosphorylatable p150^{Glued} to the centrosome restores microtubule radial array in cells with inhibited SLK (LOSK). The phosphorylation site is located in a microtubule-binding region that is variable for two isoforms (1A and 1B) of p150^{Glued} expressed in cultured fibroblast-like cells (isoform 1B lacks 20 amino acids in the basic microtubule-binding domain). The fact that SLK (LOSK) phosphorylates only a minor isoform 1A of p150^{Glued} suggests that transport and microtubule-organizing functions of dynactin are distinctly divided between the two isoforms. We also show that dynactin phosphorylation is involved in Golgi reorientation in polarized cells.**

Monitoring Editor
Yixian Zheng
Carnegie Institution

Received: Mar 11, 2013
Revised: Aug 13, 2013
Accepted: Aug 19, 2013

INTRODUCTION

Microtubules (MTs) in interphase cells are organized into a radial array with the minus ends focused in the centrosome and plus ends directed toward cell's periphery. Such an array maintains polarized transport of molecules and organelles driven by motor proteins. The molecular mechanisms that regulate radial organization of microtubules are unknown. A typical microtubule-organizing center in fibroblast-like cultured cells is represented by the centrosome, where microtubules are nucleated and anchored. γ-Tubulin ring complexes nucleate microtubules and can remain bound to their minus ends further on (Wiese and Zheng, 2000, 2006; Anders and Sawin, 2011). γ-Tubulin, however, is not the only anchor of microtubules at the centrosome. Depletion of other centrosomal proteins—ninein (Mogensen *et al.*, 2000; Dammermann and Merdes, 2002; Delgehyr *et al.*, 2005; Ibi *et al.*, 2011), 4.1R (Perez-Ferreiro *et al.*, 2004), Nlp (Casenghi *et al.*, 2005), CAP350 and FOP (Yan *et al.*, 2006), and TACC3/maskin (Albee and Wiese, 2008)—causes randomization of microtubules in cells, although the centrosome retains its nucleating activity, which can be visualized by tracking plus end–binding proteins (EB1 and EB3) at growing ends of microtubules. Inhibition of some dynein-binding proteins, such as Bicaudal D and Nudel (Fumoto *et al.*, 2006; Guo *et al.*, 2006), and dynein–dynactin complex does not affect microtubule nucleation at the centrosome (Zhapparova *et al.*, 2007;

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E13-03-0137) on August 28, 2013.

Address correspondence to: Elena S. Nadezhdina (nadezhdina@vega.protres.ru).

Abbreviations used: LOSK, long Ste20-like protein kinase; MT, microtubules; SLK, Ste20-like protein kinase.

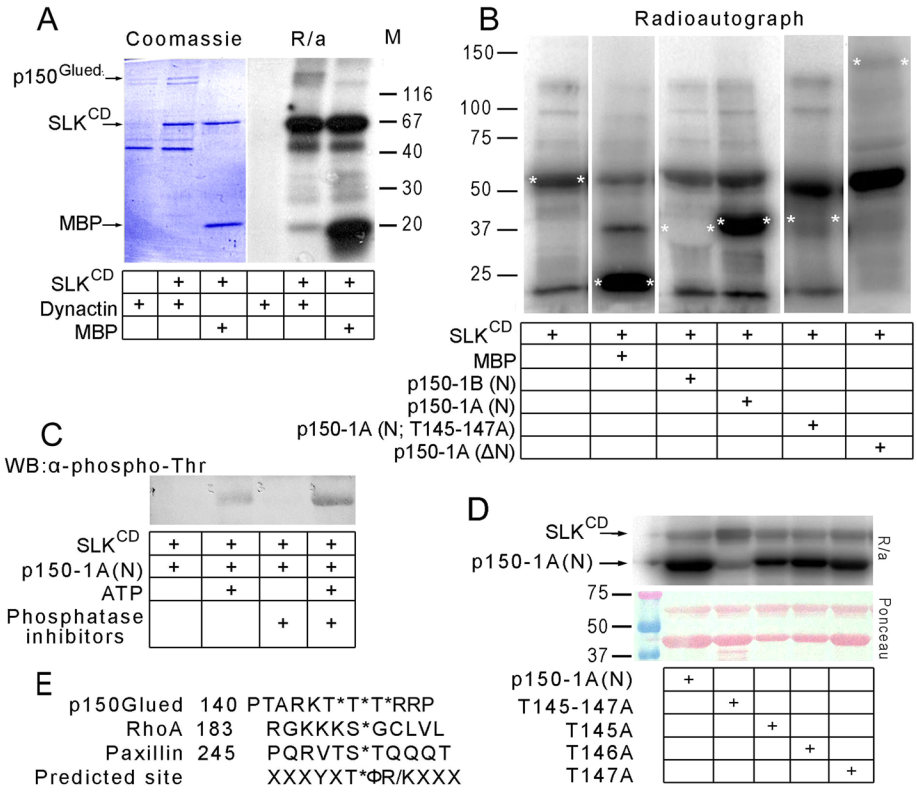


FIGURE 1: SLK (LOSK) phosphorylates dynactin in vitro. (A) A catalytic domain of SLK (SLK^{CD}) phosphorylates dynactin preparation from bovine brain. The ^{32}P label is incorporated into a 150-kDa band. Autophosphorylation of the kinase domain is also visible and is likely to cause label incorporation into the lower bands (a result of truncated protein synthesis in *E. coli* or partial protein degradation). Myelin basic protein is used as positive control. Radioautograph (R/a) and Coomassie staining of the same gel are represented. (B) Identification of the phosphorylation site. SLK^{CD} phosphorylates the N-terminal part of $p150^{Glued}$-1A (aa 1–157) but does not phosphorylate the N-terminal part of $p150^{Glued}$-1B (aa 1–137), ΔN-$p150^{Glued}$-1A (aa 151–1281), or T145-147A mutant of $p150^{Glued}$-1A (aa 1–157, T145-147A). Asterisks mark positions of recombinant proteins visualized by Ponceau staining before radioautography (Ponceau is not shown). (C) In vitro kinase reaction with GST-$p150^{Glued}$-1A N-terminal fragment in the presence and absence of cold ATP. Western blot is stained with antiphosphothreonine antibodies. The reaction is inhibited in the absence of phosphatase inhibitors. (D) Threonines in all three positions are phosphorylated by SLK. In vitro kinase assay with the GST-fused N-terminal parts of wild-type $p150^{Glued}$-1A and mutants (T145-147A, T145A, T146A, and T147A). ^{32}P is incorporated in all three threonines (145–147; (lanes 3–5). Radioautograph and Ponceau-stained membrane. Kinase domain and all fragments of $p150^{Glued}$ shown were synthesized in *E. coli* as GST fusions and purified on glutathione-agarose. (E) Phosphorylation sites for SLK in human RhoA, paxillin, and the identified site in $p150^{Glued}$-1A. Predicted site is represented according to Pike *et al.* (2008). Φ corresponds to aliphatic amino acid.

Burakov *et al.*, 2008a) but does disturb anchoring. Rapid disorganization of microtubules after the inhibition of dynein-dependent transport might result from the compromised delivery of anchoring proteins to the centrosome (Fumoto *et al.*, 2006). In case of dynactin inhibition, the centrosome still contains its major proteins—γ-tubulin, pericentrin, centrin, and ninein—but loses the ability to anchor microtubules (Burakov *et al.*, 2008a,b). These data indicate that the role of dynactin in microtubule organization is not limited to its transport function.

Dynactin is considered as a multisubunit cofactor of dynein, which ensures cargo binding and processivity of the motor protein (King and Schroer, 2000; Schroer, 2004). One of the subunits, $p150^{Glued}$, contains a MT-binding region (CAP-Gly and basic domains) and can interact with microtubules independently of dynein (Waterman-Storer *et al.*, 1995; Culver-Hanlon *et al.*, 2006; Weisbrich *et al.*, 2007). Therefore dynactin might contribute to MT organization by direct or indirect MT anchoring at the centrosome and/or regulation of dynein activity and dynein-mediated transport of other anchoring proteins to the centrosome. In either case, depletion of centrosomal dynactin results in inevitable disorganization of microtubules (Quintyne *et al.*, 1999; Kim *et al.*, 2007). Two subunits of the complex (p50 and $p150^{Glued}$) interact with centrosomal proteins (Cep135 and PCM1, respectively; Uetake *et al.*, 2004; Kodani *et al.*, 2010), but the regulation of dynactin localization at the centrosome is far from understood.

Previously we showed that depletion of centrosomal dynactin and randomization of microtubules were observed after the inhibition of the long Ste20-like kinase (LOSK; also called Ste20-like kinase [SLK]; Burakov *et al.*, 2008b). SLK (LOSK) is a Ser/Thr-protein kinase associated with the centrosome and microtubules (Zinovkina *et al.*, 1997, 1998; Itoh *et al.*, 1997; Sabourin and Rudnicki, 1999; Yamada *et al.*, 2000; Nadezhdina *et al.*, 2001). Its inhibition results in disorganization of microtubules and disturbed polarization and motility of cells (Burakov *et al.*, 2008b; Roovers *et al.*, 2009). SLK phosphorylates several cytoskeletal proteins: human RhoA (Guilluy *et al.*, 2008), paxillin (Quizi *et al.*, 2013), and, presumably, ezrin (Viswanatha *et al.*, 2012). None of them, however, is involved in centrosome-mediated microtubule organization. Given that inhibition of SLK results in a dramatic decrease of centrosomal dynactin (Burakov *et al.*, 2008b), we suggested that dynactin might mediate the effect of SLK on microtubules.

RESULTS

SLK (LOSK) phosphorylates $p150^{Glued}$

To test whether SLK can directly phosphorylate dynactin, we purified dynactin from bovine brain and incubated it with the recombinant kinase domain of SLK (SLK^{CD}) in the presence of ^{32}P-labeled ATP. The label was incorporated into a 150-kDa band (Figure 1A), also recognized by anti-$p150^{Glued}$ antibodies in Western blot (data not shown). We assume that it might have been a $p150^{Glued}$ subunit of the dynactin complex.

Dynactin phosphorylation was further studied with in vitro kinase assays using recombinant fragments of $p150^{Glued}$. Two isoforms of $p150^{Glued}$ (1A and 1B) with molecular weight 150 kDa were described previously (Dixit *et al.*, 2008; Zhapparova *et al.*, 2009). They vary in the length of the basic microtubule-binding region. We tested glutathione *S*-transferase–fused recombinant fragments of the N-terminal parts of $p150^{Glued}$-1A, with a full-length basic domain (amino acids [aa] 1–157), $p150^{Glued}$-1B, without the basic domain (aa 1–137), and the ΔN-$p150^{Glued}$ fragment (aa 151–1281) shared by the two isoforms. ^{32}P-phosphate was incorporated in only the N-terminal part of the 1A isoform (Figure 1B), indicating that the site of

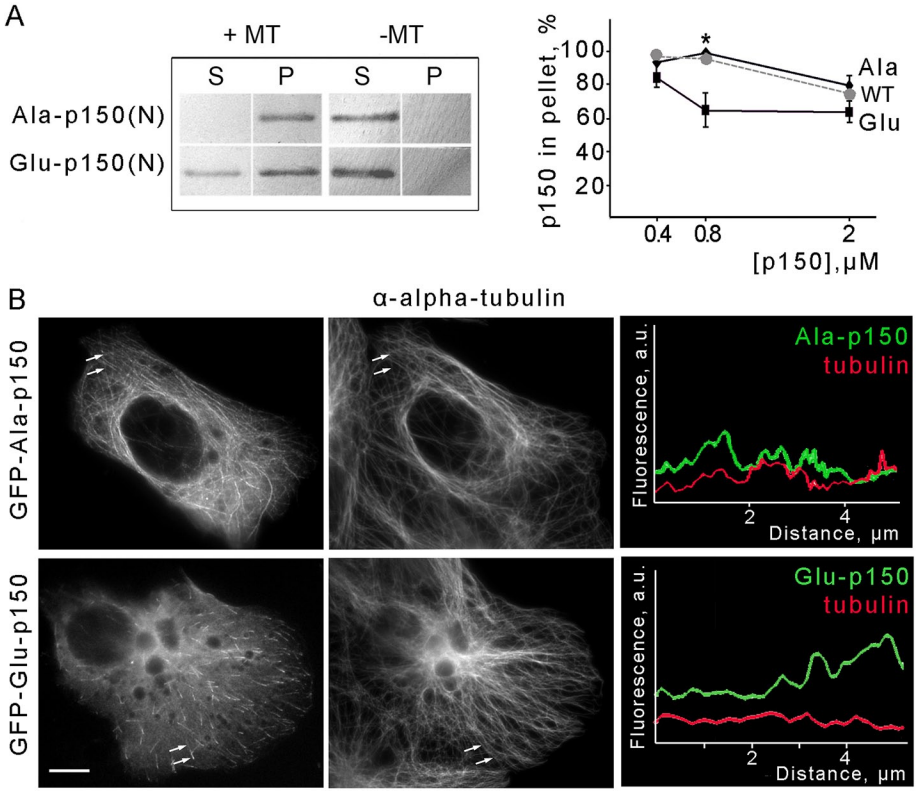


FIGURE 2: p150^{Glued} mutants vary in the affinity for microtubules in vitro and in vivo. (A) Co-pelleting of GST-fused N-terminal fragments (aa 1–157) of wild type (WT) and Ala- and Glu-p150^{Glued} with microtubules. Fragments of p150^{Glued} (0.4, 0.8, and 2 μM) were incubated with microtubules (16 μM) and pelleted through a 4 M glycerol cushion. In control (–MT), fragments of p150^{Glued} were precipitated under the same sedimentation conditions in the absence of microtubules. Protein content in supernatant (S) and pellet (P) was analyzed by Western blot with anti-p150^{Glued} antibodies. Relative content of p150^{Glued} in microtubule pellets. Error bars represent SD. *$p < 0.05$ for Ala- and Glu-p150^{Glued}. (B) Localization of full-length GFP-fused Ala- and Glu-p150^{Glued} in Vero cells. Microtubules were stained with anti-tubulin antibodies. Ala-p150^{Glued} decorates the entire microtubules, whereas Glu-p150^{Glued} is concentrated mostly at distal ends. Line scans of microtubules indicated with arrowheads. Scale bar, 10 μm.

phosphorylation was located somewhere within the variable region. Phosphorylation of the N-terminal part of p150^{Glued}-1A was also confirmed in a kinase assay with cold ATP and antiphosphothreonine antibodies (Figure 1C).

SLK belongs to the family of serine/threonine protein kinases. The variable region of p150^{Glued} does not contain serine residues but has four threonines at positions 141 and 145–147. We obtained two mutants in which threonines were substituted for alanine, T141A and T145-147A, and tested them in a kinase assay. The label was still incorporated into the T141A mutant (data not shown) but not in the T145-147A mutant (Figure 1B), indicating that the phosphorylation site was located within threonines 145–147. To define which of three threonines was phosphorylated, we obtained a series of mutants, T145A, T146A, and T147A, and performed a kinase assay (Figure 1D). Phosphate incorporated in all three mutants, demonstrating that each of three threonines could be phosphorylated (Figure 1D). Simultaneous phosphorylation of adjacent amino acids is a rare phenomenon, which was described for a few viral proteins (Scheidtmann *et al.*, 1982). It is intriguing whether SLK phosphorylates all three threonines or one or two of them and whether the phosphorylation status of dynactin varies under different conditions. We hope to elucidate this in further research. Here we studied the effect of dynactin phosphorylation using triple mutants of p150^{Glued} (T145-147A and T145-147E). The identified phosphorylation site in p150^{Glued} (140PTARKT*T*T*RRPKP) has common features with SLK sites in paxillin (Quizi *et al.*, 2013) and RhoA (Guilluy *et al.*, 2008; Figure 1E), although none of these sites coincides with the consensus site defined by peptide library analysis (X-X-X-**Y**-X-T*-Φ-**R/K**-X-X-X, where Φ is an aliphatic amino acid; Pike *et al.*, 2008; Figure 1E).

p150^{Glued} mutants vary in affinity for microtubules in vitro and in vivo

Because p150^{Glued} was phosphorylated at the MT-binding domain, we suggested that this phosphorylation might affect the interaction with microtubules. To test this, we performed a copelleting assay. GST-fused N-terminal fragments (1–157 aa) of Ala-p150^{Glued} (T145-147A) and Glu-p150^{Glued} (T145-147E) mutants were incubated with Taxol-stabilized microtubules and pelleted through a glycerol cushion (Figure 2A). The Glu mutant exhibited slightly reduced affinity for microtubules compared with the Ala mutant: at 0.8 μM concentration Ala-p150^{Glued} was found only in the microtubule pellet, whereas Glu-p150^{Glued} was also detected in the supernatant (Figure 2A). At 2 μM concentration, Ala-p150^{Glued} was found in the pellet and in the supernatant as well, indicating the saturation of microtubule binding. Wild-type p150^{Glued}-1A exhibited similar affinity for microtubules as Ala-p150^{Glued}: it bound microtubules tightly and was detected mostly in the pellet (Figure 2A and Supplemental Table S1; Zhapparova *et al.*, 2009).

Then we analyzed the interaction of mutants with microtubules in vivo. We expressed green fluorescent protein (GFP)–fused full-length p150^{Glued} mutants in Vero cells and analyzed their distribution along the microtubules (Figure 2B). Consistent with the in vitro results, the Ala mutant demonstrated higher affinity for microtubules and decorated them along the entire length (Figure 2B). The Glu mutant formed long comets at microtubule plus ends but rarely covered the entire microtubules. The localization pattern was independent of the level of recombinant protein expression (data not shown).

The Glu-mutant of p150^{Glued} exhibits higher affinity for the centrosome

The activity of protein kinase SLK and the presence of dynein–dynactin complex at the centrosome are required for the maintenance of microtubule radial array (Quintyne *et al.*, 1999; Burakov *et al.*, 2008b). Considering p150^{Glued} as a substrate of SLK, we studied its interaction with the centrosome regarding phosphorylation status. We expressed GFP-fused full-length Ala- and Glu-p150^{Glued} mutants in Vero cells and visualized the centrosome with anti–γ-tubulin antibodies (Figure 3A). It is striking that Ala-p150^{Glued}, which exhibited higher affinity for microtubules, was located at the

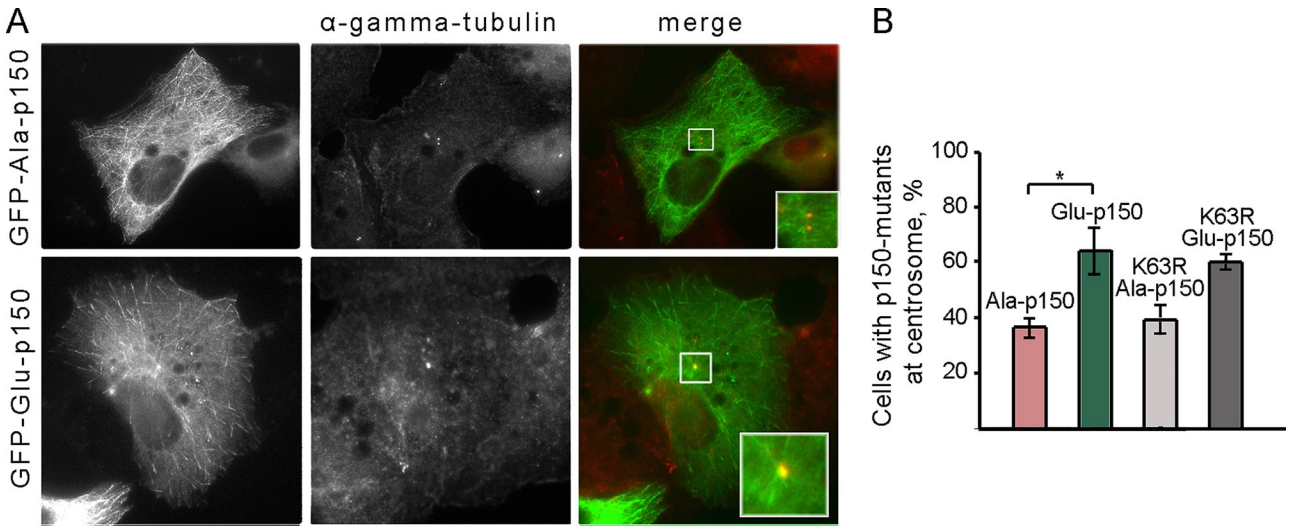


FIGURE 3: Glu-p150^{Glued} exhibits higher affinity for the centrosome compared with Ala-p150^{Glued}. (A) Expression of full-length GFP-fused Ala- and Glu-p150^{Glued} in Vero cells and immunostaining of fixed cells with anti–γ-tubulin antibodies to visualize centrosome. Boxed areas are shown at higher magnification. (B) Quantification of cells with centrosomal localization of p150^{Glued} mutants. Ala, a full-length GFP-fused Ala-p150^{Glued} (T145-147A; $n = 349$); Glu, a full-length GFP-fused Glu-p150^{Glued} (T145-147E; $n = 420$); K63R/Ala, cotransfection of K63R (dsRed-fused dominant-negative mutant of SLK^{CD}; aa 1–342) and Ala-p150^{Glued} ($n = 74$); and K63R/Glu, cotransfection of K63R and Glu-p150^{Glued} ($n = 60$). Experiments were performed in at least three repeats; *n*, total number of cells analyzed in all experiments. Error bars, SD; *$p < 0.05$ (*t* test).

centrosome in only 36% of cells. At the same time, Glu-p150^{Glued} was concentrated at the centrosome in 64% of cells, indicating that centrosomal localization of p150^{Glued} was regulated by SLK-dependent phosphorylation (Figure 3, A and B).

The Glu mutant of p150^{Glued} restores radial organization of microtubules in cells with inhibited SLK (LOSK)

To determine the effect of p150^{Glued} phosphorylation on the organization of microtubules, we examined the system of microtubules in cells with inhibited SLK. As we showed previously, expression of dominant-negative SLK (K63R) disrupts radial organization of microtubules in 80% of cells and entails marked depletion of centrosomal dynactin (Burakov *et al.*, 2008b). Here we coexpressed dsRed-tagged dominant-negative SLK and GFP fusion of either Ala- or Glu-p150^{Glued}. Glu-p150^{Glued} markedly restored radial organization of microtubules in 53% of Vero cells, whereas Ala-p150^{Glued} had no effect, and only 22% of Ala-p150^{Glued} cells contained a radial array of microtubules (Figure 4, A and B). Note that the expression of full-length p150^{Glued} itself induces randomization of microtubules in 58% of cells (Supplemental Figure S1). The effect might be caused by coiled-coil 1 region of p150^{Glued}, which is responsible for dynein–dynactin interactions (Quintyne *et al.*, 1999). Therefore the results of coexpression should be compared with corresponding controls of individual Ala- and Glu-p150^{Glued} expression. Of note, Ala-p150^{Glued} itself caused randomization of microtubules in 70% of cells (Figure 4B), indicating that nonphosphorylatable p150^{Glued} could not maintain radial organization of microtubules.

In some cases, microtubule randomization in cells might result from the expression of microtubule-binding proteins, which stabilize microtubules. To test whether such an effect was occurring in our system, we expressed GFP-fused wild-type p150^{Glued}-1A and p150^{Glued}-1B. These isoforms vary in the length of microtubule-binding region and have different affinity for microtubules (Zhapparova *et al.*, 2009). The 1A isoform, similarly to Ala-p150^{Glued}, had higher affinity for microtubules but did not cause such dramatic randomization of microtubules as the mutant protein (Supplemental Table and Supplemental Figure S1). These data indicate that binding of dynactin to microtubules is not enough for their randomization.

SLK (LOSK) regulates microtubule organization by targeting p150^{Glued} to the centrosome

The data raise a question about the exact function of dynactin phosphorylation. Phosphorylation 1) might be required only for targeting dynactin to the centrosome or 2) can affect interactions with microtubules and putative mediator proteins. If the first assumption is true, artificial targeting of nonphosphorylatable p150^{Glued} to the centrosome would restore microtubule array in cells with inhibited SLK.

To verify the hypothesis, we fused Ala-p150^{Glued} to the PACT domain of AKAP450 protein. The PACT domain is responsible for centrosomal localization of AKAP450 and pericentrin, and its fusion to GFP results in clear centrosomal localization of the latter (Gillingham and Munro, 2000). We expressed full-length GFP-fused Ala-p150^{Glued} carrying the PACT domain at the N- or C-terminus (Figure 5A). Both proteins accumulated at the centrosome of >90% cells (compare to 34% cells in the case of Ala-p150^{Glued} without PACT domain; Figure 5A). Then we coexpressed the described constructs and dominant-negative SLK and examined microtubule organization (Figure 5B). PACT-Ala-p150^{Glued} restored microtubule array in 47% cells (compare to 50 and 23% in the case of Glu-p150^{Glued} and Ala-p150^{Glued}, respectively; Figure 4B). Expression of GFP-fused PACT had no effect on microtubule organization (data not shown). The obtained data indicate that phosphorylation itself is required only for dynactin targeting to the centrosome and not for its interactions with microtubules or microtubule-interacting proteins there.

We obtained unexpected results with Ala-p150 carrying the PACT domain at the C-terminus (Figure 5, A and B). It was unable to restore a microtubule array in cells with inhibited SLK, although the

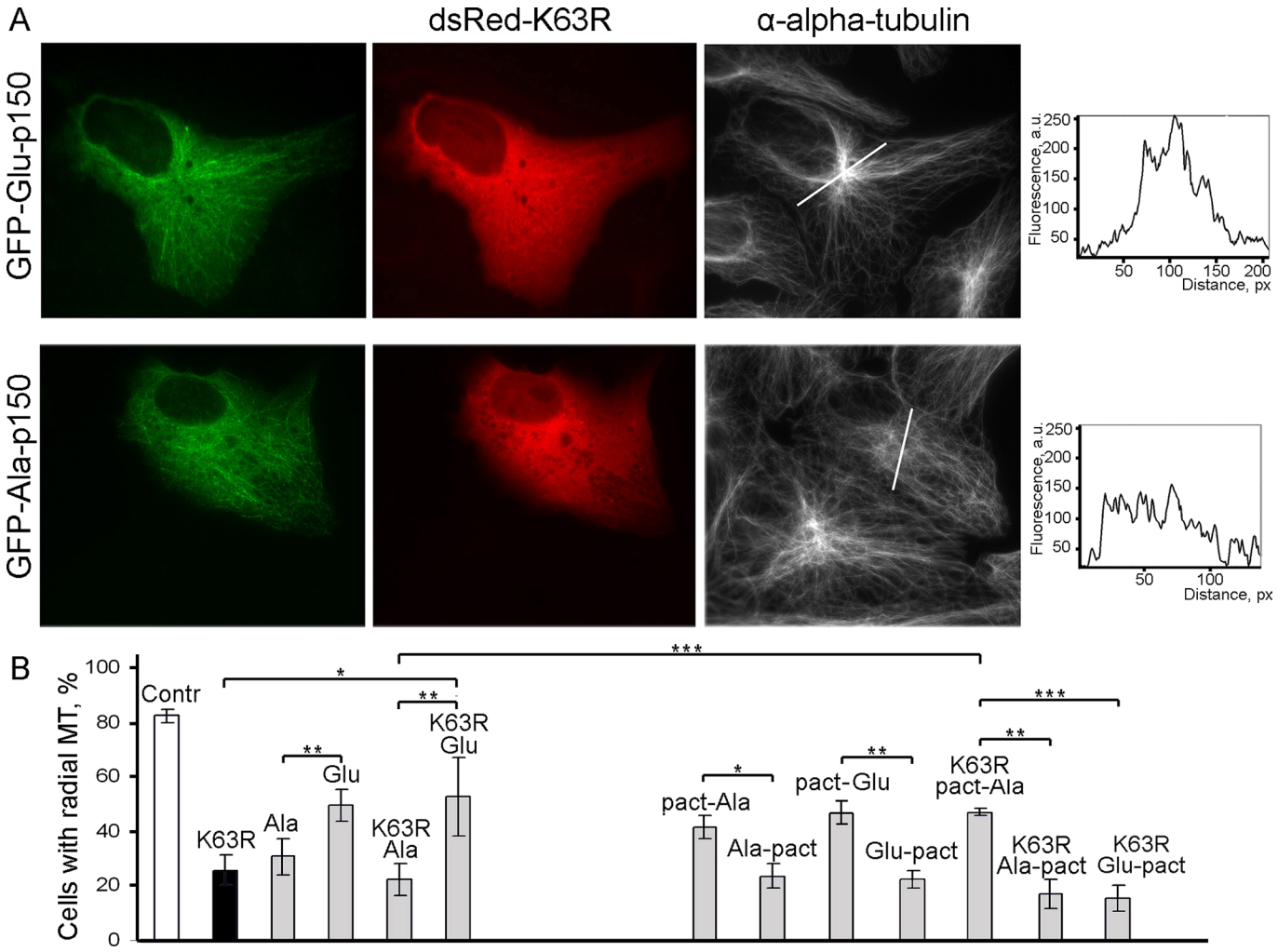


FIGURE 4: Glu-mutant of p150^{Glued} restores radial organization of microtubules in cells with inhibited SLK. (A) Coexpression of dsRed-fused, dominant-negative kinase domain of SLK (K63R-ΔT) and full-length GFP-fused Ala- or Glu-p150^{Glued} in Vero cells and immunostaining of fixed cells with anti-tubulin antibodies. Expression of Glu-p150^{Glued} restores radial microtubule array in cells with inhibited SLK, whereas expression of Ala-p150^{Glued} enhances microtubule randomization in such cells. Microtubule organization was estimated by measuring fluorescence intensity along lines drawn through the cell's center. Scans of fluorescence intensity along such lines are shown. (B) Quantification of cells with radial microtubule array. Contr, control nontransfected cells ($n = 568$); K63R, dsRed-fused dominant-negative mutant of SLK (aa 1–342; $n = 948$); Ala, full-length GFP-fused Ala-p150^{Glued} (T145-147A; $n = 389$); Glu, full-length GFP-fused Glu-p150^{Glued} (T145-147E; $n = 225$); K63R/Ala, cotransfection of K63R and Ala-p150^{Glued} ($n = 400$); K63R/Glu, cotransfection of K63R and Glu-p150^{Glued} ($n = 862$); pact-Ala, PACT-GFP-Ala-p150^{Glued} ($n = 104$); Ala-pact, GFP-Ala-p150^{Glued}-PACT ($n = 77$); pact-Glu, PACT-GFP-Glu-p150^{Glued} ($n = 68$); Glu-pact, GFP-Glu-p150^{Glued}-PACT ($n = 273$); K63R/pact-Ala, cotransfection of K63R and PACT-GFP-Ala-p150^{Glued} ($n = 374$); K63R/Ala-pact, cotransfection of K63R and GFP-Ala-p150^{Glued}-PACT ($n = 179$); K63R/Glu-pact, cotransfection of K63R and GFP-Glu-p150^{Glued}-PACT ($n = 273$). Experiments were performed in at least three repeats; *n*, total number of cells analyzed in all experiments. Error bars, SD; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (*t* test).

protein exhibited clear centrosomal localization (Figures 5A and 4B). Moreover, microtubules were disorganized in 80% of cells expressing the Ala-p150-PACT chimera, indicating either involvement of p150^{Glued} C-tail in the interaction with some proteins or the importance of proper orientation of dynactin in the pericentriolar matrix.

To verify the importance of C-terminal interactions for microtubule organization, we expressed Glu-p150^{Glued} fused to the PACT domain at the C-terminus. As we showed in previous experiments, expression of Glu-p150^{Glued} successfully restored microtubule array in cells with inhibited SLK. Expression of Glu-p150^{Glued}-PACT, however, caused microtubule randomization and did not restore the radial array in cells with inhibited SLK (Figures 5B and 4B).

In cells, p150^{Glued} functions as a part of dynactin complex, and inhibition of SLK results in the release of all dynactin subunits from the centrosome (Burakov *et al.*, 2008). Here we demonstrated that phosphorylation of p150^{Glued} is required for its centrosomal localization and for the maintenance of microtubule radial array. Next we studied whether other dynactin subunits were implicated in this process. The ability of p150^{Glued} mutants to interact with other dynactin subunits was verified by immunoprecipitation (Supplemental Figure S2). Then we immunostained cells expressing dominant-negative SLK and Glu-p150^{Glued} for the dynamitin (p50) subunit. Dynamitin was recruited to the centrosome in only half of cells with centrosomal localization of Glu-p150^{Glued} (data not

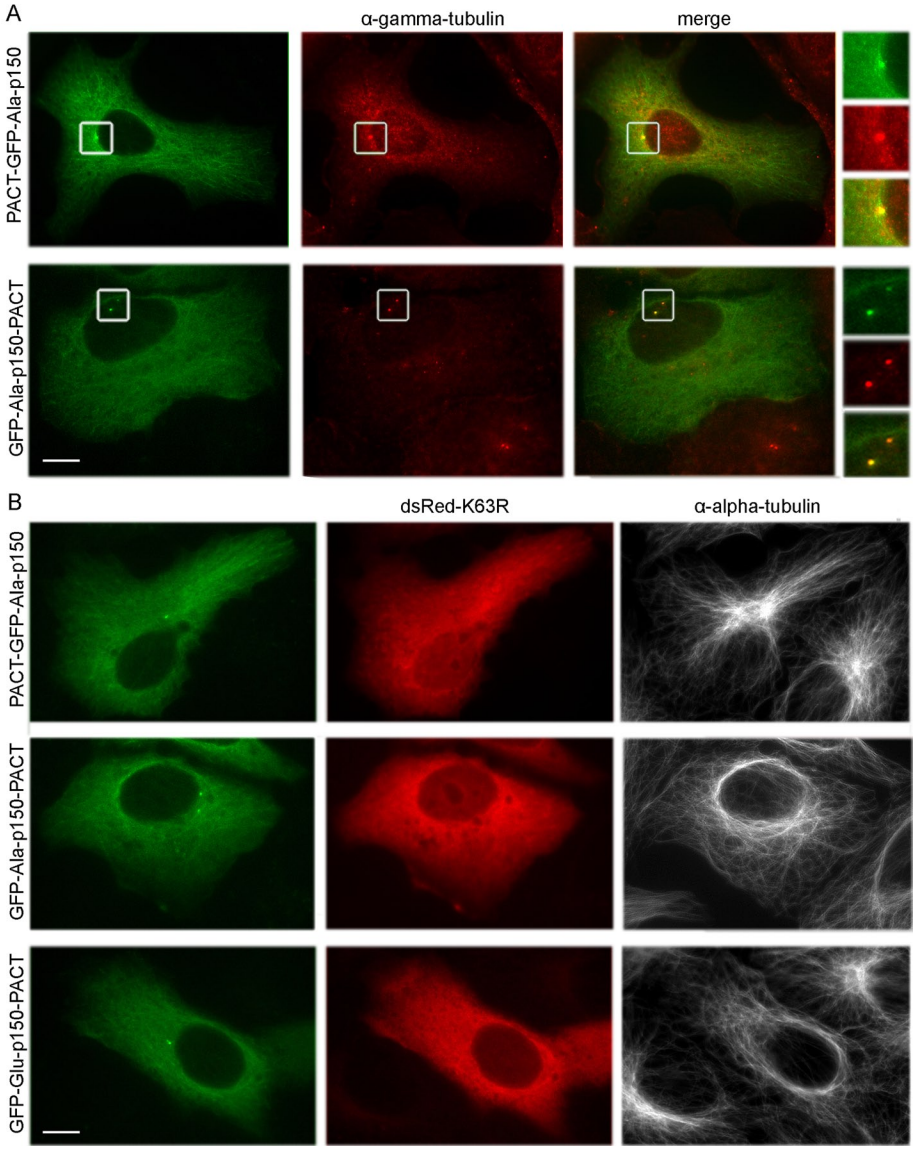


FIGURE 5: SLK (LOSK) regulates microtubule organization by targeting p150^{Glued} to the centrosome. (A) Expression of PACT fusions in Vero cells and immunostaining of fixed cells with anti–γ-tubulin antibodies to visualize centrosome. Boxed areas are shown at higher magnification. PACT at either end of Ala-p150^{Glued} ensures centrosomal localization of the protein in >90% of cells. (B) Coexpression of dsRed-fused, dominant-negative kinase domain of SLK (K63R) and p150^{Glued} mutants fused with PACT domain and immunostaining of fixed cells with anti-tubulin antibodies. PACT at the N-terminus of Ala-p150^{Glued} rescues radial microtubule array in cells with inhibited SLK. PACT at the C-terminus of Ala-p150^{Glued} or Glu-p150^{Glued} does not restore radial microtubule array in cells with inhibited SLK.

shown). The absence of other dynactin subunits seems to have no effect on microtubule radial organization once Glu-p150^{Glued} is located at the centrosome.

Glu-p150^{Glued} rescues Golgi reorientation in polarized cells with inhibited SLK (LOSK)

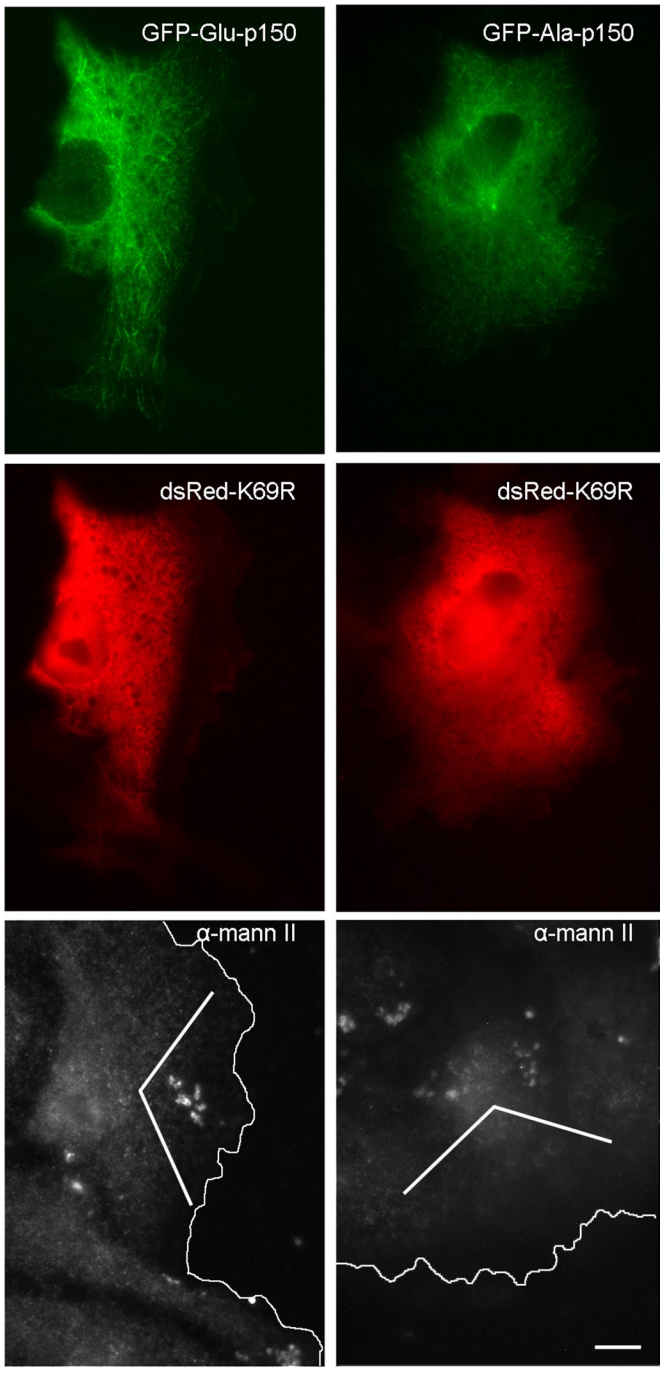
Together with the maintenance of microtubule array, SLK was shown to be required for Golgi reorientation in polarized cells (Burakov *et al.*, 2008b). We examined whether this effect depended on dynactin phosphorylation. Polarized cells were obtained at the edge of an experimental wound scratched in a monolayer of Vero cells. Normally, compact Golgi cisternae localize in the perinuclear region, and during polarization they are translocated toward the leading edge. Inhibition of SLK results in Golgi mislocalization in 80% of cells (Burakov *et al.*, 2008b; Figure 6, A and B). Coexpression of dominant-negative SLK and Glu-p150^{Glued} restored Golgi orientation in 60% of cells. The observed orientation of Golgi was independent of microtubule organization: only 33% of cells expressing Ala-p150^{Glued} had radial array of microtubules (Figure 4B), but >60% of such cells contained normally oriented Golgi. The p150^{Glued} mutants themselves had almost no effect on Golgi orientation.

Together with reoriented Golgi, polarized migrating fibroblast-like cells usually have their centrosomes translocated toward the leading edge (Euteneuer and Schliwa, 1992). Such localization facilitates microtubule growth toward the leading edge. We examined centrosome positioning in cells coexpressing dominant-negative SLK and a p150^{Glued} mutant. In all cases the centrosome was oriented as in the nontransfected control (data not shown). The result indicates that Golgi and the centrosome, which usually migrate together, are uncoupled in cells with inhibited SLK.

DISCUSSION

Here we unraveled a new regulatory mechanism of microtubule anchoring at the centrosome. We demonstrated that the protein kinase SLK (LOSK), required for the maintenance of microtubule array, phosphorylates the p150^{Glued} subunit of the dynactin complex. A phosphomimicking mutant of p150^{Glued} exhibits high affinity for the centrosome and restores microtubule array in cells with inhibited SLK. This mutant also restores Golgi polarization in cells with inhibited SLK at the edge of an experimental wound.

Phosphorylation status of p150^{Glued} depends on cell signaling pathways (Farshori and Holzbaur, 1997). Although Farshori and Holzbaur (1997) detected only phosphoserine residues in the phosphorylated dynactin, p150^{Glued} phosphorylated at threonines is associated with the centrosome and thus might comprise a minor fraction of the whole dynactin pool. Phosphorylation at Ser-19 (presumably by cAMP-dependent protein kinase) regulates p150^{Glued} interaction with microtubules (Vaughan *et al.*, 2002). In prophase mammalian cells, polo-like kinase Plk1 phosphorylates p150^{Glued} at Ser-179 located distally to the basic domain (Li *et al.*, 2010) and thus regulates nuclear envelope breakdown. In mitotic *Drosophila* cells, the dynamics of p150^{Glued} is regulated by Aurora A, which phosphorylates serines in the N-terminal microtubule-binding domain (Romé *et al.*, 2010). Note that *Drosophila* p150^{Glued} contains a short basic domain, which lacks the variable region with threonines (Zhapparova *et al.*, 2009).



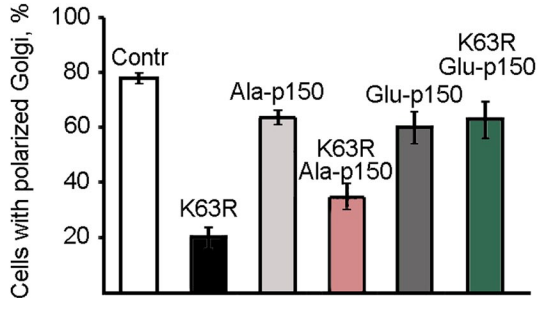


FIGURE 6: Glu-p150^{Glued} rescues Golgi reorientation in polarized cells with inhibited SLK (LOSK). (A) Coexpression of dsRed-fused, dominant-negative kinase domain of SLK (K63R) and GFP-fused Ala- or Glu-p150^{Glued} in polarized Vero cells and immunostaining of fixed cells with anti–mannosidase II antibodies to visualize the Golgi. Polarized cells were obtained at the edge of an experimental wound in a monolayer of Vero cells. Expression of Glu-p150^{Glued} restores normal reorientation of the Golgi toward the leading edge of the cell with inhibited SLK. (B) Quantification of cells with polarized Golgi. Contr, control nontransfected cells (n = 157); K63R, dsRed-fused, dominant-negative mutant of SLK (aa 1–342; n = 75); Ala, full-length GFP-fused Ala-p150^{Glued} (T145-147A; n = 66); Glu, full-length GFP-fused Glu-p150^{Glued} (T145-147E; n = 70); K63R/Ala, cotransfection of K63R and Ala-p150^{Glued} (n = 69); K63R/Glu, cotransfection of K63R and Glu-p150^{Glued} (n = 81). Experiments were performed in at least three repeats; n, total number of cells analyzed in all experiments. Error bars, SD. ***$p < 0.001$ (t test).

Dynactin can fulfill two possible functions: 1) deliver microtubule-anchoring proteins to the centrosome within the dynein–dynactin complex (transport function) and 2) anchor microtubules directly via its MT-binding subunit p150^{Glued} or indirectly via some dynactin-binding proteins or even dynein (anchoring function). SLK does not affect the transport function of dynactin, and the major proteins involved in microtubule anchoring retain their centrosomal localization—γ-tubulin, pericentrin, ninein (Burakov *et al.*, 2008b), and EB1 (data not shown). Our results, however, indicate that dynactin phosphorylation is definitely required for its microtubule-anchoring function. It seems that the cell contains at least two pools of dynactin with distinct functions. Such functional diversity might be explained by structural differences of p150^{Glued} isoforms that vary in the microtubule-binding region (Dixit *et al.*, 2008; Zhapparova *et al.*, 2009). Fibroblasts and other nonneuronal cells express mostly p150^{Glued}-1B, which lacks a 20-aa region in the basic MT-binding domain. A full-length 1A isoform is expressed in such cells in trace amounts and is located mostly in the centrosomal area, where its functions are unknown (Zhapparova *et al.*, 2009). The fact that the phosphorylation site is within the variable region of two isoforms suggests that they might fulfill different functions in cells. The dominant isoform (p150^{Glued}-1B) might be responsible for intracellular transport, and the minor one (1A) might be implicated in microtubule organization.

The precise role of dynactin in microtubule anchoring at the centrosome is unknown: dynactin can directly hold microtubules or engage mediator proteins. We demonstrate here that phosphorylation is essential for targeting p150^{Glued} to the centrosome, although it does not affect centrosomal function. It seems that SLK phosphorylates dynactin and recruits it to the centrosome, where the increased amount of dynactin anchors more microtubules and maintains microtubule radial organization more efficiently. Centrosomal proteins that interact with the phosphorylated basic domain of p150^{Glued} are unknown, and further experiments are required for their identification.

Experiments with overexpression of Glu-p150^{Glued} suggest that centrosomal localization of other dynactin subunits is not essential for microtubule radial organization when p150^{Glued} is targeted to the centrosome. In vivo, however, the integrity of the dynactin complex might be required for its transport to the centrosome and its anchoring there. The mechanism of dynactin retention at the centrosome is debatable. Until recently, a "shoulder" part of dynactin was considered to be responsible for binding to the centrosome. Dynamitin (p50) was shown to interact with centrosomal protein Cep135 (Uetake *et al.*, 2004), and separation of "shoulder" and "arm" induced by overexpression of the p24 subunit resulted in the retention of Arp1 filament and release of p150^{Glued} from the centrosome (Quintyne *et al.*, 1999). Recent data (Kodani *et al.*, 2010) indicate

that p150^{Glued} interacts with PCM-1 and thus can bind the centrosome. Alternatively, p150^{Glued} might have some other partners at the centrosome besides PCM-1.

Although p150^{Glued} can directly interact with microtubules, our experiments masking the C-terminus of p150^{Glued} with the PACT domain indicate that dynactin requires some additional proteins to hold a microtubule at the centrosome. The C-tail of p150^{Glued} interacts with cargo proteins during vesicular transport: Rab7-interacting lysosomal protein (Johansson *et al.,* 2007), transport protein particle TRAPPC9 (Zong *et al.,* 2012), and COPII proteins Sec23/24 (Watson *et al.,* 2005). It is unlikely, however, that these proteins are involved in microtubule organization at the centrosome. The C-terminus of p150^{Glued} also might be important for proper positioning of dynactin in a multicomponent and multilayer pericentriolar material (Lawo *et al.,* 2012).

Note that not only is phosphorylation of p150^{Glued} required for microtubule organization, but it is also involved in cell polarization. In nonneuronal cells the 1A isoform of p150^{Glued} usually accumulates at the centrosome, whereas during cell polarization it binds to microtubules directed toward the leading edge (Zhapparova *et al.,* 2009). Our results demonstrate that SLK-dependent phosphorylation is required for proper orientation of the Golgi in polarized cells. The observed phenomenon might be explained by the fact that formation of the Golgi and its positioning in polarized cells depend on microtubules that grow from the centrosome (Vinogradova *et al.,* 2012). Cells with inhibited SLK activity contain few such microtubules. Alternatively, the amount of phosphorylated p150^{Glued} might be increased at microtubules directed toward the leading edge, since isoform 1A is actively recruited there during cell polarization (Zhapparova *et al.,* 2009). Phosphorylated dynactin might specifically interact with Golgi proteins and thus ensure reorientation of the organelle. We could not, however, detect any differences in the localization of phosphomimicking p150^{Glued} at microtubules in the leading and rear parts of the cell, which might result from high levels of protein expression.

Overall our data indicate that SLK regulates the organization of microtubules and cell polarization through phosphorylation of p150^{Glued}-1A, which does not affect the transport functions of dynactin.

MATERIALS AND METHODS

DNA constructs

Full-length human cDNA encoding p150^{Glued}-1B and mouse cDNA encoding p150^{Glued}-1A (described in Zhapparova *et al.,* 2009) were cloned into a pEGFP-C1 vector (Clontech, Mountain View, CA). N-terminal fragments encoding aa 1–157 of p150^{Glued}-1A, 1–137 of p150^{Glued}-1B, and 151–1281 of a ΔN fragment of p150^{Glued}-1A were cloned into pGEX-4T3 (GE Healthcare, Piscataway, NJ). High-fidelity PCR Enzyme Mix, restriction endonucleases, and other enzymes used for molecular cloning were from Fermentas (Vilnius, Lithuania). Primers were purchased from Syntol (Moscow, Russia). Site-directed mutagenesis was performed with a QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA). In GST-fused N-terminal fragments of p150^{Glued}-1A, each threonine in positions 145–147 was substituted for an alanine residue to obtain T145A, T146A, and T147A mutants; threonine in position 141 was substituted for alanine to obtain the T141A mutant; and all threonines in positions 145–147 were substituted for alanines to obtain the T145-147A mutant. In GFP-fused full-length p150^{Glued}-1A, threonines in positions 145–147 were simultaneously substituted for three alanine or three glutamate residues to obtain Ala-p150^{Glued} and Glu-p150^{Glued} mutants, respectively. GFP-fused catalytic domain of SLK (SLK^{CD}, aa 1–342) and a dominant-negative mutant (K63R, aa 1-342) were described in Burakov *et al.* (2008b). Dominant-negative mutant fused to dsRed was obtained by subcloning into a dsRed-C1 vector (Clontech).

For cloning of the PACT domain, total RNA was isolated from cultured HeLa cells using an RNeasy Kit (Qiagen, Hilden, Germany). First-strand cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen) and random hexanucleotide primers (Syntol). PACT domain (aa 3702–3789) of AKAP450 (AJ131693.1) was amplified using the primers 5′- TATGGTAAATACTTGAGGGCAGAAAG-3′ and 5′-TGACTCGATGCCACCGTCGAAC-3′. The obtained PACT-domain DNA was amplified with corresponding primers and subcloned into pEGFPC1 vector at *Eco*RI/*Sal*I sites (GFP-PACT). PACT-domain DNA was subcloned into pEGFPC1 vector carrying Ala-p150^{Glued} either upstream of GFP (at *Age*I site, PACT-Ala-p150^{Glued}) or downstream of Ala-p150^{Glued} (Ala-p150^{Glued}-PACT). Glu-p150^{Glued}-PACT was obtained as a result of PACT subcloning into pEGFPC1 vector carrying Glu-p150^{Glued} downstream of dynactin. All constructs were verified with automated sequencing, and the length of corresponding protein products was confirmed with SDS–PAGE and Western blot.

Mammalian cell growth, transfection, fixation, immunostaining, and wound-healing assay

Green monkey kidney Vero epithelia-like cells were cultured in DMEM/F12 (1:1) medium (Paneco, Moscow, Russia) supplemented with 7.5% fetal bovine serum (Paneco) at 37°C and 5% CO_2.

Transfection was performed using TransIT-LT1 reagent (Mirus Bio, Madison WI) or Lipofectamine 2000 (Invitrogen, Carlsbad, CA).

For immunostaining, cells were fixed with methanol for 10 min at –20°C, followed by 3% paraformaldehyde for 20 min at 4°C. The following antibodies were used: mouse monoclonal antibody to p150^{Glued} (610473; BD Biosciences, Franklin Lakes, NJ), p50/dynamitin (611002; BD Biosciences), tubulin DM-1A (Sigma-Aldrich, St. Louis, MO); rat monoclonal anti–tubulin YOL1/34 antibody (Abcam, Cambridge, United Kingdom); and rabbit polyclonal antibodies to mannosidase II (12277; Abcam), phosphothreonine (71-8200; Invitrogen), and γ-tubulin (kindly provided by R. Uzbekov, Lomonosov Moscow State University, Russia). Fluorochrome-conjugated (fluorescein isothiocyanate, tetramethylrhodamine isothiocyanate, pentamethincyanine) secondary antibodies (MultiLabeling type) were obtained from Jackson ImmunoResearch Laboratories (Newmarket, United Kingdom). Images of immunostained cells were taken using a Carl Zeiss Axiovert 200M microscope supplied with a 12-bit AxioCamHR camera and AxioVision software (Carl Zeiss, Jena, Germany). The data were analyzed using ImageJ (National Institutes of Health, Bethesda, MD) and Excel software (Microsoft, Redmond, WA.

For wound-healing assay, Vero cells were grown to a monolayer, then scratched with a pipet tip, incubated at 37°C for 2 h, and fixed as described.

Tubulin purification and microtubule copelleting assay

Tubulin was isolated from rat brains as described in Zhapparova *et al.* (2009). For copelleting experiments, 6 mg/ml rat tubulin was incubated in BRB buffer (80 mM 1,4-piperazinediethanesulfonic acid [PIPES], pH 6.8, 1 mM $MgCl_2$, 1 mM ethylene glycol tetraacetic acid) with 1 mM GTP for 20 min at 37°C; then 2 μM Taxol (Sigma-Aldrich) was added. The mixture was incubated at 37°C for 15 min, after which the Taxol concentration was increased to 20 μM and the mixture was incubated at 37°C for another 15 min. We mixed 16 μM microtubules, 1 mM GTP, and 15 μM Taxol in BRB buffer with GST-dynactin fragments, incubated at 37°C for 30 min, and applied over a warm 4 M glycerol cushion with 1 mM GTP and 5 μM Taxol in BRB. Microtubules were pelleted in a TLS55 rotor (Beckman Coulter,

Brea, CA) at 50,000 rpm and 25°C for 30 min. Supernatants were collected and mixed with 4× sample buffer (SB), and cushions were washed three times with BRB and discarded. The pellets (mostly invisible) were resuspended in an equal volume of 2× SB.

Immunoprecipitation, recombinant protein production, SDS–PAGE, and Western blot analysis

For immunoprecipitation, human embryonic kidney HEK293T cells were transfected and harvested in PHEM buffer (50 mM PIPES, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES], 1 mM EDTA, 2 mM $MgSO_4$, pH 7.0) supplemented with 0.5% Nonidet P-40, 0.5% Triton X-100, and 0.25% sodium deoxycholate. After centrifugation (TLS55 rotor [Beckman Coulter], 32,000 rpm, 4°C, 20 min), supernatant was incubated with protein A–Sepharose (P3391; Sigma-Aldrich) or MabSelect-Sepharose (GE Healthcare) and antibodies for immunoprecipitation against p50/dynamitin (sc-135135; Santa Cruz Biotechnology, Santa Cruz, CA) or GFP (GMA0311; Protein Synthesis, Moscow, Russia) for 3 h at 4°C. The results were analyzed with Western blot using mouse monoclonal anti-$p150^{Glued}$ (610473; BD Biosciences), anti-dynamitin/p50 (611002; BD Biosciences), anti-GFP (AMA 0311; Protein Synthesis) antibodies and rabbit polyclonal anti-GFP (AB121; Evrogen, Moscow, Russia) and anti-ACTR1B (ab84809, Abcam) antibodies.

Fragments of GST-fused $p150^{Glued}$ and SLK^{CD} were expressed in *Escherichia coli* and purified using a glutathione-agarose column (Sigma-Aldrich) according to the manufacturer's instructions. Purified N-terminal fragments of $p150^{Glued}$ were used in microtubule copelleting and in vitro phosphorylation experiments. SDS–PAGE was performed in 7.5, 10, or 6–12% gradient gels. Nitrocellulose membranes (Bio-Rad, Hercules, CA) were blocked in Tris-buffered saline supplemented with 0.05% Tween-20 and 5% fat-free milk overnight and subsequently incubated with primary antibodies and goat anti-rabbit or anti-mouse immunoglobulin G antibodies conjugated to alkaline phosphatase (KPL, Gaithersburg, MD). The results were visualized with 5-bromo-4-chloro-3′-indolyphosphate/nitro-blue tetrazolium solution (KPL).

Preparation of dynactin isolated from bovine brain was kindly provided by S. Kuznetsov (University of Rostock, Rostock, Germany).

In vitro kinase assay

GST-fused SLK^{CD} and N-terminal fragments of $p150^{Glued}$ were expressed in *E. coli* and purified on glutathione-agarose. For in vitro kinase assay, 5 µg of kinase and dynactin fragments or 2.5 µg of myelin basic protein (Sigma-Aldrich) were mixed with γ-^{32}P-ATP (10 µCi; FEI Research Center, Obninsk, Russia) in kinase buffer (50 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 100 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol) and incubated at 25°C for 1 h. For the experiments with brain dynactin, 0.2 mM heparin was added to inhibit contaminant endogenous casein kinase activity in dynactin preparation. The results were analyzed with SDS–PAGE and autoradiography. In vitro kinase assay with cold ATP was performed with the same amount of proteins; the reaction was incubated overnight at 4°C. The results were analyzed with Western blot stained with antiphosphothreonine antibodies (Invitrogen).

ACKNOWLEDGMENTS

We are grateful to colleagues who provided reagents and especially thank Rustem Uzbekov and Sergei Kuznetsov. We thank Irina Tsimbarevich for help in experiments and Anton Burakov for fruitful discussions. Some preliminary experiments were performed by the late Olga Kovalenko. The work was financially supported by Russian Foundation of Basic Research Grants 11-04-01022, 12-04-31380, and 12-04-33178. N.V. was supported by RF Presidential Program in Support of Young Scientists Grant MK-5961.2012.4 and the Dmitry Zimin Dynasty Foundation.

REFERENCES

Albee AJ, Wiese C (2008). *Xenopus* TACC3/maskin is not required for microtubule stability but is required for anchoring microtubules at the centrosome. Mol Biol Cell 19, 3347–3356.

Anders A, Sawin KE (2011). Microtubule stabilization in vivo by nucleation-incompetent γ-tubulin complex. J Cell Sci 124, 1207–1213.

Burakov A, Kovalenko O, Semenova I, Zhapparova O, Nadezhdina E, Rodionov V (2008a). Cytoplasmic dynein is involved in the retention of microtubules at the centrosome in interphase cells. Traffic 4, 472–480.

Burakov A, Zhapparova O, Kovalenko O, Zinovkina L, Potekhina E, Shanina N, Weiss D, Kuznetsov S, Nadezhdina E (2008b). Ste20-related protein kinase LOSK (SLK) controls microtubule radial array in interphase. Mol Biol Cell 19, 1952–1961.

Casenghi M, Barr F, Nigg E (2005). Phosphorylation of Nlp by Plk1 negatively regulates its dynein-dynactin-dependent targeting to the centrosome. J Cell Sci 118, 5101–5108.

Culver-Hanlon T, Lex S, Stephens A, Quintyne N, King S (2006). A microtubule-binding domain in dynactin increases dynein processivity by skating along microtubules. Nat Cell Biol 8, 264–270.

Dammermann A, Merdes A (2002). Assembly of centrosomal proteins and microtubule organization depends on PCM-1. J Cell Biol 159, 255–266.

Delgehyr N, Silllibourne J, Bornens M (2005). Microtubule nucleation and anchoring at the centrosome are independent processes linked by ninein function. J Cell Sci 118, 1565–1575.

Dixit R, Levy J, Tokito M, Ligon L, Holzbaur E (2008). Regulation of dynactin through the differential expression of $p150^{Glued}$ isoforms. J Cell Biol 283, 33611–33619.

Euteneuer U, Schliwa M (1992). Mechanism of centrosome positioning during the wound response in BSC-1 cells. J Cell Biol 116, 1157–1166.

Farshori P, Holzbaur EL (1997). Dynactin phosphorylation is modulated in response to cellular effectors. Biochem Biophys Res Commun 232, 810–816.

Fumoto K, Hoogenraad CC, Kikuchi A (2006). GSK-3beta-regulated interaction of BICD with dynein is involved in microtubule anchorage at centrosome. EMBO J 25, 5670–5682.

Gillingham AK, Munro S (2000). The PACT domain, a conserved centrosomal targeting motif in the coiled-coil proteins AKAP450 and pericentrin. EMBO Rep 1, 524–529.

Guilluy C, Rolli-Derkinderen M, Loufrani L, Bourge A, Henrion D, Sabourin L, Loirand G, Pacaud P (2008). Ste20-related kinase SLK phosphorylates Ser188 of RhoA to induce vasodilation in response to angiotensin II type 2 receptor activation. Circ Res 102, 1265–1274.

Guo J, Yang Z, Song W, Chen Q, Wang F, Zhang Q, Zhu X (2006). Nudel contributes to microtubule anchoring at the mother centriole and is involved in both dynein-dependent and -independent centrosomal protein assembly. Mol Biol Cell 17, 680–689.

Ibi M *et al.* (2011). Trichoplein controls microtubule anchoring at the centrosome by binding to Odf2 and ninein. J Cell Sci 124, 857–864.

Itoh S, Kameda Y, Yamada E, Tsujikawa K, Mimura T, Kohama Y (1997). Molecular cloning and characterization of a novel putative STE20-like kinase in guinea pigs. Arch Biochem Biophys 340, 201–207.

Johansson M, Rocha N, Zwart W, Jordens I, Janssen L, Kuijl C, Olkkonen VM, Neefjes J (2007). Activation of endosomal dynein motors by stepwise assembly of Rab7-RILP-p150Glued, ORP1L, and the receptor betalll spectrin. J Cell Biol 176, 459–471.

Kim H, Ling S, Rogers G, Kural C, Selvin P, Rogers S, Gelfand V (2007). Microtubule binding by dynactin is required for microtubule organization but not cargo transport. J Cell Biol 176, 641–651.

King S, Schroer T (2000). Dynactin increases the processivity of the cytoplasmic dynein motor. Nat Cell Biol 2, 20–24.

Kodani A, Tonthat V, Wu B, Sutterlin C (2010). Par6 alpha interacts with the dynactin subunit $p150^{Glued}$ and is a critical regulator of centrosomal protein recruitment. Mol Biol Cell 21, 3376–3385.

Lawo S, Hasegan M, Gupta GD, Pelletier L (2012). Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material. Nat Cell Biol 14, 1148–1158.

Li H, Liu XS, Yang X, Song B, Wang Y, Liu X (2010). Polo-like kinase 1 phosphorylation of p150Glued facilitates nuclear envelope breakdown during prophase. Proc Natl Acad Sci USA 107, 14633–14638.

Mogensen M, Malik A, Piel M, Bouckson-Castaing V, Bornens M (2000). Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. J Cell Sci 113, 3013–3023.
Nadezhdina ES, Zinovkina LA, Fais D, Chentsov Iu S (2001). Spermatozoa of the loach *Misgurnus fossilis* as a test system for identification of new centrosome proteins [in Russian]. Ontogenez 32, 41–50.
Perez-Ferreiro C, Vernos I, Correas I (2004). Protein 4.1R regulates interphase microtubule organization at the centrosome. J Cell Sci 117, 6197–6206.
Pike AC, Rellos P, Niesen FH, Turnbull A, Oliver AW, Parker SA, Turk BE, Pearl LH, Knapp S (2008). Activation segment dimerization: a mechanism for kinase autophosphorylation of non-consensus sites. EMBO J 27, 704–714.
Quintyne NJ, Gill SR, Eckley DM, Crego CL, Compton DA, Schroer TA (1999). Dynactin is required for microtubule anchoring at centrosomes. J Cell Biol 147, 321–334.
Quizi JL, Baron K, Al-Zahrani KN, O'Reilly P, Sriram RK, Conway J, Laurin AA, Sabourin LA (2013). SLK-mediated phosphorylation of paxillin is required for focal adhesion turnover and cell migration. Oncogene 32, 4656–4663.
Romé P, Montembault E, Franck N, Pascal A, Glover DM, Giet R (2010). Aurora A contributes to p150(^{Glued}) phosphorylation and function during mitosis. J Cell Biol 189, 651–659.
Roovers K, Wagner S, Storbeck CJ, O'Reilly P, Lo V, Northey JJ, Chmielecki J, Muller WJ, Siegel PM, Sabourin LA (2009). The Ste20-like kinase SLK is required for ErbB2-driven breast cancer cell motility. Oncogene 28, 2839–2848.
Sabourin L, Rudnicki M (1999). Induction of apoptosis by SLK, a Ste20-like kinase. Oncogene 18, 7566–7575.
Scheidtmann KH, Echle B, Walter G (1982). Simian virus 40 large T antigen is phosphorylated at multiple sites clustered in two separate regions. J Virol 44, 116–133.
Schroer T (2004). Dynactin. Annu Rev Cell Dev Biol 20, 759–779.
Uetake Y, Terada Y, Matuliene J, Kuriyama R (2004). Interaction of Cep135 with a p50 dynactin subunit in mammalian centrosomes. Cell Motil Cytoskeleton 58, 53–66.
Vaughan PS, Miura P, Henderson M, Byrne B, Vaughan KT (2002). A role for regulated binding of p150(^{Glued}) to microtubule plus ends in organelle transport. J Cell Biol 158, 305–319.
Vinogradova T, Paul R, Grimaldi AD, Loncarek J, Miller PM, Yampolsky D, Magidson V, Khodjakov A, Mogilner A, Kaverina I (2012). Concerted effort of centrosomal and Golgi-derived microtubules is required for proper Golgi complex assembly but not for maintenance. Mol Biol Cell 23, 820–833.
Viswanatha R, Ohouo PY, Smolka MB, Bretscher A (2012). Local phosphocycling mediated by LOK/SLK restricts ezrin function to the apical aspect of epithelial cells. J Cell Biol 199, 969–984.
Waterman-Storer CM, Karki S, Holzbaur EL (1995). The p150^{Glued} component of the dynactin complex binds to both microtubules and the actin-related protein centractin (Arp-1). Proc Natl Acad Sci USA 92, 1634–1638.
Watson P, Forster R, Palmer KJ, Pepperkok R, Stephens DJ (2005). Coupling of ER exit to microtubules through direct interaction of COPII with dynactin. Nat Cell Biol 7, 48–55.
Weisbrich A, Honnappa S, Jaussi R, Okhrimenko O, Frey D, Jelesarov I, Akhmanova A, Steinmetz M (2007). Structure-function relationship of CAP-Gly domains. Nat Struct Mol Biol 14, 959–967.
Wiese C, Zheng Y (2000). A new function for the gamma-tubulin ring complex as a microtubule minus-end cap. Nat Cell Biol 2, 358–364.
Wiese C, Zheng Y (2006). Microtubule nucleation: gamma-tubulin and beyond. J Cell Sci 119, 4143–4153.
Yamada E, Tsujikawa K, Itoh S, Kameda Y, Kohama Y, Yamamoto H (2000). Molecular cloning and characterization of a novel human Ste20-like kinase, hSLK. Biochim Biophys Acta 1495, 250–262.
Yan X, Habedanck R, Nigg E (2006). A complex of two centrosomal proteins, CAP350 and FOP, cooperates with EB1 in microtubule anchoring. Mol Biol Cell 17, 634–644.
Zhapparova ON, Bryantseva SA, Dergunova LV, Raevskaya NM, Burakov AV, Bantysh OB, Shanina NA, Nadezhdina ES (2009). Dynactin subunit p150^{Glued} isoforms notable for differential interaction with microtubules. Traffic 10, 1635–1646.
Zhapparova ON, Burakov AV, Nadezhdina ES (2007). The centrosome keeps nucleating microtubules but looses the ability to anchor them after the inhibition of dynein-dynactin complex. Biochemistry (Mosc) 72, 1233–1240.
Zinovkina L, Poltaraus A, Solovyanova O, Nadezhdina E (1997). Chinese hamster protein homologous to human putative protein kinase KIAA0204 is associated with nuclei, microtubules and centrosomes in CHO-K1 cells. FEBS Lett 414, 135–139.
Zinovkina LA, Poltaraus AB, Solov'ianova OB, Nadezhdina ES (1998). A proposed new mammalian cell protein kinase, associated with microtubules. Mol Biol (Mosk) 32, 341–348.
Zong M, Satoh A, Yu MK, Siu KY, Ng WY, Chan HC, Tanner JA, Yu S (2012). TRAPPC9 mediates the interaction between p150 and COPII vesicles at the target membrane. PLoS One 7, e29995.