Oncogene (2008) 27, 3494–3500

www.nature.com/onc

SHORT COMMUNICATION

The Src-like adaptor protein regulates PDGF-induced actin dorsal ruffles in a c-Cbl-dependent manner

A Sirvent[1], C Leroy[1], A Boureux[2], V Simon and S Roche

CRBM, CNRS UMR5237, UMI and II, Montpellier, France

The Src-like adaptor protein (SLAP) belongs to the subfamily of adapter proteins that negatively regulate cellular signalling initiated by tyrosine kinases. SLAP has a unique, myristylated N-terminus, followed by SH3 and SH2 domains with high homology to Src family tyrosine kinases (SFK) and a unique C-terminal tail, which is important for c-Cbl binding. We have previously shown that SLAP negatively regulates platelet-derived growth factor (PDGF)-induced mitogenesis in fibroblasts and we now report that it regulates F-actin assembly for dorsal ruffles formation. c-Cbl mediated SLAP inhibition towards actin remodelling. Moreover, SLAP enhanced PDGF-induced c-Cbl phosphorylation by SFK. In contrast, SLAP mitogenic inhibition was not mediated by c-Cbl, but it rather involved a competitive mechanism with SFK for PDGF-receptor (PDGFR) association and mitogenic signalling. Accordingly, phosphorylation of the Src mitogenic substrates Stat3 and Shc were reduced by SLAP. Thus, we concluded that SLAP regulates PDGFR signalling by two independent mechanisms: a competitive mechanism for PDGF-induced Src mitogenic signalling and a non-competitive mechanism for dorsal ruffles formation mediated by c-Cbl.
Oncogene (2008) **27,** 3494–3500; doi:10.1038/sj.onc.1211011; published online 14 January 2008

Keywords: SLAP; Src; PDGF; Cbl; DNA synthesis; F-actin assembly

The Src-like adaptor protein (SLAP) is ubiquitously expressed at the mRNA level with a strong expression in spleen and lung (Pandey *et al*., 1995). Gene knockout in mice pointed to a crucial role for SLAP in thymocytes development (Sosinowski *et al*., 2001). The adapter and E3 ubiquitin ligase protein c-Cbl (Schmidt and Dikic, 2005) has been reported to bind to the SLAP C-terminus for negative regulation of T-cell antigen receptor signalling including ubiquitination and downregulation of the CD3 complex (Myers *et al*., 2006). We have previously reported that SLAP also negatively regulates mitogenesis induced by growth factors in fibroblasts including platelet-derived growth factor (PDGF) (Roche *et al*., 1998a). Further experimental data suggested that it regulates mitogenesis by inhibition of a Src-signalling pathway (Manes *et al*., 2000). Whether a SLAP–c-Cbl complex also regulates growth factor receptor signalling, however, is unknown.

In addition to mitogenesis, PDGF induces morphological changes leading to F-actin assembly for both lateral and dorsal/circular ruffles formation. While lateral ruffles mediate directional cell migration, dorsal ruffles were linked to cell invasion into the extracellular matrix (Suetsugu *et al*., 2003). This cytoskeletal rearrangement is regulated by cortical actin polymerization through the activation of the Arp2/3 complex and an Rac/WAVE1-specific pathway (Suetsugu *et al*., 2003). Ras and Rab5 pathways have also been documented (Lanzetti *et al*., 2004), and we have recently shown that this response implicates Src family tyrosine kinases (SFK). In addition, our results suggest that in contrast to Src mitogenic function, SFK signal transduction promoting actin assembly does not require receptor association (Veracini *et al*., 2006). Rather, it involves the lipid second messenger sphingosine-1-phosphate, which may activate SFK through a seven-transmembrane receptor of the EDG family and an associated heterotrimeric Gi protein.

SLAP negatively regulates PDGF-induced dorsal ruffles

Owing to its homology with SFK, we investigated the role of SLAP on actin remodelling. We found that 65% of NIH 3T3 cells exhibited at least one dorsal ruffle after 5 min of PDGF stimulation. Interestingly, SLAP reduced this response by 70% (Figure 1b). A structure–function analysis implicated both the SLAP SH2 and C-terminus. Next, the role of the endogenous protein was investigated. As SLAP is weakly expressed in NIH-3T3 cells, we used IMR90 human lung fibroblasts that express higher level of this adapter (not shown). PDGF induced dorsal ruffles in 30% of these cells, whereas and downregulation of SLAP level enhanced this response by two (Figures 1c–e). This potentiating effect was specific to dorsal ruffles as lateral ruffles were not

Correspondence: Dr S Roche, CRBM, CNRS UMR5237, 1919 route de Mende, Montpellier, Cedex 05 34293, France.
E-mail: serge.roche@crbm.cnrs.fr
[1]These authors contributed equally to this work
[2]Current address: IGH UPR1142-UMII, place Eugène Bataillon, Montpellier 34095, France.
Received 30 July 2007; revised 25 October 2007; accepted 4 December 2007; published online 14 January 2008

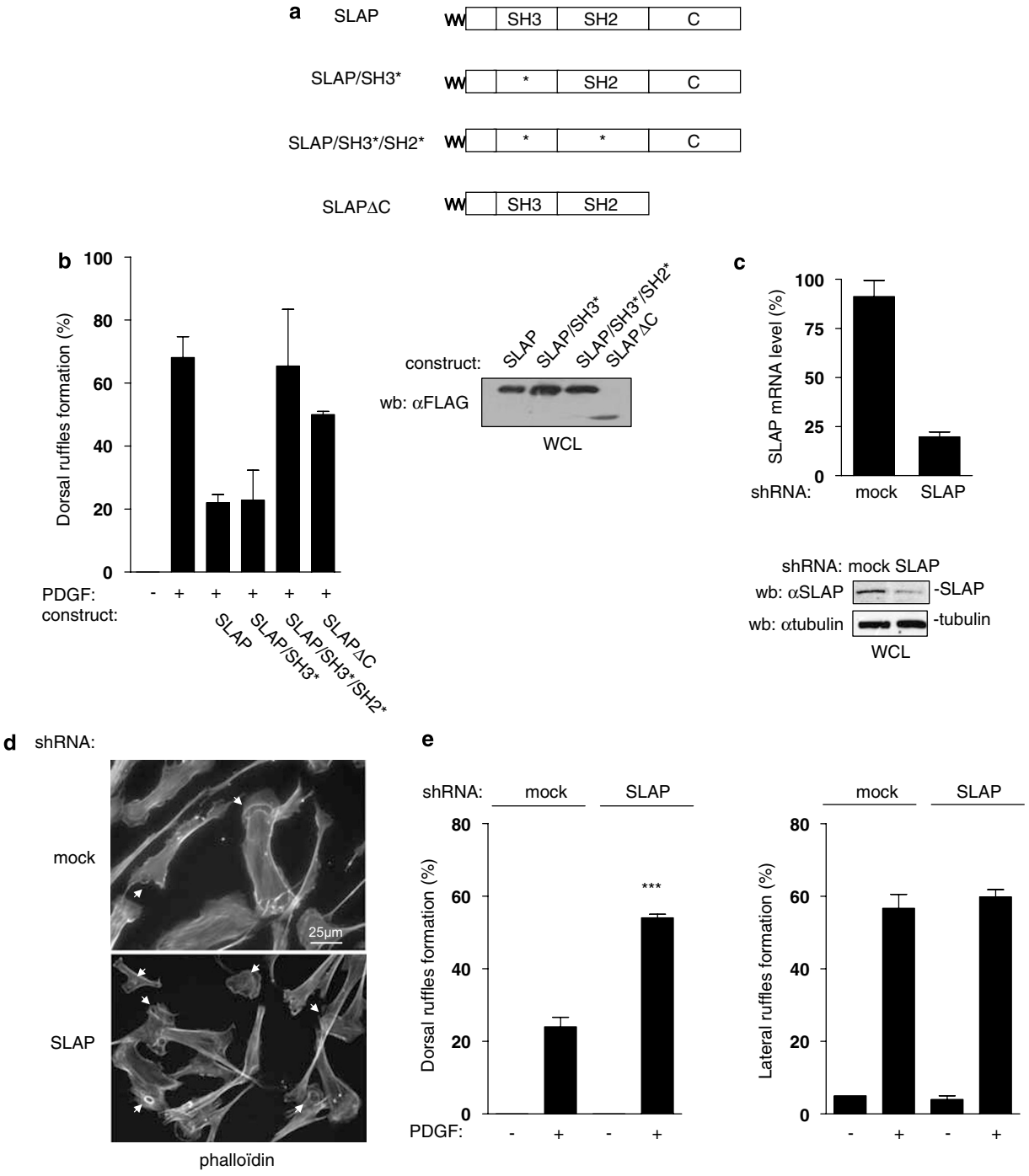

Figure 1 SLAP negatively regulates PDGF-induced dorsal ruffles. (**a**) SLAP constructs: (\/\/\/), myristylation; *, point mutations in the SH3 (W58A) or SH2 (R111L) domain (SH3* and SH2*); ΔC, C-terminal truncation. (**b**) SLAP inhibits PDGF-induced dorsal ruffles. Left panel: statistical analysis of dorsal ruffles formation in PDGF-stimulated cells expressing indicated SLAP constructs. Right panel: level of SLAP mutants. (**c**) SLAP mRNA and protein levels in IMR90 cells infected with retroviruses expressing shRNA specific to luciferase (mock) or SLAP sequence (GACCTGGTGAACCACTATT) and assessed by Q-PCR (forward CCGGAGG GACTGGATAGC and reverse ACAGCCAGCCATGGTAAAC primers) and western-blotting (sc-1215, Santa Cruz Biotechnology, Santa Cruz, CA, USA) from a whole-cell lysate. Tubulin level is also shown. (**d**) and (**e**) SLAP depletion potentiates PDGF-induced dorsal ruffles. An example (**d**) and statistical analysis (**e**) of ruffles formation in cells stimulated with PDGF or unstimulated cells that were transduced with indicated shRNA. Cells grown on coverslips were transfected or not with indicated construct, serum-starved and stimulated with PDGF–BB (Abcys) (20 ng ml^{-1}) for 5 min. Cells were fixed and preceded for actin staining using Rhodamin–phalloïdin and ectopic protein expression. Ruffles formation (%) = (number of ruffles-positive transfected cells) (number of transfected cells) × 100. The mean ± s.d. from 3–5 independent experiments is shown. SLAP constructs (Manes *et al*., 2000) were subcloned into pBABE. Cell culture, transfection, infection, immunofluorescence analysis, Q-PCR and biochemistry have been described by Veracini *et al*. (2006). White arrows indicate cells with dorsal ruffles. ***$P<0.001$ using a student's *t*-test. PDGF, platelet-derived growth factor; Q-PCR, quantitative-PCR; shRNA, short hairpin RNA; SLAP, Src-like adaptor protein.

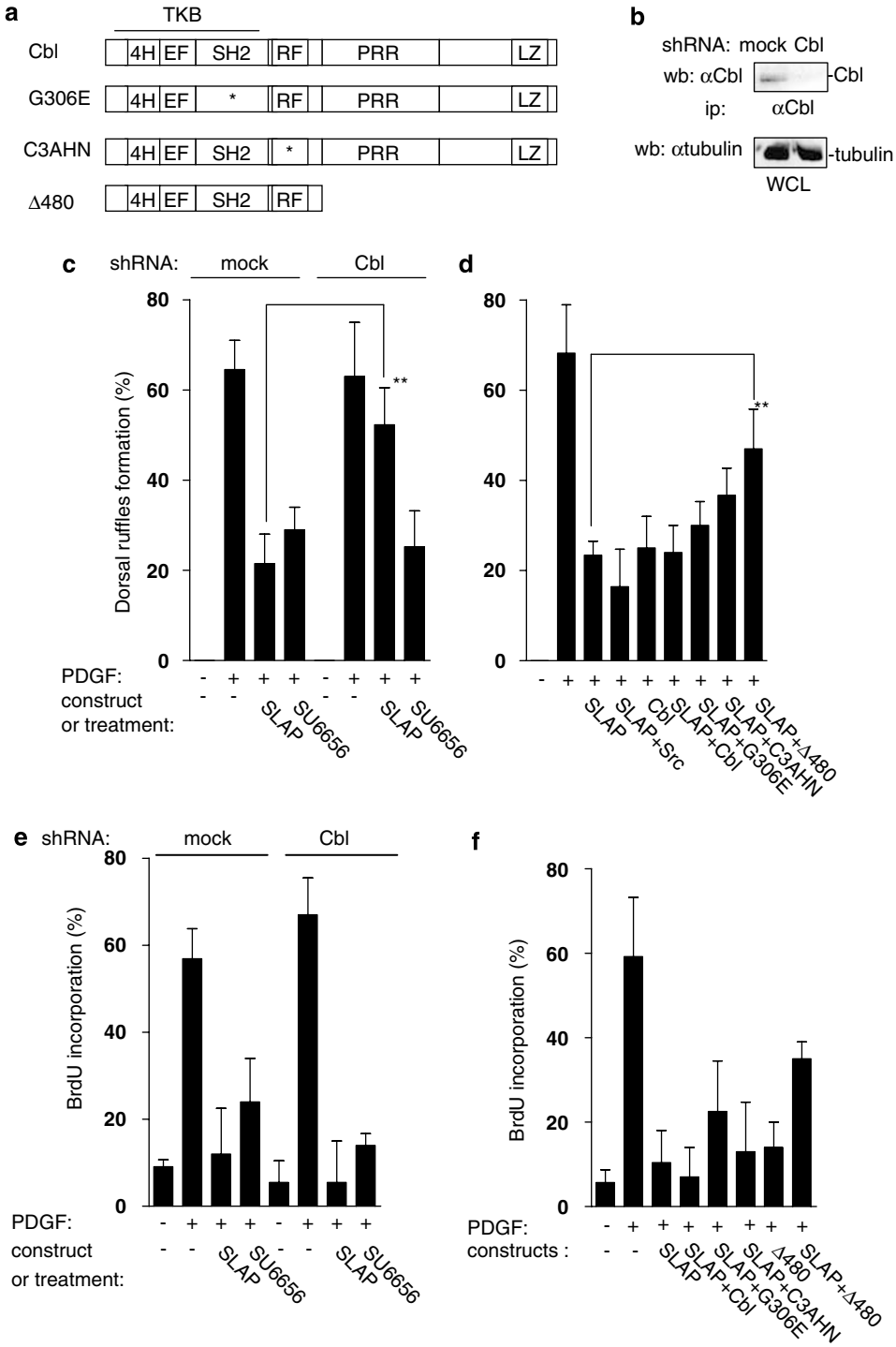

Figure 2 c-Cbl mediates SLAP activity towards actin remodelling but not mitogenesis. (**a**) c-Cbl constructs: 4H, four-helix bundle; EF, EF hand; *, point mutations in TKB or RF domains; PRR, proline-rich repeat; LZ, leucine zipper; G306E (a gift from Dr Gross), TKB domain mutant, C3AHN (a gift form Professor Band), multiple point mutations in RF domain; Δ480 (a gift from Professor Langdon), truncation at amino acid 480. (**b**) c-Cbl level in NIH-3T3 cells infected with retroviruses expressing scramble (mock) or shRNA specific to the Cbl sequence (ACACTTTCCGGATTACTA) and assessed by western blotting of immunoprecipitated c-Cbl (sc-170, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The level of tubulin from a whole-cell lysate is shown. (**c**) SLAP does not inhibit dorsal ruffles in c-Cbl-depleted cells. (**d**) SLAP inhibition towards dorsal ruffles induction is overcome in cells with inactive c-Cbl. (**e**) SLAP still inhibits mitogenesis in c-Cbl-depleted cells. (**f**) SLAP still inhibits mitogenesis in cells coexpressing c-Cbl inactive mutants. Indicated quiescent cells that were transfected or not with indicated constructs, treated with SU6656 or untreated (2 μM) (Calbiochem, San Diego, CA, USA) and stimulated with PDGF–BB for 5 min (ruffles formation) or 18 h in the presence of BrdU (0.1 mM, Sigma) (BrdU incorporation). Cells were fixed and preceded for immunostaining as described by Veracini *et al*. (2006). BrdU incorporation (%) = (number of BrdU-positive transfected cells) (number of transfected cells) × 100. The mean ± s.d. from 3–5 independent experiments is shown. $^{**}P<0.01$ using a student's *t*-test. BrdU, bromo-deoxyuridine; RF, ring finger; shRNA, short hairpin RNA; SLAP, Src-like adaptor protein; TKB, tyrosine kinase-binding.

affected by SLAP depletion. Thus, we concluded that SLAP negatively regulates PDGF-induced dorsal ruffles.

c-Cbl mediates SLAP activity towards dorsal ruffles induction

The mechanism by which SLAP regulates actin remodelling was next investigated. As both SLAP and SFK require their SH2 domain in this cellular process (Veracini *et al*., 2006), we sought an SLAP-competitive mechanism that prevents SFK signalling. However, Src coexpression had no rescuing effect, even when expressed at higher level, suggesting that SLAP does not act at the Src level (Figure 2d). As c-Cbl has been implicated in F-actin assembly (Scaife *et al*., 2003; Swaminathan *et al*., 2007), we then addressed the role of

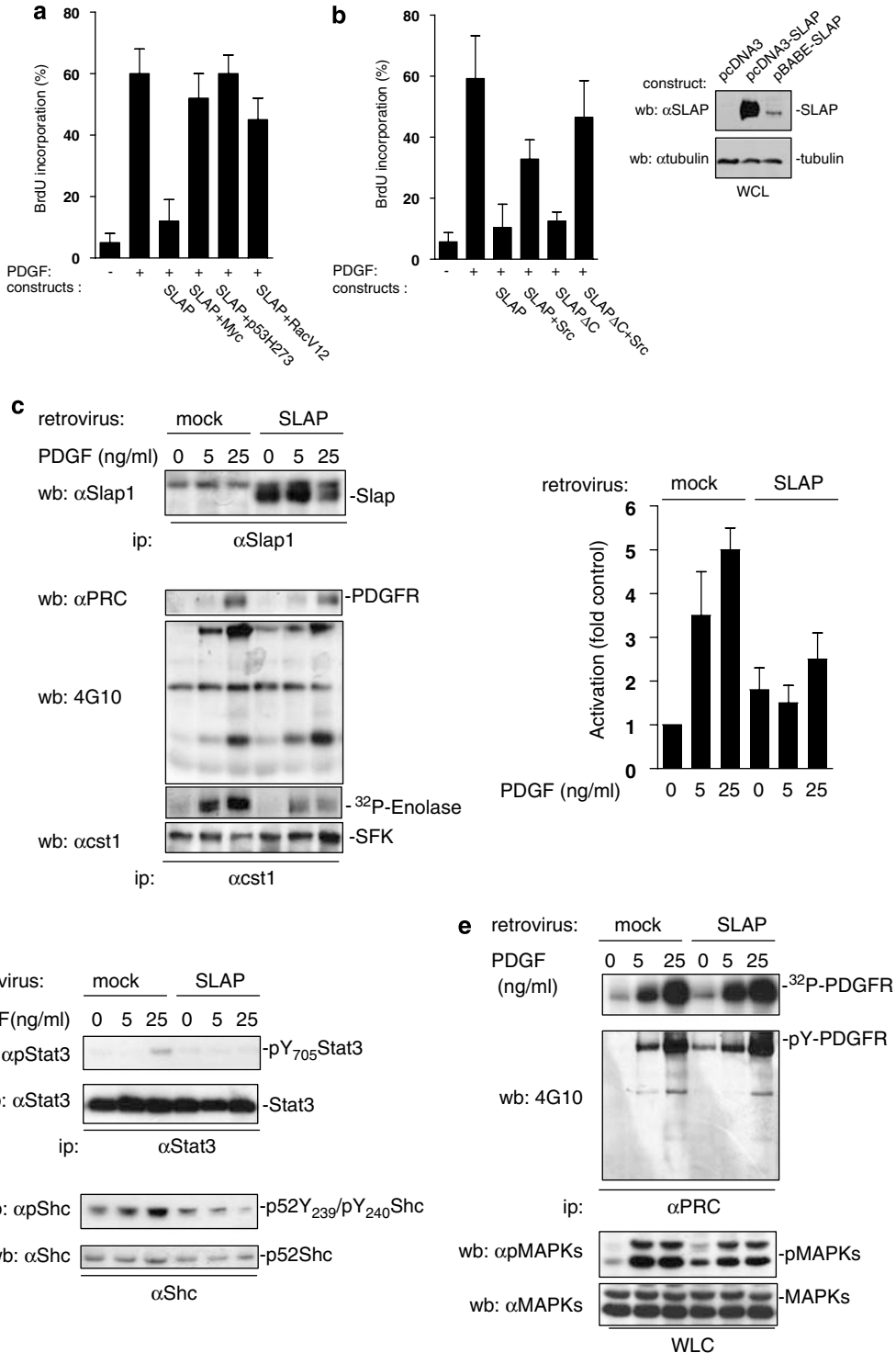

Figure 3

this adapter on SLAP morphological activity. To this end, c-Cbl was depleted from cells with a short hairpin RNA that reduced 80% of protein level (Figure 2b). Interestingly, SLAP cytoskeletal inhibition was not observed anymore in c-Cbl-deficient cells (Figure 2c). In contrast, PDGF-induced F-actin assembly still required SFK activity as shown by the inhibitory effect of SU6656, a pharmacological inhibitor of SFK (Figure 2c). This indicates that c-Cbl is required for this SLAP activity and that SFK use additional substrates for promoting dorsal ruffles. The role of c-Cbl was next confirmed by a dominant-negative approach. A c-Cbl mutant bearing deletion at amino acid 480 (Δ480) has been shown to potentiate dorsal ruffles (Scaife *et al*., 2003), in agreement with a dominant-negative activity towards c-Cbl-regulating F-actin assembly. Indeed, this mutant significantly restored ruffles in cells expressing SLAP (Figure 2d). Δ480 had lost its capacity to bind to SH3-containing proteins and to be phosphorylated by SFK, implicating either molecular event for c-Cbl-mediating SLAP inhibitory effect. In contrast, c-Cbl mutants bearing inactive E3-ligase activity (C3AHN) or tyrosine kinase domain (G306E) had a lower rescuing effect (Figure 2d). We concluded that this SLAP function is mediated by c-Cbl, implicating its C-terminal domain. Moreover, the absence of inhibitory effect observed with SLAPΔC suggests that the interaction with c-Cbl may be necessary for *in vivo* activity (Figure 1b).

Cbl does not mediate SLAP mitogenic activity

We next addressed whether c-Cbl plays a similar role in SLAP mitogenic inhibition. First, cells with reduced-c-Cbl gave a significant higher PDGF response, in agreement with a negative function for this adapter on mitogenic signalling (Broome *et al*., 1999; Miyake *et al*., 1999). Nevertheless, SLAP retroviral expression still inhibited this response (Figure 2e), suggesting that c-Cbl does not mediate its mitogenic activity. Similarly, SFK activities were necessary for mitogenesis (Figure 2e) in agreement with the requirement of additional Src substrates in this signalling process (Bromann *et al*., 2004). We next confirmed this data with a dominant-negative approach. G306E exhibits dominant-negative activity towards c-Cbl mitogenic function (Broome *et al*., 1999; Miyake *et al*., 1999), therefore, this mutant was coexpressed with SLAP for c-Cbl inhibition. However, it did not overcome the block induced by SLAP (Figure 2f), confirming the c-Cbl-independent nature of this SLAP signalling. It should be mentioned that Δ480 gave a partial rescuing effect, while inhibiting mitogenesis *per se* (Figure 2f). It thus may affect SLAP activity by a mechanism independent of c-Cbl.

SLAP mitogenic inhibition involves a SFK-competitive mechanism

We next investigated the mechanism by which SLAP inhibits the PDGF mitogenic response. Our previous studies indicated that SLAP interferes with Src signalling, including the association of Src-SH2 with the PDGF receptor (PDGFR) at phospho-Tyr579 (Roche *et al*., 1998b; Manes *et al*., 2000). SFK activation induces an Rac/Myc-signalling cascade necessary for the induction of DNA synthesis, which can be suppressed by a p53-dependent activity (Bromann *et al*., 2004). PDGF mitogenic response was inhibited by a moderate expression of SLAP (Figure 3a; Figure 3b, right panel). Moreover, this inhibition was bypassed when constitutive active elements of the Src pathway, that is, Myc and active V12Rac, and the dominant-negative p53H273 are coexpressed (Figure 3a). More importantly, Src also significantly rescued this mitogenic blockade (Figure 3b), suggesting that SLAP acts, at least in part, at the Src level. We attributed our inability to previously rescue SLAP inhibition (Manes *et al*., 2000) to a higher expression of this adapter (Figure 3b, right panel).

The impact of SLAP on Src mitogenic signaling was next confirmed at the molecular level. SLAP exhibited a strong antiproliferative effect in mouse fibroblasts (Roche *et al*., 1998a). Nevertheless, we could generate NIH-3T3 cells stably expressing SLAP when transduced with retroviral infection (Figure 3c). PDGF-induced SFK association with the receptor and kinase activation were both reduced by SLAP (Figure 3c). Accordingly, phosphorylation of Src mitogenic substrates Stat3 and Shc (Bromann *et al*., 2004) were inhibited (Figure 3d). Specificity was shown by the inability of SLAP to affect PDGFR activation (Figure 3e). Moreover, this adapter

Figure 3 SLAP inhibits SFK mitogenic signalling. (**a**) SLAP inhibits PDGF-induced Src mitogenic signalling (**b**) at the Src level. BrdU-incorporation assays in quiescent NIH-3T3 cells transfected with indicated constructs and stimulated with PDGF–BB or unstimulated. Src, Myc and RacV12 constructs have been described by Boureux *et al*. (2005) and p53H273 was a kind gift from Dr Hibner. Right panel: SLAP level in HEK 293 cells transfected with indicated constructs. (**c**) PDGF-induced SFK activation and (**d**) phosphorylation of Src mitogenic substrates were reduced in NIH-3T3 cells stably expressing SLAP. (**e**) SLAP does not affect all PDGFR-signalling pathways. Cell lysates were made from quiescent cells that were infected as indicated and stimulated for 5 min with indicated concentrations of PDGF–BB. The level of immunoprecipitated SLAP (αSLAP1; Manes *et al*., 2000), immunoprecipitated SFK with associated tyrosine-phosphorylated proteins and PDGFR are shown (left). An example (left) and statistical analysis (right) (mean ± s.d., $n = 3$) of *in vitro* SFK activities (relative to activities from quiescent mock-infected cells) is shown and was assessed by the capacity to purified SFK to phosphorylate denatured enolase (^{32}P-enolase) as in (Veracini *et al*., 2006). (**d**) Levels of immunoprecipitated Stat3 and Shc and their phosphorylation on Tyr705 and Tyr239/240, respectively are shown from indicated cell-lysates. (**e**) Kinase activity (^{32}P-PDGFR) and tyrosine phosphorylation content of immunoprecipitated PDGFR is shown from indicated cell-lysates. The levels of phosphorylated MAPKs (p-MAPKs) and MAPKs are also shown. Used antibodies are described by Veracini *et al*., (2006). BrdU, bromo-deoxyuridine; PDGFR, PDGF-receptor; PDGF, platelet-derived growth factor; SFK, Src family tyrosine kinases; SLAP, Src-like adaptor protein; MAPKs, mitogen-activated protein kinases.

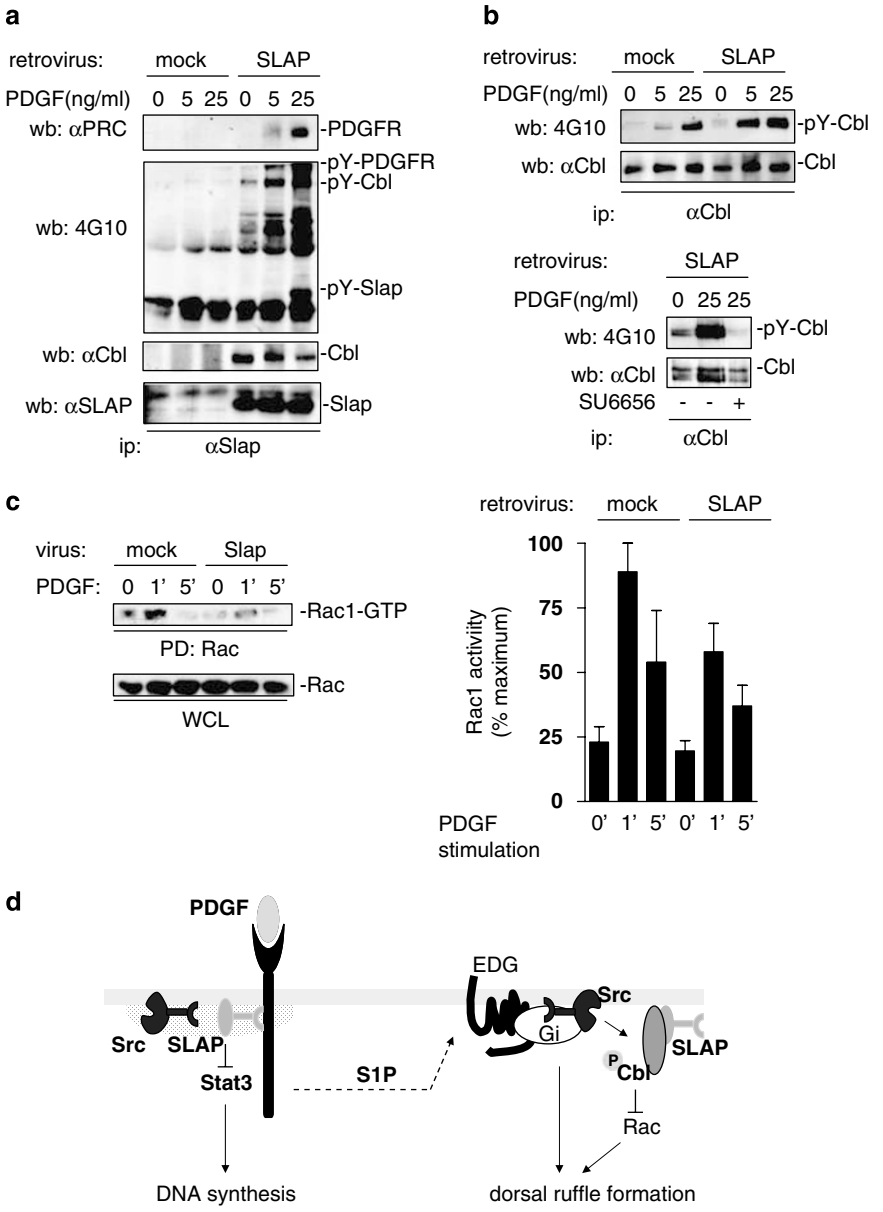

Figure 4 SLAP associates with c-Cbl and inhibits Rac activation. (**a**) SLAP associates with PDGFR and c-Cbl. (**b**) SLAP potentiates PDGF-induced c-Cbl phosphorylation in an SFK-dependent manner. Cell-lysates were prepared from quiescent cells that were infected with as indicated and stimulated for 5 min with indicated concentrations of PDGF–BB. Left: levels of immunoprecipitated SLAP with associated tyrosine-phosphorylated proteins, PDGFR and c-Cbl are shown. Right: the level of immunoprecipitated c-Cbl and its tyrosine phosphorylation content is shown from indicated cells stimulated with PDGF–BB and treated with SU6656 or untreated (2 μM). (**c**) SLAP affects PDGF-induced Rac activation. An example (left) and statistical analysis (right) (mean ± s.e., $n = 3$) of Rac1-GTP level (% of maximum) in cells stimulated with PDGF (10 ng ml^{-1}) is shown and was assessed as described by Boureux *et al*. (2005). (**d**) A model for SLAP-interfering with PDGF-induced SFK signalling leading to DNA-synthesis and dorsal ruffles. Cholesterol-enriched microdomains are shown in grey. S1P: sphingosine 1 phosphate, endothelial differentiation gene (EDG) receptors; Gi: heterotrimeric protein of the Gi sub-family; PDGF, platelet-derived growth factor; SLAP, Src-like adaptor protein.

had a low impact on Ras-induced MAPKs activation, indicating that it does not inhibit all signalling pathways (Figure 3e).

SLAP associates with c-Cbl and affects Rac activation

SLAP associated with the activated PDGFR in agreement with a reduction of SFK–PDGFR complex formation, and it was tyrosine phosphorylated upon PDGF stimulation (Figure 4a). Moreover, SLAP is associated with a tyrosine-phosphorylated protein of 120 kDa that was further identified as c-Cbl (Figure 4a). However, SLAP–c-Cbl complex formation was independent of growth factor stimulation. Nevertheless, we found that SLAP potentiated PDGF-induced c-Cbl tyrosine phosphorylation that was regulated by SFK (Figure 4b). As this phosphorylation has been implicated

in c-Cbl activities (Schmidt and Dikic, 2005), we suggest that SLAP promotes c-Cbl functions for negative regulation of F-actin assembly. C-Cbl also inhibits Rac activity, required for dorsal ruffle formation (Scaife *et al*., 2003; Swaminathan *et al*., 2007). Accordingly, PDGF-induced Rac activation was also affected by SLAP. This suggests that dorsal ruffle inhibition by SLAP implicates a c-Cbl/Rac pathway.

Here, we show that in addition to mitogenesis, SLAP negatively regulates PDGF-induced dorsal ruffles and this activity is mediated by c-Cbl. We propose that SLAP promotes Src-induced c-Cbl phosphorylation at the C-terminus for inhibition of Rac activation. Given the proposed function of dorsal ruffles formation in cell invasion (Suetsugu *et al*., 2003), we suggest that SLAP may play a role in this biological process induced by growth factors. Nevertheless, additional cytoskeletal function for SLAP–c-Cbl may be expected (Teckchandani *et al*., 2005). c-Cbl has been also implicated in PDGFR β-degradation for downregulation of mitogenesis (Broome *et al*., 1999; Miyake *et al*., 1999). However, our data suggest that in contrast with F-actin assembly, c-Cbl does not mediate SLAP mitogenic inhibition. Therefore, c-Cbl may use distinct mechanisms for PDGFR association and ubiquitination (Reddi *et al*., 2007). Thus, we propose that SLAP dictates c-Cbl-specificity towards PDGFR signalling. Finally, this report suggests the existence of two distinct pools of SLAP for regulation of SFK signalling. SFK regulates mitogenesis by the direct association of the receptor in cholesterol-enriched microdomains whereas SFK-promoting dorsal ruffles is localized at the actin cytoskeleton (Veracini *et al*., 2006). Therefore, we suggest that SLAP negatively regulates mitogenesis by directly interfering with SFK–PDGFR complex formation in caveolae, whereas an SLAP–c-Cbl complex regulates actin remodelling by targeting SFK-induced Rac activation. As SFK plays crucial roles in neoplastic transformation (Ishizawar and Parsons, 2004), we anticipate negative functions for SLAP in human cancer.

Acknowledgements

We thank H Band, I Gross, U Hibner, WY Langdon and N Morin for various reagents. This work was supported by grants of the CNRS, University of Montpellier I and II, INCa and ARC. AS was supported by SERVIER laboratories and CL by INCa. SR is an INSERM investigator.

References

Boureux A, Furstoss O, Simon V, Roche S. (2005). c-Abl tyrosine kinase regulates a Rac/JNK and a Rac/Nox pathway for DNA synthesis and c-myc expression induced by growth factors. **118**: 3717–3726.

Bromann PA, Korkaya H, Courtneidge SA. (2004). The interplay between Src family kinases and receptor tyrosine kinases. *Oncogene* **23**: 7957–7968.

Broome MA, Galisteo ML, Schlessinger J, Courtneidge SA. (1999). The proto-oncogene c-Cbl is a negative regulator of DNA synthesis initiated by both receptor and cytoplasmic tyrosine kinases. *Oncogene* **18**: 2908–2912.

Ishizawar R, Parsons SJ. (2004). c-Src and cooperating partners in human cancer. *Cancer Cell* **6**: 209–214.

Lanzetti L, Palamidessi A, Areces L, Scita G, Di Fiore PP. (2004). Rab5 is a signalling GTPase involved in actin remodelling by receptor tyrosine kinases. *Nature* **429**: 309–314.

Manes G, Bello P, Roche S. (2000). Slap inhibits the Src-mitogenic function but does not affect Src-induced cell transformation. *Mol Cell Biol* **20**: 3396–3406.

Miyake S, Mullane-Robinson KP, Lill NL, Douillard P, Band H. (1999). Cbl-mediated negative regulation of platelet-derived growth factor receptor-dependent cell proliferation. A critical role for Cbl tyrosine kinase-binding domain. *J Biol Chem* **274**: 16619–16628.

Myers MD, Sosinowski T, Dragone LL, White C, Band H, Gu H *et al*. (2006). Src-like adaptor protein regulates TCR expression on thymocytes by linking the ubiquitin ligase c-Cbl to the TCR complex. *Nat Immunol* **7**: 57–66.

Pandey A, Duan H, Dixit VM. (1995). Characterization of a novel Src-like adapter protein that associates with the Eck receptor tyrosine kinase. *J Biol Chem* **270**: 19201–19204.

Reddi AL, Ying G, Duan L, Chen G, Dimri M, Douillard P *et al*. (2007). Binding of Cbl to a PLCgamma 1-docking site on PDGFRbeta provides a dual mechanism of negative regulation. *J Biol Chem* **282**: 29336–29347.

Roche S, Alonso G, Kazlauskas A, Dixit VM, Courtneidge SA, Pandey A. (1998a). Src-like adaptor protein (Slap) is a negative regulator of mitogenesis. *Curr Biol* **8**: 975–978.

Roche S, Downward J, Raynal P, Courtneidge SA. (1998b). A function for phosphatidylinositol 3-kinase beta (p85alpha-p110beta) in fibroblasts during mitogenesis: requirement for insulin- and lysophosphatidic acid-mediated signal transduction. *Mol Cell Biol* **18**: 7119–7129.

Scaife RM, Courtneidge SA, Langdon WY. (2003). The multi-adaptor proto-oncoprotein Cbl is a key regulator of Rac and actin assembly. *J Cell Sci* **116**: 463–473.

Schmidt MH, Dikic I. (2005). The Cbl interactome and its functions. *Nat Rev Mol Cell Biol* **6**: 907–919.

Sosinowski T, Killeen N, Weiss A. (2001). The Src-like adaptor protein downregulates the T cell receptor on CD4+CD8+ thymocytes and regulates positive selection. *Immunity* **15**: 457–466.

Suetsugu S, Yamazaki D, Kurisu S, Takenawa T. (2003). Differential roles of WAVE1 and WAVE2 in dorsal and peripheral ruffle formation for fibroblast cell migration. *Dev Cell* **5**: 595–609.

Swaminathan G, Feshchenko EA, Tsygankov AY. (2007). c-Cbl-facilitated cytoskeletal effects in v-Abl-transformed fibroblasts are regulated by membrane association of c-Cbl. *Oncogene* **26**: 4095–4105.

Teckchandani AM, Panetti TS, Tsygankov AY. (2005). c-Cbl regulates migration of v-Abl-transformed NIH 3T3 fibroblasts via Rac1. *Exp Cell Res* **307**: 247–258.

Veracini L, Franco M, Boureux A, Simon V, Roche S, Benistant C. (2006). Two distinct pools of Src family tyrosine kinases regulate PDGF-induced DNA synthesis and actin dorsal ruffles. *J Cell Sci* **20**: 2921–2934.