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Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10

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The stimulation of glucose uptake by insulin in muscle and adipose tissue requires translocation of the GLUT4 glucose transporter protein from intracellular storage sites to the cell surface[1–6]. Although the cellular dynamics of GLUT4 vesicle trafficking are well described, the signalling pathways that link the insulin receptor to GLUT4 translocation remain poorly understood. Activation of phosphatidylinositol-3-OH kinase (PI(3)K) is required for this trafficking event, but it is not sufficient to produce GLUT4 translocation[7]. We previously described a pathway involving the insulin-stimulated tyrosine phosphorylation of Cbl, which is recruited to the insulin receptor by the adapter protein CAP[8,9]. On phosphorylation, Cbl is translocated to lipid rafts. Blocking this step completely inhibits the stimulation of GLUT4 translocation by insulin[10]. Here we show that phosphorylated Cbl recruits the CrkII–C3G complex to lipid rafts, where C3G specifically activates the small GTP-binding protein TC10. This process is independent of PI(3)K, but requires the translocation of Cbl, Crk and C3G to the lipid raft. The activation of TC10 is essential for insulin-stimulated glucose uptake and GLUT4 translocation. The TC10 pathway functions in parallel with PI(3)K to stimulate fully GLUT4 translocation in response to insulin.

Many receptor tyrosine kinases use small GTP-binding proteins as molecular switches to convert proximal tyrosine phosphorylation into the activation of serine/threonine kinase cascades[11]. In addition, small GTP-binding proteins are critical for intracellular vesicle trafficking[12]. We have examined the modulation of insulin-stimulated glucose uptake and GLUT4 translocation by several small GTP-binding proteins[1]. Expression of wild-type, dominant-interfering and constitutively active mutants of Ras, Rap1, Rac1, TC21, RhoA and RhoD in 3T3L1 adipocytes had no discernible effect on this biological response (data not shown). In contrast, expression of wild-type TC10 (TC10/WT) and a dominant-interfering TC10 mutant (TC10/T31N) significantly inhibited insulin-stimulated glucose uptake (Fig. 1a).

As the transfection efficiency was about 50%, it is likely that expression of the TC10/T31N mutant almost completely inhibited glucose uptake in the transfected cell population. Out of the known GTP-binding proteins, Cdc42 has the greatest degree of sequence similarity to TC10, with an overall sequence identity of 69% and similarity of 83%. However, expression of wild-type Cdc42 (Cdc42/WT) or the dominant-interfering Cdc42 mutant (Cdc42/T17N) had no statistically significant effect on insulin-stimulated glucose uptake (Fig. 1a).

To determine whether TC10 inhibits insulin-stimulated glucose uptake through a blockade of GLUT4 translocation, we microinjected 3T3L1 adipocytes along with expression plasmids for TC10 or Cdc42 (Fig. 1b). The cells were microinjected with the carboxy-terminal domain of Ras fused to the maltose-binding protein (MBP–Ras) as a marker for plasma-membrane in injected cells[2–4]. As GLUT4 is localized predominantly in intracellular vesicles in the basal state, there is only a low level of GLUT4 immunofluorescence in the plasma-membrane sheets derived from both the microinjected and non-microinjected cells. As typically observed, insulin produced the robust appearance of plasma-membrane GLUT4 immunofluorescence in both the microinjected and surrounding non-injected cells. Microinjection of either TC10/WT or TC10/T31N inhibited insulin-stimulated translocation of GLUT4, in

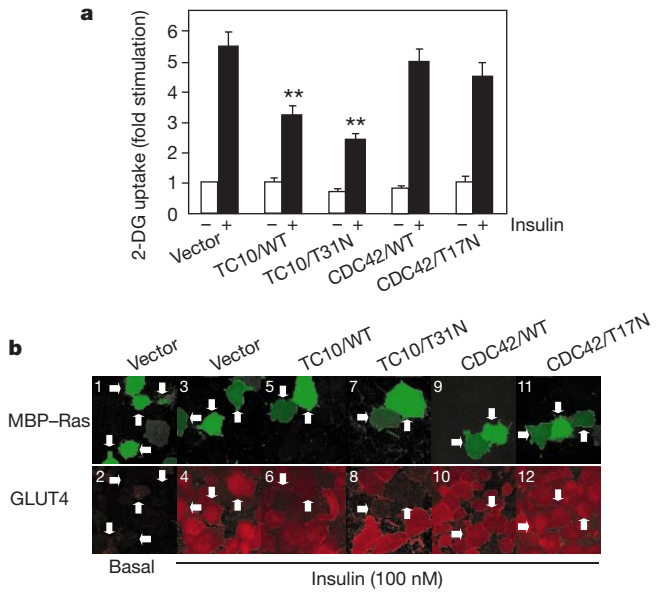

Figure 1 Expression of TC10 inhibits insulin-stimulated glucose uptake and GLUT4 translocation in 3T3L1 adipocytes. **a**, Differentiated 3T3L1 adipocytes were transfected with TC10 and Cdc42 mutants. The cells were left untreated or were stimulated with 100 nM insulin for 30 min. The rate of [^{3}H]2-deoxyglucose (2-DG) uptake was determined. Results are the mean ± s.e. of 3–5 independent experiments from individual experiments performed in triplicate with a transfection efficiency of ~50%. Two asterisks, $P < 0.01$. **b**, Differentiated 3T3L1 adipocyte nuclei were microinjected with 0.2 mg ml^{-1} of MBP–Ras plus either the empty vector or the cDNAs encoding TC10/WT, TC10/T31N, CDC42/WT or CDC42/T17N. The cells were allowed to recover for 24 h, and left untreated or treated with 100 nM insulin for 30 min.

comparison to the surrounding non-injected cells. Microinjection of Cdc42/WT or Cdc42/T17N had no significant effect on insulin-stimulated GLUT4 translocation. We scored 30–60 plasma-membrane sheets per condition from 4 independent experiments, which showed that the complementary DNAs for TC10/WT, TC10/T31N, Cdc42/WT and Cdc42/T17N inhibited insulin-stimulated GLUT4 translocation in 66 ± 8, 75 ± 8, 7 ± 3 and 8 ± 4% of the microinjected 3T3L1 adipocytes, respectively.

As a control for GLUT4 translocation specificity, we also examined the effect of TC10 and Cdc42 expression on insulin-stimulated GLUT1 translocation (Supplementary Information Fig. 1). Although insulin has a more modest effect on the translocation of GLUT1 as compared with GLUT4 (refs 5, 6), microinjection of both the TC10 and Cdc42 cDNAs had no effect.

To confirm the effect of TC10 on GLUT4 translocation, we co-expressed TC10 with a GLUT4–enhanced green fluorescent protein (EGFP) fusion construct in 3T3L1 adipocytes (Supplementary Information Fig. 2). Co-expression of GLUT4–EGFP with TC10/WT or TC10/T31N markedly decreased the number of the cells responding to insulin. In contrast, overexpression of Cdc42/WT or Cdc42/T17N had no effect on insulin-stimulated GLUT4–EGFP translocation. In addition, expressing the constitutively active mutant TC10/Q75L and GTP-cycling mutant TC10/F42L resulted in a substantial inhibition of insulin-stimulated GLUT4 translocation. As a measure of GLUT4 vesicle specificity, expression of TC10 or Cdc42 had no significant effect on the insulin-stimulated translocation of the cation-independent mannose-6-phosphate receptor (data not shown).

The inhibition of insulin-stimulated glucose uptake and GLUT4 translocation through overexpression of the TC10/T31N dominant-interfering mutant is consistent with its role as a signalling intermediate; however, it was surprising that overexpression of the wild-type TC10 protein also inhibited this biological response. TC10 has a lower intrinsic GTPase activity than has Cdc42 (ref. 13). If a GTPase-activating activity limits the process, overexpression of TC10 would cause increased basal TC10-GTP, and thus behave like a constitutively active mutant. Indeed, both constitutively active and dominant-negative rhoA or Rac1 proteins perturbed tight junction gate function[14]. Thus, the overexpressed mutant GTPases might displace cycling endogenous GTPases from targets. As mutant proteins remain bound to the effectors, overexpression results in quick domination of the mutant phenotype. Overexpressing wild-type TC10 may alter binding stoichiometry and/or mistarget the effectors to wrong locations and thereby prevent the appropriate propagation of the signal.

Several studies have shown that in the active (GTP-bound) con-

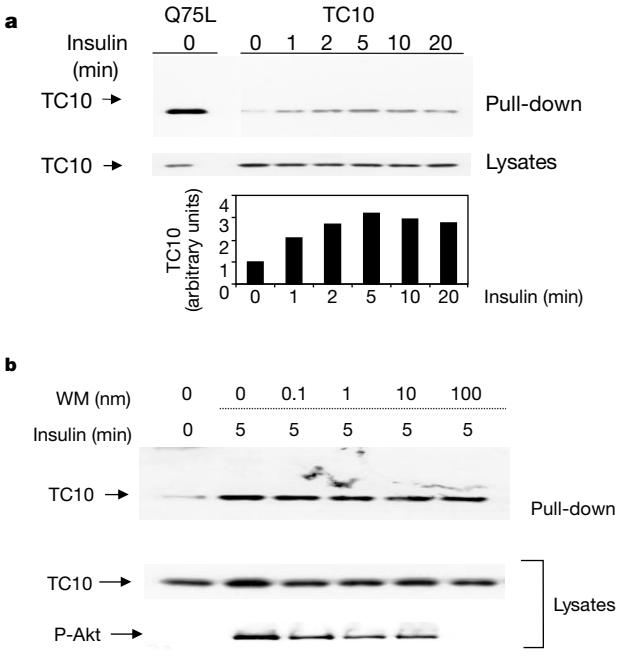

Figure 2 Insulin activates TC10 through a PI(3)K-independent pathway in 3T3L1 adipocytes. **a**, Fully differentiated 3T3L1 adipocytes were electroporated with 60 μg of a cDNA encoding HA-tagged TC10. Cells were starved for 3 h before treatment with 100 nM insulin. Bottom, lysates from different time points were immunoblotted with anti-HA antibody. Top, lysates were incubated with GST–Pak1 for 1 h, followed by 3 washes and immunoblotted with anti-HA (top). **b**, 3T3L1 adipocytes were electroporated with HA-tagged TC10, incubated for 30 min with increasing doses of wortmannin (WM; 0–100 nM), and treated with insulin for 5 min. Top, GST–Pak1 pull-down assay probed with anti-HA. Middle, anti-HA immunoblots with lysates from different treatments. Bottom, phospho-Akt blot with the same lysates.

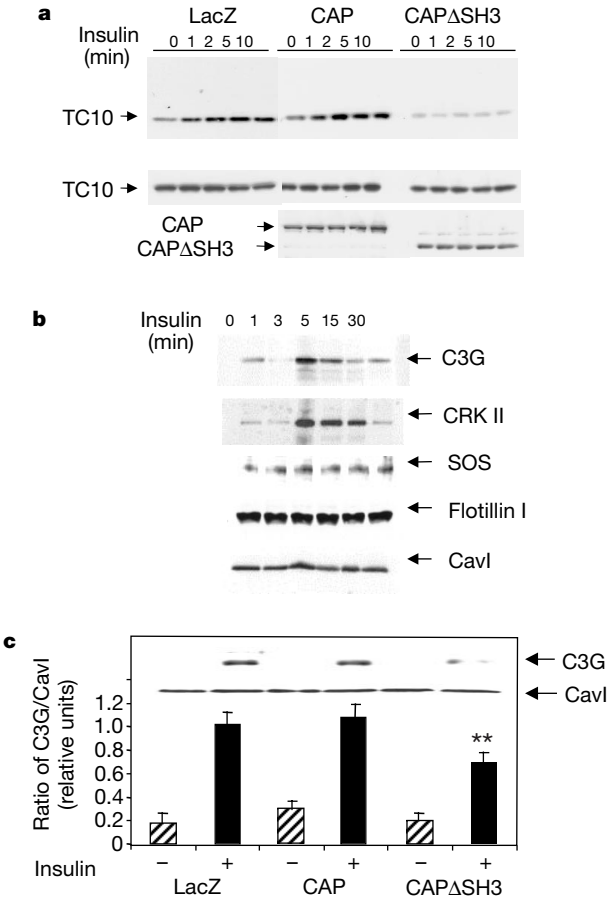

Figure 3 TC10 is activated by insulin through a CAP-dependent pathway. **a**, 3T3L1 adipocytes were co-electroporated with 60 μg of HA–TC10 and 500 μg of LacZ control, or Flag-tagged CAP or CAPΔSH3. Cells were starved for 3 h before collection. Top, HA immunoblot of pull-down samples from different time points. Bottom, HA and Flag immunoblots with 50 μg of lysate. **b**, 3T3L1 adipocytes were treated with 100 nM insulin for various times. Cells were lysed at 4 °C in the presence of Triton X-100. Triton-insoluble complexes were prepared[10], and 20 μg of the triton-insoluble material was suspended in Laemmli sample buffer, separated by SDS–PAGE, transferred to nitrocellulose and blotted for C3G, CRKII, caveolin I (Cav1) and flotillin. **c**, 3T3L1 adipocytes were transfected by electroporation with LacZ, CAP or CAPΔSH3, and stimulated with 100 nM insulin for 5 min. The transfection efficiency was 50%. Cells were lysed at 4 °C in the presence of TritonX-100. Triton-insoluble complexes were suspended in sample buffer, resolved by SDS–PAGE, transferred to nitrocellulose and immunoblotted with anti-C3G and caveolin I antibodies. A representative blot is shown. Films were scanned and quantified, and the ratio of total C3G to caveolin I was calculated. Two asterisks, $P < 0.01$; $n = 4$.

formation, Rac and Cdc42 associate specifically with the Cdc42/Rac interactive binding (CRIB) motif of the p21-activated protein kinase 1, Pak1 (refs 15–17). TC10 also binds the CRIB domain of effector proteins in a GTP-dependent manner[13]. To confirm that the Pak1 CRIB domain specifically interacts with these proteins, we transfected Chinese hamster ovary (CHO) cells with HA-epitope tagged wild-type Rac1, Cdc42 or TC10 cDNAs (Supplementary Information Fig. 3). The extracts were incubated with GDP or GTP-γS, and precipitated with a gluthathione *S*-transferase (GST)–Pak1 fusion protein. The fusion protein quantitatively precipitated the GTPγS-bound forms of Rac, Cdc42 or TC10, showing that GST–Pak1 specifically recognizes the GTP-bound, activated state of these Rho family GTPases.

To examine whether TC10 can be activated in 3T3L1 adipocytes, we electroporated cells with a cDNA encoding HA–TC10 and treated them with insulin (Fig. 2a). Treating the cells with insulin caused the activation of TC10, which was maximal by about 5 min and declined thereafter. The activation of TC10 by insulin was specific, as insulin had no effect on GTP loading of Cdc42 (Supplementary Information Fig. 4). To determine whether activation of TC10 is unique for insulin, we treated HA–TC10-electroporated cells with platelet-derived growth factor (PDGF). In contrast to insulin, TC10 was not activated by PDGF (Supplementary Information Fig. 5). Together, these data provide strong evidence that TC10 is activated by insulin through a specific and unique signalling pathway.

Several studies have shown that PI(3)K activation is necessary, but not sufficient for insulin-stimulated GLUT4 translocation[18–20]. To determine whether TC10 activation requires PI(3)K, 3T3L1 adipocytes were electroporated with HA–TC10, followed by incubation with increasing doses of wortmannin, a PI(3)K inhibitor. Cells were then stimulated with insulin, and lysates were analysed for Akt phosphorylation and TC10 activation. The stimulation of Akt phosphorylation by insulin was completely blocked by incubation with wortmannin[21,22] (Fig. 2b). In contrast, TC10 activation was unaffected by wortmannin, indicating that TC10 activation does not occur downstream of PI(3)K.

We have reported that the insulin-stimulated tyrosine phosphorylation of Cbl results in its recruitment to flotillin-enriched plasma-membrane subdomains through the adapter protein CAP[10]. Expression of a dominant-interfering CAP mutant (CAPΔSH3) has no effect on PI(3)K activation, but prevents the recruitment of tyrosine-phosphorylated Cbl to lipid rafts, and concomitantly inhibits insulin-stimulated glucose uptake and GLUT4 translocation[10]. To assess the potential role of the CAP–Cbl complex in TC10 activation, we co-transfected 3T3L1 adipocytes with HA–TC10 and either wild-type CAP or CAPΔSH3 (Fig. 3a). The insulin-stimulated activation of TC10 was unaffected by the co-expression of the wild-type CAP protein; however, expression of CAPΔSH3 slightly reduced the basal level of activated TC10, and completely prevented the insulin-stimulated increase in TC10 GTP loading. These data show that the CAP–Cbl axis provides a necessary signal parallel to the PI(3)K pathway for the activation of TC10 by insulin.

Stimulation of the tyrosine phosphorylation of Cbl by insulin generates a specific docking site for the amino-terminal Src homology (SH)-2 domain of the small adapter protein CrkII (ref. 8). Furthermore, the SH3 domain of CrkII interacts directly with the proline-rich domain of the guanyl nucleotide exchange factor C3G[23]. As tyrosine-phosphorylated Cbl is translocated into lipid rafts in response to insulin, we thought that Cbl might also recruit the CrkII–C3G complex into this plasma-membrane region. To test this possibility, 3T3L1 adipocytes were stimulated with insulin, and the lipid raft plasma-membrane domain was isolated as a Triton X-100 insoluble fraction (Fig. 3b). Immunoblotting with an anti-caveolin antibody showed that caveolin I and flotillin were found in the Triton-insoluble fraction, and that their distribution was unaffected by insulin. In contrast, both CrkII and C3G migrated in the Triton-soluble fraction, but were recruited into the Triton-insoluble faction in response to insulin. This effect was maximal at 3 min, and was reversed by 30 min. The time course of CrkII and C3G movement to the Triton-insoluble fraction paralleled the insulin-stimulated recruitment of Cbl[10] and the activation of TC10 (Fig. 2). As a control, insulin had no effect on the subcellular localization of the Ras nucleotide exchange factor SOS. This protein was found largely in the Triton-soluble fraction, but small amounts could be seen in the insoluble fraction on overexposure of the gels. Furthermore, the translocation of neither CrkII nor C3G was affected by pretreatment of cells with wortmannin (data not shown).

As expression of the dominant-interfering mutant CAPΔSH3 prevented the recruitment of tyrosine-phosphorylated Cbl to the caveolin/flotillin-enriched subdomain[10], we evaluated its effect on the recruitment of C3G to the Triton-insoluble complex (Fig. 3c). Insulin stimulated a marked recruitment of C3G into the Triton-insoluble fraction in both LacZ- and CAP-expressing cells; however, expression of CAPΔSH3 decreased the insulin-stimulated recruitment of C3G by roughly 40%. Taking into account a 50% transfection efficiency, this corresponds to a near complete block of C3G recruitment in the transfected cell population. Thus, insulin stimulates the translocation of C3G into caveolin-enriched compartments through a process that requires CAP function.

The temporal and spatial correlation of TC10 activation with that

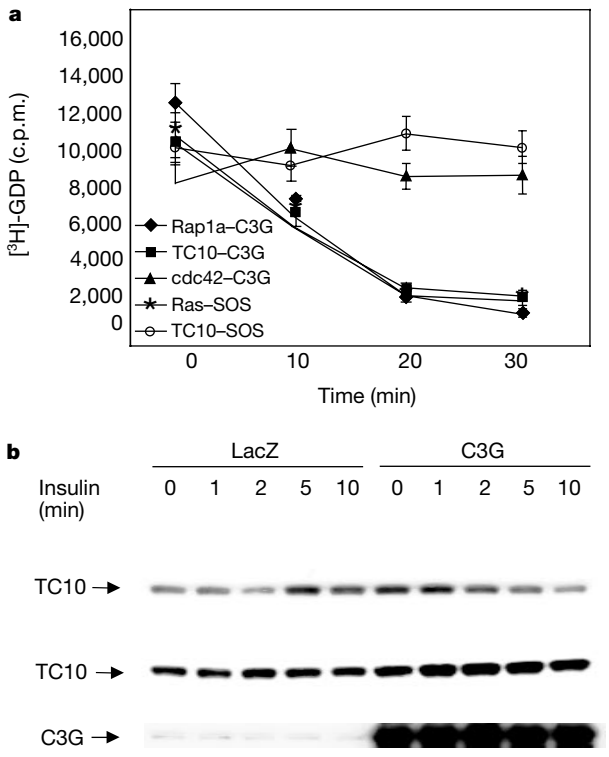

Figure 4 C3G catalyses guanine nucleotide exchange for TC10. **a**, 293T cells were transfected with constructs encoding Myc–C3G and Flag–SOS. Cells were immunoprecipitated with anti-Flag or anti-Myc antibodies, and immunoprecipitates were assayed for exchange activity using GST fusion proteins of [^{3}H]GDP-loaded TC10, cdc42, Rap1a and Ras in the presence of GTP. Aliquots were withdrawn at various time points and scintillation counting was performed. Results are the mean ± s.e. of triplicate determinations and are representative of three experiments. **b**, 3T3L1 adipocytes were co-electroporated with HA–TC10 and 200 μg of a LacZ control or a C3G expression vector. Top, HA immunoblot of pull-down samples from different time points. Bottom, HA and C3G immunoblots with 50 μg of lysate.

of C3G recruitment implicates C3G as a possible insulin-regulated guanyl nucleotide exchange factor (GEF) for TC10. To assess the relative GEF activity of C3G for TC10, both C3G and SOS were expressed in 293T cells, and immunoprecipitation was carried out. GTP-loading assays were performed on the immunoprecipitates using Ras, Rap1 or TC10 as substrate (Fig. 4a). As previously reported[24], SOS had a relatively high activity for Ras, but was a poor GEF for Rap1. In contrast, C3G effectively catalysed the exchange of GTP for GDP on Rap1, but was ineffective with Ras. SOS was also a very weak GEF for TC10, whereas C3G displayed profound GTP/GDP exchange activity for TC10. However, C3G did not show exchange activity with Cdc42 as substrate.

To examine the specific exchange activity of C3G for TC10, we deleted the putative GEF catalytic domain that exhibits sequence similarity to the *Saccharomyces cerevisiae* CDC25 exchange factor (C3GΔcdc25). The C3GΔcdc25 mutant failed to exchange GTP for GDP on Rap1 as well as TC10 (Supplementary Information Fig. 6). Thus, C3G can function as a specific GEF for TC10 *in vitro*, with activity comparable to that seen with its established target Rap1.

To determine whether C3G can function as a TC10 GEF *in vivo*, we co-transfected 3T3L1 adipocytes with HA–TC10 either with or without an expression vector encoding C3G (Fig. 4b). Transfection with C3G cDNA resulted in a marked increase in C3G protein over endogenous levels, but did not significantly affect the co-expression of HA–TC10. In the absence of C3G overexpression, TC10 was predominantly in the inactive GDP-bound state, but was activated in response to insulin. In cells overexpressing C3G, however, TC10 was quantitatively converted to the active GTP-bound state in the absence of insulin. Under these conditions, TC10 was activated maximally, as insulin did not further increase the amount of GTP-bound TC10.

The activation of TC10 by wild-type C3G suggests that overexpression of C3G may result in its being targeted to lipid rafts in an insulin-independent manner. Consistent with this hypothesis, a significant fraction of the wild-type C3G was detected in the Triton-insoluble fraction, although most of the overexpressed C3G protein was cytosolic (Supplementary Information Fig. 7). Thus, the excess expression of wild-type C3G results in its hormone-independent mistargeting to lipid rafts, where it can catalyse nucleotide exchange on TC10.

Although our data suggest that TC10 activation provides a critical component in the stimulation of glucose transport by insulin, it is also likely that the pathway will not be sufficient for insulin action, as glucose transport also requires the activation of PI(3)K and downstream protein kinases. To examine this issue, we evaluated the impact of TC10 activation on glucose transport by overexpressing C3G in 3T3L1 adipocytes. We transfected cells with C3G as above, and assessed the dose dependence of insulin-stimulated glucose transport. Insulin stimulated glucose uptake in 3T3L1 adipocytes, with an EC_{50} (effector concentration at half-maximal response) of about 0.2 nM (Fig. 5a). Expression of C3G resulted in a 4–5-fold leftward shift of the dose–response curve, without any change in the maximal response. This increase in insulin sensitivity suggests that the TC10 pathway has an important role in insulin signal transduction.

Previous studies have shown that expression of a constitutively active PI(3)K catalytic subunit only results in a partial translocation of GLUT4 (refs 18–20). As TC10 activation occurs independently of PI(3)K, we examined whether these two pathways together are sufficient to stimulate GLUT4 translocation fully (Fig. 5b). As typically observed, in the basal state roughly 20% of GLUT4–EGFP-expressing cells displayed a plasma-membrane fluorescence, which increased to 80% after insulin stimulation. Consistent with previous reports[18,19], co-expression of GLUT4–EGFP with a constitutively active p110 (p110CAAX) resulted in partial GLUT4 translocation that was further stimulated on the addition of insulin. Overexpressing C3G alone did not affect translocation in the basal state, in agreement with glucose transport data (see Fig. 5a); however, overexpressing both C3G and active p110 resulted in 75% of the transfected cells displaying the GLUT4 translocation, which is essentially identical to that induced by insulin. The ability of C3G and p110CAAX to mimic in full the effect of insulin strongly suggests that the combination of these two pathways is sufficient to mediate insulin-stimulated GLUT4 translocation.

These observations lead us to propose a new model for the spatial compartmentalization of signals that regulate glucose transport in adipocytes (Supplementary Information Fig. 8). The insulin receptor can catalyse the tyrosine phosphorylation of a number of substrates, including Cbl and the insulin receptor substrate (IRS) family. On tyrosine phosphorylation, IRS proteins can interact with PI(3)K, leading to the localized generation of phosphatidylinositol trisphosphate ($InsP_3$) and the subsequent activation/localization of $InsP_3$-dependent protein kinases. In parallel, a second pathway is activated by the insulin receptor through the tyrosine phosphorylation of Cbl. The tyrosine-phosphorylated Cbl protein, complexed with the adapter protein CAP, translocates to a lipid raft subdomain in the plasma membrane; the C-terminal domain of CAP binds Cbl, while the N-terminal domain specifically interacts with the lipid raft-localized protein, flotillin. The tyrosine phosphorylation of Cbl directs its association with the CrkII–C3G complex, resulting in its co-recruitment to the lipid raft. Because TC10 is also localized in these plasma-membrane subdomains, C3G is in close enough

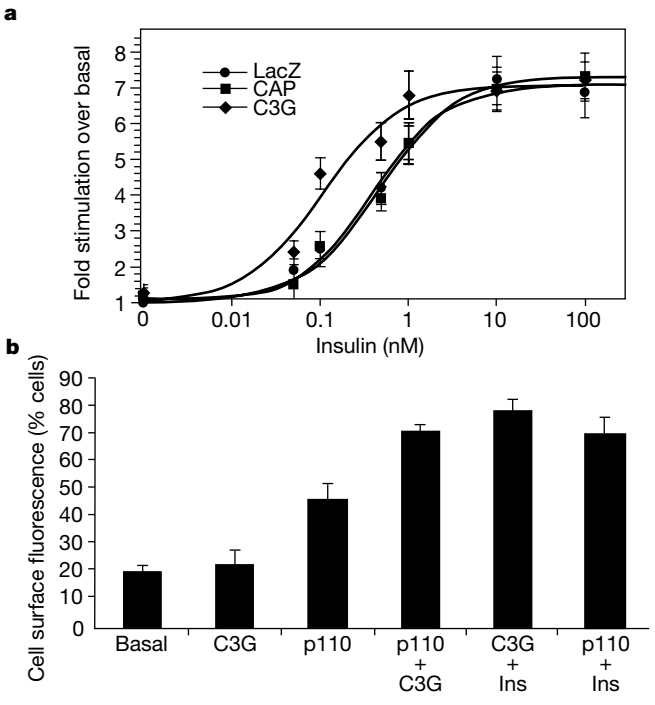

Figure 5 Overexpression of C3G and constitutively active p110 in 3T3L1 adipocytes mimics the effect of insulin on GLUT4 translocation. **a**, 3T3-L1 adipocytes were transfected by electroporation with LacZ, CAP or C3G containing constructs. [^{14}C]2-deoxyglucose uptake was measured after exposure to various doses of insulin. Fold stimulation by insulin is calculated as the insulin-stimulated level divided by the basal value. Each point was performed in triplicate; means ± s.e. are shown. **b**, 3T3-L1 adipocytes were transfected with 25 μg of GLUT4–EGFP, either alone or with 200 μg C3G or 200 μg p110CAAX (a gift from A. Klippel), or with a combination of both p110CAAX and C3G. Cells were incubated in the absence or presence of 100 nM insulin (Ins) for 30 min. The number of cells displaying plasma-membrane rim fluorescence was then determined. Values are mean ± s.e. from three independent experiments in which 75–100 individual cells were counted.

proximity to catalyse the exchange of GTP for GDP, resulting in the activation of TC10.

How does TC10 affect glucose transport? Our data lead us to speculate that TC10 may mediate the activation of two separate downstream pathways by insulin. Insulin-activated TC10 might directly produce a cytoskeletal rearrangement to facilitate the exocytosis of GLUT4. Evidence suggests that actin filaments may have a crucial role in the exocytotic recruitment of GLUT4 to the plasma membrane from an intracellular pool in isolated adipocytes[25]. Alternatively, TC10 might be crucial in the regulation of GLUT4 docking and fusion with the plasma membrane. Insulin can modulate the binding of the vesicle-SNARE protein VAMP2 with the target-SNARE syntaxin 4 to control the docking and fusion of GLUT4 vesicles through a PI(3)K-independent pathway. Thus, it is tempting to speculate that the activation of TC10 is directly upstream of this event. Future studies will be needed to address these possibilities. □

Methods

Transfection of CHO/IR and 3T3L1 adipocytes

CHO/IR cells and differentiated 3T3L1 adipocytes were transiently transfected as described[1,10].

Guanine nucleotide exchange assay

We transfected 293T cells with Myc–C3G or Flag–SOS plasmid DNA. Transfected cells were cultured for 48 h, gathered and lysed in 25 mM HEPES pH 7.4, 150 mM NaCl, 1.5 mM $MgCl_2$, 0.5% Triton X-100 and protease inhibitors. Myc–C3G and Flag–SOS proteins were immunoprecipitated with Myc antibody or Flag antibodies and protein-A/G–Sepharose. Guanine nucleotide exchange assays were performed essentially as described[26]. We constructed C3G and C3GΔcdc25 by cloning the *Nco*I–*Xba*I and *Nco*I–*Sma*I fragments, respectively, of the coding sequence of C3G into a pCS2-mt vector with a 5′ Myc epitope tag.

Affinity precipitation of TC10 using GST–Pak1 PBD

We modified the method from ref. 15. Cells were incubated with binding buffer (25 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 30 mM $MgCl_2$, 40 mM NaCl and 0.5% Nonidet P-40) in the presence of 7 μg of GST–Pak1 pzi-binding domain (PBD) agarose (Upstate) for 1 h at 4 °C. The beads were washed three times with 1% Nonidet P-40 washing buffer. The beads were suspended in sample buffer, separated by 4–20% SDS–PAGE, transferred to a nitrocellulose membrane, and blotted with anti-HA monoclonal antibody.

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The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors

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Migration is a basic feature of many cell types in a wide range of species[1]. Since the 1800s, cell migration has been proposed to occur in the nervous and immune systems[2,3], and distinct molecular cues for mammalian neurons and leukocytes have been identified. Here we report that Slit, a secreted protein previously known for its role of repulsion in axon guidance and neuronal migration, can also inhibit leukocyte chemotaxis induced by chemotactic factors. Slit inhibition of the chemokine-induced chemotaxis can be reconstituted by the co-expression of a chemokine receptor containing seven transmembrane domains and Roundabout (Robo), a Slit receptor containing a single transmembrane domain. Thus, there is a functional interaction between single and seven transmembrane receptors. Our results reveal the activity of a neuronal guidance cue in regulating leukocyte migration and indicate that there may be a general