Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 359 (2007) 121–128
BBRC
www.elsevier.com/locate/ybbrc

Bi-directional transport of GLUT4 vesicles near the plasma membrane of primary rat adipocytes

Ying-Ke Xu, Ke-Di Xu, Jie-Yue Li, Lin-Qing Feng, Di Lang, Xiao-Xiang Zheng *

Key Laboratory for Biomedical Engineering of Ministry of China, Department of Biomedical Engineering, Zhejiang University, Zhejiang 310027, PR China

Received 9 May 2007
Available online 22 May 2007

Abstract

Insulin stimulates glucose uptake into adipocytes by mobilizing intracellular membrane vesicles containing GLUT4 proteins to the plasma membrane. Here we applied time-lapse total internal reflection fluorescence microscopy to study moving parameters and characters of exogenously expressed GLUT4 vesicles in basal, insulin and nocodazole treated primary rat adipocytes. Our results showed that microtubules were essential for long-range transport of GLUT4 vesicles but not obligatory for GLUT4 distribution in rat adipocytes. Insulin reduced the mobility of the vesicles, made them tethered/docked to the PM and finally had constitutive exocytosis. Moreover, long-range bi-directional movements of GLUT4 vesicles were visualized for the first time by TIRFM. It is likely that there are interactions between insulin signaling and microtubules, to regulating GLUT4 translocation in rat adipocytes.

Keywords: GLUT4 vesicles; Microtubule; Bi-directional transport; TIRF; Rat adipocyte

Glucose transporter 4, the primarily GLUT isoform in muscle and fat cells, is responsible for insulin-stimulated glucose uptake into these tissues and maintaining the physiological glucose homeostasis [1]. In unstimulated adipocytes, GLUT4 is excluded from the PM and retained by multistep process involving slow exocytosis and rapid endocytosis [2,3]. Insulin considerably increases GLUT4 exocytosis, resulting in a net redistribution of GLUT4 to the PM [3,4].

In adipocytes, GLUT4 is carried to the PM by specialized tubulo-vesicular compartments (referred to as GLUT4 vesicles here) packed with the transporters [5]. Diverse microscopy approaches have showed that GLUT4 localizes to several elements of the recycling pathways, including the endosomal recycling compartment, the Golgi complex and the *trans*-Golgi network (TGN) [6]. The intercellular itinerary of GLUT4 is rather complex, with GLUT4 cycling directly between the endosomes and the TGN or with a specialized intracellular compartment that constitutes the insulin-responsive pool of GLUT4 [3,7,8]. Given the uncertainty of the physical characterization of the intracellular compartments that sequestered GLUT4 and the complexity of the GLUT4 recycling pathways, the basal and insulin-stimulated GLUT4 movements in adipocytes still require further investigation.

Microtubules play central roles in the cellular transport and trafficking of many different types of vesicles [9]. Live cell TIRFM studies in primary rat adipocytes demonstrate that GLUT4 vesicles have long-range movements along microtubules [10]. There are accumulating evidences favor that insulin-stimulated GLUT4 distribution in adipocytes are dependent on microtubule structure [11–13]. Furthermore, pharmacological disruption of the microtubules or expression of mutant kinesins have been shown to partially inhibit GLUT4 recycling and insulin-stimulated GLUT4 translocation [11,13,14]. Kinesin and dynein motors, the two types of molecular motors which participate in the long-range transport of the cargos by microtubules are

Abbreviations: GLUT4, glucose transporter 4; PM, plasma membrane; TIRF, total internal reflection fluorescence; TIRFM, TIRF microscopy; ROI, region of interest.

* Corresponding author. Fax: +86 571 8795 3860.
E-mail address: zxx@mail.bme.zju.edu.cn (X.-X. Zheng).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.05.075

uni-directional orientated, with the kinesins to move cargos towards the cell periphery, and dyneins to bring cargos back [15]. When kinesins and dyneins are simultaneously binding to the cargos or the activities or existence of the motors are spatially and temporally coordinated, cargoes could be moved bi-directionally along the microtubules. To date, a number of cargoes have been shown to display bi-directional motion, like the secretory vesicles, mitochondria, pigment granules, viruses, intermediate filaments and mRNA particles [16]. In rat adipocytes, the roles that the microtubules play on the intracellular GLUT4 translocation and insulin-stimulated glucose transport still remained controversial [17,18].

Here we used a GFP-based labeling system and time-lapse TIRFM to investigate the movement of exogenously expressed GLUT4 vesicles in primary rat adipocytes. Our goal was to determine the basic parameters of GLUT4 vesicles movement in basal, insulin and microtubule network disruption conditions, thus to ascertain the possible mechanisms underlying the microtubule-dependent transport and regulated GLUT4 vesicles distribution in rat adipocytes.

Materials and methods

Materials. The construct HA-GLUT4-GFP was kindly provided by Dr. Samuel Cushman. For transfection experiments, the plasmid was purified in milligram quantities using a Qiagen maxiprep kit (Valencia, CA). All reagents were obtained from Sigma (St. Louis, MO) unless otherwise noted.

Preparation and electroporation of isolated rat adipocytes. All procedures used in this study were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (China Ministry of Health) and complied with the NIH guidelines on handling of experimental animals. Adipocytes were prepared from epididymal fat pads of male Sprague–Dawley rats (150–200 g) as described [19]. Briefly, epididymal fat pads were removed, minced and digested with type I collagenase (Invitrogen, Carlsbad, CA). The preparation of the cells was carried out at 37 °C in KRBH buffer, pH 7.4, containing 10 mM $NaHCO_3$, 30 mM Hepes, 200 nM adenosine, and 1% (w/v) BSA. Isolated cells were then washed three times with KRBH and twice with DMEM (Gibco, Carlsbad, CA) supplemented with 5% (w/v) BSA and 200 nM PIA. The cells were transfected by electroporation as previously described [19]. Briefly, 200 μL of the cell suspension were added to 200 μL of DMEM containing 100 μg of sheared herring sperm DNA and 5 μg of the construct. Electroporation was carried out in 0.4 cm gap-width cuvettes with three square wave pulses of 12 ms at 200 V using GenePulser Xcell electroporation system (Bio-Rad, Richmond, CA). After electroporation, the cells were cultured at 37 °C, 5% CO_2 in DMEM containing 3.5% BSA, PIA, ampicillin and gentamycin. The cells used for experiments were within 16–24 h of incubation.

TIRF microscopy. The Prism-less TIRFM setup was based on an Olympus IX81 inverted microscope, equipped with an argon laser (excitation λ=488 nm, 10 mW), a 60×1.45 NA oil immersion objective (PlanApoN, Olympus). Illumination angle was set to produce the shallowest evanescent illumination (≈100 nm). Time-lapse digital images of ROI were acquired on a cooled CCD camera (iXon, ANDOR Technology), 5–20 frames per second, under the control of Andor iQ software (Version 1.5, Media Cybernetics, Inc.).

The procedure for monitoring the GFP-labeled GLUT4 vesicle motion in primary rat adipocytes is described elsewhere [10], but made some modification. Briefly, the cells were washed, incubated with 100 nM insulin or nocodazole with indicated time in KRBH buffer, 37 °C, pH 7.4, containing 200 nM adenosine, 5% (w/v) BSA. After treatment, a 15 μL volume of the cell suspension in KRBH buffer supplemented with 2% Ficoll-400 (Pharmacia, USA) was placed over a glass slide, and then a 20×20 mm coverslip was placed over the slide and squashed slightly to enable a good contact between the cells and the coverslip. The slide was then sealed with colorless nail polish and put into a thermostat-controlled chamber in 37 °C throughout the experiments.

Immunofluorescent staining of fixed cells. After treatment, cells were washed with KRBH buffer and fixed with 4% paraformaldehyde in PBS for 20 min at room temperature (RT). The fixative was removed by three washes with PBS, and the aldehyde-induced nonspecific bindings were quenched with 50-mM glycine in PBS. Fixed cells were permeabilized and blocked with 0.1% Triton X-100, 1% BSA, and 3% normal goat serum in PBS for 45 min at RT, and then incubated with the appropriate antibodies. Microtubules were detected using mouse anti-β-tubulin (1:200 dilution, Molecular Probes) followed by Alexa Fluor 488-conjugated anti-mouse IgG (1:100 dilution). F-actin was stained by Alexa Fluor 488 phalloidin (1:200 dilution). Finally, the cells were washed, mounted and observed with TIRF microscope.

Imaging date analysis. Image J 1.37 (National Institute of Health) was applied to detect and calculate the number of vesicles in individual TIRF images. Gaussian fit procedure was implemented to measure the true coordinates and central intensity of a vesicle. For tracking the long-range movement of GLUT4 vesicles in an image stack, the starting point was designated visually and the tracked vesicle in the subsequent images was located through the identical Gaussian fit procedure. The trajectory of the movement was then made by lining the located vesicles over time. Meanwhile, many characteristic parameters such as the velocity and displacement can also be computed. To implement these tracking methods, MATLAB 7.1 (The MathWorks Inc., USA) has been utilized.

Results

Movements of GLUT4 vesicles near the PM of rat adipocytes

Application of TIRFM made us possible to visualize single GLUT4 vesicles near the PM of rat adipocytes (see supplemental materials). In basal state, GLUT4 vesicles near the PM were highly mobile. We detected 16.0 ± 3.4 vesicles per 100 μm^2 area of the PM (12 cells, SD) in the TIRF zone. The vesicles had long-range (>10 μm) lateral movements along predefined trajectories (Fig. 1A). Sometimes, they were moved on the same trajectories one after another (Fig. 1C and video 1). The vesicles tended to intermittently stop for a period of time at some special places, usually two or more vesicles were stopped at the same position. The time of stopping were varied on different positions or different vesicles on the same position, usually from hundreds of millisecond to 1.5 min (video 2).

Constitutive exocytosis of GLUT4 vesicles could be detected when treated with insulin (video 3, see supplemental materials). Long-range movements of GLUT4 vesicles in TIRF zone were reduced and most of the vesicles were joggled, showing restricted motions after insulin stimulation (Fig. 1B). After 5-min of insulin stimulation, we detected 16.5 ± 2.8 vesicles per 100 μm^2 area of the PM (12 cells, SD) in the TIRF zone. The vesicles appeared in the TIRF zone seldom moved out, with the time on, they tended to move into clusters, which displayed bright and large dots in TIRF images. Insulin stimulation induced

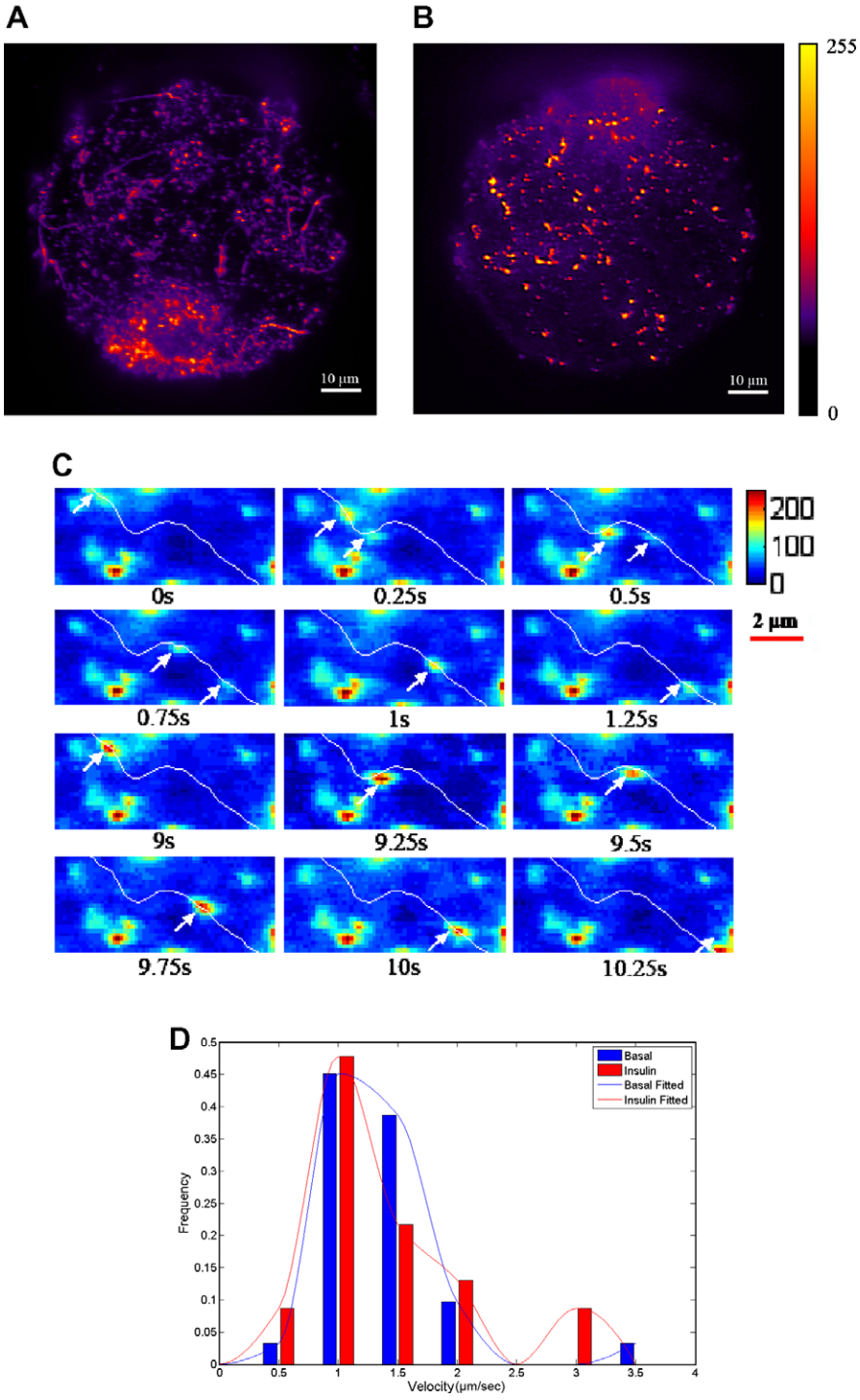

Fig. 1. Movements of GLUT4 vesicles in basal and insulin-stimulated adipocytes. Projection images of basal (A) and 30 min of insulin-stimulation adipocytes (B) made from 1.5-min-long recordings. (C) Sequential images showed three vesicles (white arrows) taking the same trace (white line). (D) Histograms of the mean velocities of long-range moving (>10 μm) GLUT4 vesicles in basal and insulin-stimulated adipocytes.

docking of vesicles to the PM, but not the velocity of the moving vesicles. The mean velocity of long-range (>10 μm) moving GLUT4 vesicles in basal and insulin-treated conditions were compared in Fig. 1D. The distribution of the velocity did not have dominated difference between the two treatments, both showing distinct peaks at 1 and 1.5 μm/s.

Long-range movements of GLUT4 vesicles are dependent on microtubule network

To determine the role of intact microtubule cytoskeleton in intracellular GLUT4 transport, we examined the effect of nocodazole on the integrity of the microtubule network of rat adipocytes by morphological study. The microtubules

were observed as filamentous networks developed near the PM, dense bundles and extensive network of microtubules could be clearly visualized under TIRFM. Treatment with nocodazole resulted in a dose-dependent disappearance of these filamentous signals, but without affecting the cell morphology (Fig. 2A). The concentration of 100 μM completely disrupted the microtubule network of primary rat adipocytes. The filamentous and extensive networks of microtubules were disappeared and the tubulins were aggregated into short and dense structures. How-

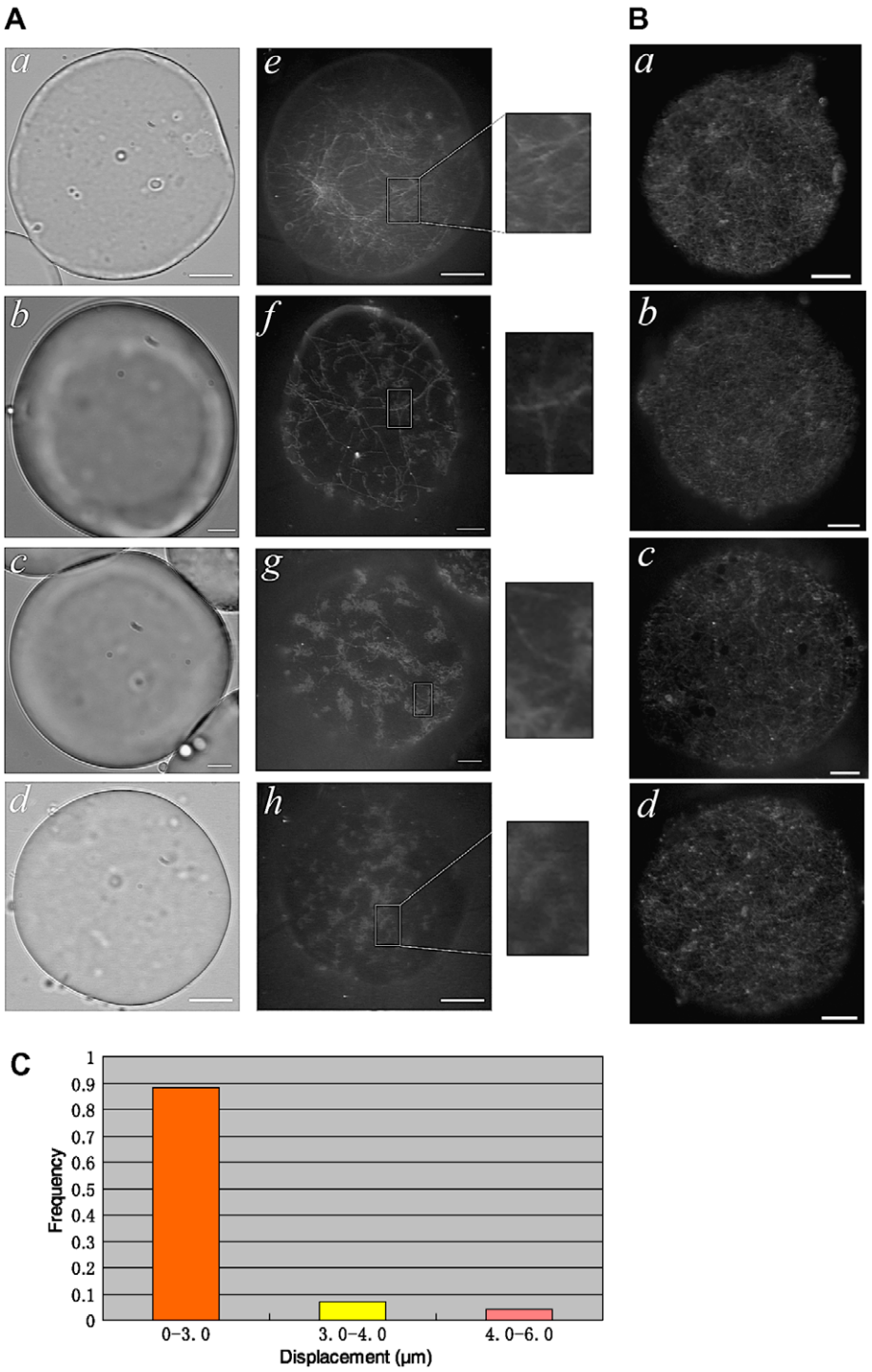

Fig. 2. Role of microtubules in long-range movements of GLUT4 vesicles. (A) Dose-dependent disruption of the microtubules near the PM by nocodazole. Isolated rat adipocytes in KRBH buffer were incubated for 30 min without (a and e) or with 1 (b and f), 10 (c and g), or 100 μM (d and h) of nocodazole. The cells were fixed and the microtubules were visualized with anti-β-tubulin antibody as described under Materials and methods. (a–d) Images obtained in DIC. (e–h) Images obtained by TIRFM. (B) Visualization of cortical actin after nocodazole treatments by TRIFM. Adipcoytes in KRBH buffer were incubated without (a) or with 1 (b), 10 (c), 100 μM (d) of nocodazole for 30 min at 37 °C. The cells were then fixed and immunostained for F-actin. (C) Distribution of the displacements of moving GLUT4 vesicles after treatment with 100 μM of nocodazole for 30 min. Bars, 10 μm.

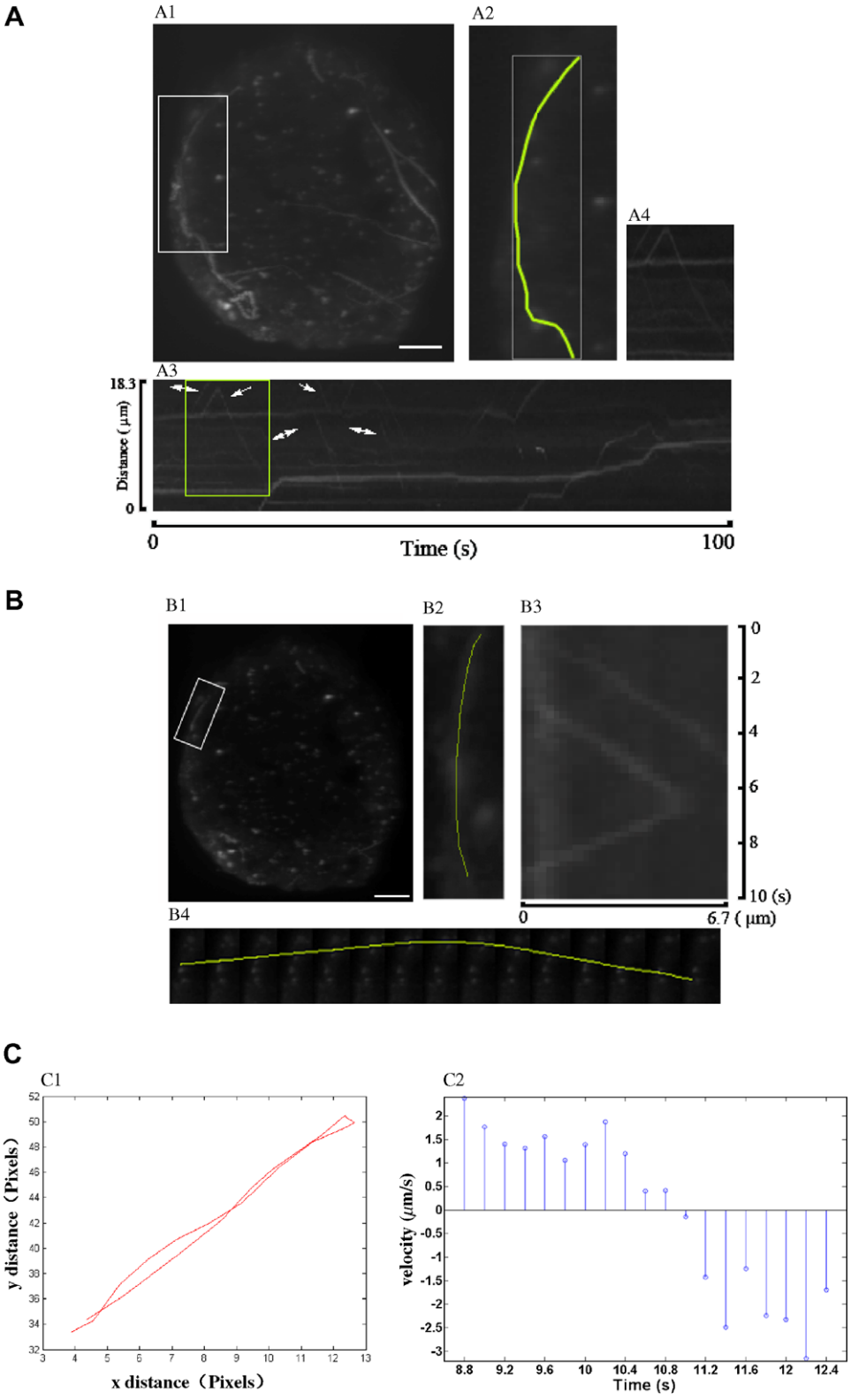

Fig. 3. Visualization of long-range bi-directional transport of GLUT4 vesicles in rat adipocytes. Kymography studies of sequential images made from 100 s of recording (A) and segmented images (B) to investigate the moving characters of interesting vesicles. Bars, 10 μm. For research purpose, projection images (A1 and B1) of sequential images were made to visualize the moving trajectories. (A2 and B2) are a higher magnification of the ROIs and corresponding lines (yellow lines) used to measure the movements. (A3 and B3) are kymographs of single moving GLUT4 vesicles. The white rectangle in (B1) demarcates the region used to generate the montage in (B4). (B4) A long-range bi-directional moving vesicle, underlined in yellow. (C) Single vesicle tracking and its velocity measurement. (C1) The trajectories of bi-directional moving vesicle made by computer-based tracking. Note the difference in scale of the *x*–*y* axes. The velocities of the moving vesicle were plotted in (C2). Kymographs and movies were created with Andor iQ software. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ever, nocodazole treatments had little effects on the integrity of the cortical actin network (Fig. 2B).

These morphological evidence were corroborated by time-lapse videos made using TIRFM. Depolymerization of the microtubule network by 100 μM nocodazole for 30 min almost completely abolished lateral long-range movements of GLUT4 vesicles in rat adipocytes (video 4). Comparing with those cells of basal state, nocodazole-treated cells had fewer vesicle dots in TIRF zone, 13.3 ± 3.2 (10 cells, SD) VS 16.0 ± 3.4 vesicles ($P > 0.1$) per 100 μm^2 area of the PM. By computer-based single vesicle tracking, the maximum displacements of the vesicles in nocodazole-treated cells were calculated. The displacements (70 vesicles in 5 cells, 2.13 ± 0.87 μm, SD) less than 4 μm consisted of approximately 96% of the moving vesicles (Fig. 2C).

GLUT4 vesicles displayed bi-directional movements along the microtubules in basal adipocytes

By stacks projection, the trajectories of moving vesicles could be clearly depicted (Fig. 3A1). In kymographs, the vesicles which took the same pathways exhibited parallel trajectories (Fig. 3A3, two-head arrows), whereas bi-directional moving vesicles showed "V" or inverted "V" shaped trajectories (one-head arrows). The kymograph of a bi-directional moving vesicle was given in Fig. 3B3, which showed that preceding the bi-directional moving one, there was another vesicle and they were moving on the same trajectory (video 5). The montage shown in Fig. 3B4 demonstrated that the tendency of the moving vesicle fitted greatly to the yellow line. Furthermore, single vesicle tracking was applied to analyze the x–y plane trajectories of the bi-directional moving vesicle (Fig. 3C1). Under the circumstance that the fluorescence of the moving vesicle was seldom varied, we assume that the forward and backward trajectories were mostly overlapped. The velocity distributions of the vesicle were nearly symmetrical, except that the mean velocity of backward moving was slightly exceeding the forward (2.08 VS 1.34 μm/s).

Discussion

It has been well established that insulin treatment increases the amount of GLUT4 transporters present in the PM of rat adipocytes [19–22]. The exocytic steps regulated by insulin are not known and potentially include: mobilization of a pool of GLUT4 vesicles, increased movement of GLUT4 vesicles, increased docking or fusion of GLUT4 vesicles with the PM. In basal state, the vesicles usually had long-range movements in the TIRF zone, with their trajectories extensively spread on the entire PM (Fig. 1A). Insulin stimulation reduced the mobility of the vesicles and made them tightly docked to the PM. In our studies, we demonstrated that insulin halted the long-range movements of GLUT4 vesicles and increased their docking to the PM. The docking and fusion of GLUT4 vesicles with target membrane is mediated by specific SNARE proteins [1]. In rat adipocytes, the vesicles were found to be preferable stopped at some specific places on the PM and their time of stopping were shown to be strictly regulated. It is still unclear how docking and fusion of GLUT4 with the PM were regulated by insulin. But studies in 3T3-L1 adipocytes revealed that the vesicle and target-SNARE proteins that mediate fusion of GLUT4 vesicles are localized in specific intracellular membranes [22]. Furthermore, the protein Munc18 in adipocytes which mediates the assembly of SNARE proteins has been showed in chromaffin cells to regulate the docking time of vesicles [23]. Thereby, it was reasonable to hypothesize that insulin could regulate individual vesicles, to determine when, and how long their staying in specific positions on the PM. Meanwhile, the temporal and spatial recruitments of various effector molecules, including molecular tethers and some other signaling molecules involved should not be neglected in this process.

The microtubule network has been shown to be necessary to get full GLUT4 redistribution in response to insulin stimulation [3,11,12,14,24]. In the current studies, we believe that long-range movements of GLUT4 vesicles in rat adipocytes are dependent on the microtubules. First, GFP tagged GLUT4 vesicles were showed to be moved in long-range trajectories, which were clearly visualized by projection images produced from sequential TIRF images (Fig. 1). Second, treatment with nocodazole resulted in a dose-dependent disruption of the microtubules near the PM. At a concentration of 100 μM, nocodazole almost abolished the long-range movements of GLUT4 vesicles. In addition, we postulate that microtubule network is facilitate for long-range transport of GLUT4 vesicles but not obligatory for GLUT4 redistribution in rat adipocytes. This hypothesis is based on several observations. First, the differences of GLUT4 vesicles per 100 μm^2 area of the PM of the basal state, insulin or nocodazole-treated cells were not significant. Second, the frequency of long-range movements of GLUT4 vesicles was reduced after insulin stimulation, but not the overall velocity of those vesicles (Fig. 1D). Third, depolymerization of microtubules by nocodazole did not affect the cortical actin arrangement near the PM, and could not abolish the short-range movements (<4 μm) of GLUT4 vesicles (Fig. 2). From the results stated above, we suggested that actin network may also play a role in intercellular GLUT4 transport and redistribution, what have been shown to be existed in 3T3-L1 adipocytes previously [13]. Taken together, these results indicated that long-range movements of GLUT4 vesicles are essentially microtubule-dependent in rat adipocytes and microtubules are not obligatory for GLUT4 distribution in rat adipocytes.

In neurons, directional transport of vesicles is strictly regulated in order to get fully distribution of neurotransmitter receptors, ion channels or specific mRNAs, what are fundamental for neuronal function and survival [25]. In adipocytes, lots of studies have favored that there are interactions between microtubule network and insulin

signaling [26]. Understanding the precise mechanism by which the interaction with the microtubules is very important and requires further study. In our studies, by single vesicle tracking and kymography studies, long-range bi-directional movements of GLUT4 vesicles in basal adipocytes were lively visualized (Fig. 3). Its mean velocity was corresponding to those reported for microtubule-based transport [9,27]. Surprisingly, the long-range bi-directional movements were only being found in cells of basal state but not in insulin-stimulated ones in our studies.

These observations are quite novel and raise a series of hypotheses concerning the mechanisms of GLUT4 translocation. First, kinesin and dynein motors may collectively bind to GLUT4 vesicles and insulin signaling could temporally and spatially coordinate their activities in rat adipocytes. In a number of cases, there have been shown that both dynein and kinesin are found on the same vesicle at the same time and the bi-directional movements of cargos along the microtubules are under tight regulation [28,29]. Actually, *in vitro* studies have revealed that insulin stimulation leads to dissociation of dynein from microtubules and increases the binding of KIF3 to microtubules in 3T3-L1 adipocytes [30,31]. Second, some effector molecules or signaling molecules could be transported via microtubules to the neighborhood of vesicles, to specify the vesicles to be hauled, docked or moved. Third, given the heterogeneity of GLUT4 vesicles in rat adipocytes (we found existence of spherical and tubular GFP tagged GLUT4 vesicles in rat adipocytes), different subpopulations of GLUT4 vesicles may display different moving modes and employ different molecular motors. The precise mechanism for bi-directional transport is still unclear, and requires further studies in the future.

Acknowledgments

We are indebted to Dr. Samuel Cushman for generously providing the HA-GLUT4-GFP construct as well as advice on electroporation procedure. This work was supported by the Key Laboratory for Biomedical Engineering of Ministry of China.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.05.075

References

[1] R.T. Watson, M. Kanzaki, J.E. Pessin, Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes, Endocr. Rev. 25 (2004) 177–204.
[2] G.D. Holman, L. Lo Leggio, S.W. Cushman, Insulin-stimulated GLUT4 glucose transporter recycling. A problem in membrane protein subcellular trafficking through multiple pools, J. Biol. Chem. 269 (1994) 17516–17524.
[3] O. Karylowski, A. Zeigerer, A. Cohen, T.E. McGraw, GLUT4 is retained by an intracellular cycle of vesicle formation and fusion with endosomes, Mol. Biol. Cell 15 (2004) 870–882.
[4] S. Satoh et al., Use of bismannose photolabel to elucidate insulin-regulated GLUT4 subcellular trafficking kinetics in rat adipose cells. Evidence that exocytosis is a critical site of hormone action, J. Biol. Chem. 268 (1993) 17820–17829.
[5] S. Rea, D.E. James, Moving GLUT4: the biogenesis and trafficking of GLUT4 storage vesicles, Diabetes 46 (1997) 1667–1677.
[6] N.J. Bryant, G. Roland, D.E. James, Regulated transport of the glucose transporter GLUT4, Nat. Rev. Mol. Cell. Biol. 3 (2002) 267–277.
[7] S. Martin et al., Effects of insulin on intracellular GLUT4 vesicles in adipocytes: evidence for a secretory mode of regulation, J. Cell Sci. 113 (2000) 3427–3438.
[8] A. Zeigerer et al., GLUT4 retention in adipocytes requires two intracellular insulin-regulated transport steps, Mol. Biol. Cell 13 (2002) 2421–2435.
[9] J.P. Caviston, E.L. Holzbaur, Microtubule motors at the intersection of trafficking and transport, Trends Cell Biol. 16 (2006) 530–537.
[10] V.A. Lizunov, H. Matsumoto, J. Zimmerberg, S.W. Cushman, V.A. Frolov, Insulin stimulates the halting, tethering, and fusion of mobile GLUT4 vesicles in rat adipose cells, J. Cell Biol. 169 (2005) 481–489.
[11] L.M. Fletcher, G.I. Welsh, P.B. Oatey, J.M. Trvaré, Role for the microtubule cytoskeleton in GLUT4 vesicle trafficking and in the regulation of insulin-stimulated glucose uptake, Biochem. J. 352 (2000) 267–276.
[12] A.L. Olson, A.R. Trumbly, G.V. Gibson, Insulin-mediated GLUT4 translocation is dependent on the microtubule network, J. Biol. Chem. 276 (2001) 10706–10714.
[13] J. Huang, T. Imamura, J.L. Babendure, J.C. Lu, J.M. Olefsky, Disruption of microtubules ablates the specificity of insulin signaling to GLUT4 translocation in 3T3-L1 adipocytes, J. Biol. Chem. 280 (2005) 42300–42306.
[14] S. Semiz et al., Conventional kinesin KIF5B mediates insulin stimulated GLUT4 movements on microtubules, EMBO J. 22 (2003) 2387–2399.
[15] R. Mallik, S.P. Gross, Molecular motors: strategies to get along, Curr. Biol. 14 (2004) R971–R982.
[16] M.A. Welte, Bidirectional transport along microtubules, Curr. Biol. 14 (2004) R525–R537.
[17] J.C. Molero, J.P. Whitehead, T. Meerloo, D.E. James, Nocodazole inhibits insulin-stimulated glucose transport in 3T3-L1 adipocytes via a microtubule-independent mechanism, J. Biol. Chem. 276 (2001) 43829–43835.
[18] S. Shigematsu, A.H. Khan, M. Kanzaki, J.E. Pessin, Intracellular insulin-responsive glucose transporter (GLUT4) distribution but not insulin-stimulated GLUT4 exocytosis and recycling are microtubule dependent, Mol. Endocrinol. 16 (2002) 1060–1068.
[19] K. Dawson, A. Aviles-Hernandez, S.W. Cushman, D. Malide, Insulin-regulated trafficking of dual-labeled glucose transporter 4 in primary rat adipose cells, Biochem. Biophys. Res. Commun. 287 (2001) 445–454.
[20] D. Malide, N.K. Dwyer, E.J. Blanchette-Mackie, S.W. Cushman, Immunocytochemical evidence that GLUT4 resides in a specialized translocation post-endosomal VAMP2-positive compartment in rat adipose cells in the absence of insulin, J. Histochem. Cytochem. 45 (1997) 1083–1096.
[21] L.B. Liu, W. Omata, I. Kojima, H. Shibata, Insulin recruits GLUT4 from distinct compartments via distinct traffic pathways with differential microtubule dependence in rat adipocytes, J. Biol. Chem. 278 (2003) 30157–30169.
[22] L.H. Chamberlain, G.W. Gould, The vesicle- and target-SNARE proteins that mediate GLUT4 vesicle fusion are localized in detergent-insoluble lipid rafts present on distinct intracellular membranes, J. Biol. Chem. 277 (2002) 49750–49754.
[23] R.F. Toonen et al., Dissecting docking and tethering of secretory vesicles at the target membrane, EMBO J. 25 (2006) 3725–3737.

[24] A. Guilherme et al., Perinuclear localization and insulin responsiveness of GLUT4 requires cytoskeletal integrity in 3T3-L1 adipocytes, J. Biol. Chem. 275 (2000) 38151–38159.
[25] N. Hirokawa, R. Takemura, Molecular motors and mechanisms of directional trasport in neurons, Nat. Rev. Neurosci. 6 (2005) 201–214.
[26] C.A. Eyster, Q.S. Duggins, G.J. Gorbsky, A.L. Olson, Microtubule network is required for insulin signaling through activation of Akt/protein kinase B: evidence that insulin stimulates vesicle docking/fusion but not intracellular mobility, J. Biol. Chem. 281 (2006) 39719–39727.
[27] H. Higuchi, S.A. Endow, Directionality and processivity of molecular motors, Curr. Opin. Cell Biol. 14 (2002) 50–57.
[28] L.A. Ligon, M. Tokito, J.M. Finklestein, F.E. Grossman, E.L.F. Holzbaur, A direct interaction between cytoplasmic dynein and kinesin I may coordinate motor activity, J. Biol. Chem. 279 (2004) 19201–19208.
[29] S.W. Deacon, A. Nascimento, A.S. Serpinskaya, V.I. Gelfand, Regulation of bidirectional melanosome transport by organelle bound MAP kinase, Curr. Biol. 15 (2005) 459–463.
[30] J. Huang, T. Imamura, J.M. Olefsky, Insulin can regulate GLUT4 internalization by signaling to Rab5 and the motor protein dynein, Proc. Natl. Acad. Sci. USA 98 (2001) 13084–13089.
[31] T. Imamura et al., Insulin-Induced GLUT4 Translocation Involves Protein Kinase C-λ-mediated functional coupling between Rab4 and the motor protein kinesin, Mol. Cell. Biol. 23 (2003) 4892–4900.