Rab10 delivers GLUT4 storage vesicles to the plasma membrane

Yu Chen and Jennifer Lippincott-Schwartz*
The Eugene Kennedy Shriver National Institute of Child Health and Human Development; National Institutes of Health; Bethesda, MD USA

The glucose transporter, GLUT4, redistributes to the plasma membrane (PM) upon insulin stimulation, but also recycles through endosomal compartments. Different Rab proteins control these transport itineraries of GLUT4. However, the specific roles played by different Rab proteins in GLUT4 trafficking has been difficult to assess, primarily due to the complexity of endomembrane organization and trafficking. To address this problem, we recently performed advanced live cell imaging using total internal reflection fluorescence (TIRF) microscopy, which images objects ~150 nm from the PM, directly visualizing GLUT4 trafficking in response to insulin stimulation. Using IRAP-pHluorin to selectively label GSVs undergoing PM fusion in response to insulin, we identified Rab10 as the only Rab protein that binds this compartment. Rab14 was found to label transferrin-positive, endosomal compartments containing GLUT4. These also could fuse with the PM in response to insulin, albeit more slowly. Several other Rab proteins, including Rab4A, 4B and 8A, were found to mediate GLUT4 intra-endosomal recycling, serving to internalize surface-bound GLUT4 into endosomal compartments for ultimate delivery to GSVs. Thus, multiple Rab proteins regulate the circulation of GLUT4 molecules within the endomembrane system, maintaining optimal insulin responsiveness within cells.

Insulin stimulates GLUT4 redistribution to the plasma membrane (PM) in adipocytes and muscle cells. This results in increased glucose influx into these cells, leading to reduction of circulating glucose level in the blood. GLUT4 redistribution to the PM involves physical trafficking of GLUT4 storage vesicles (GSVs) to the PM and is regulated by a signaling cascade of PI3K, AKT/PKB and AS160.1-3 In this cascade, PI3K and AKT/PKB are activated in response to insulin stimulation, causing phosphorylation of the Rab GTPase activation protein (GAP) AS160 by AKT.4 Akt phosphorylation inactivates the AS160 GAP domain, making it unable to stimulate hydrolysis of GTP on the Rab.5,7 In response, GSVs redistribute from internal sites to the PM, where fusion finally occurs. The negative regulatory role of AS160 Rab GAP domain in insulin-stimulated GSV delivery to the PM implicated Rab proteins as key regulators (downstream of AS160) for PM delivery of GSVs.8,9

After being delivered to the PM during insulin stimulation, GLUT4 is endocytosed into the endosomal system and recycles through early endosomes, recycling endosomes and a part of the trans-Golgi network before being reloaded into GSVs.10,11 This complex intracellular trafficking itinerary results in GLUT4 having a broad distribution pattern, with steady-state localization in many intracellular compartments. As these different compartments are characterized by having different Rab proteins associated with them,5,12,13 it has been difficult to determine which compartment(s) and its associated Rab protein(s) specifically responds to insulin through inactivation of AS160 by AKT.14-16

To identify the specific Rab protein(s) mediating delivery of GSVs to the PM after insulin stimulation, we employed insulin-responsive aminopeptidase (IRAP, which has the same intracellular distribution as...
GLUT4 vesicles in the TIRF zone. Endosomal GLUT4 vesicles associated by endocytic Rab proteins (Rab4A, Rab4B and Rab8A) are abundant in the TIRF zone. After insulin stimulation, GSVs labeled by Rab10 start to move into the TIRF zone and fuse at the PM. Because of their efficient fusion, each GSV only appears transiently in the TIRF zone, leading to GSVs making a very small fraction of GLUT4 vesicles in the TIRF zone during insulin stimulation.

We next investigated whether it is Rab10 or Rab14 that directly mediates GSV translocation to the PM in response to insulin. Rab14 was localized on compartments containing GLUT4 and TIRF, suggesting it regulated movement of GLUT4 within endosomes. On the other hand, Rab10 was only localized on TIR negative structures that contained GLUT4. This suggested it was responsible for GSV delivery to the PM. Trafficking of GLUT4 through both Rab10 and Rab 14 compartments appeared to be important under insulin stimulation since loss of Rab10 and Rab14 additively inhibited GLUT4 delivery to the PM, and re-addition of either Rab10 or Rab14 partially restored GLUT4 translocation. Given Rab10’s role in mediating GSV delivery to the PM, its regulatory role was further explored. Rab10 was associated with GSVs as the vesicles moved into the TIRF zone under insulin stimulation. This indicated its association with GSVs occurs early during insulin signaling and deep inside the cells. A Rab10 constitutively active mutant was found to be associated with GSVs and induced GSV recruitment to the TIRF zone in the absence of insulin stimulation. This indicated Rab10 activation is sufficient to recruit GSVs to the cell periphery.

These findings have helped clarify the exact roles played by different Rab proteins in trafficking of GLUT4 proteins during insulin stimulation. They have further emphasized the heterogeneity of GLUT4 compartments at the cell periphery. Indeed, due to the proximity of endosomal GLUT4 compartments to the PM, most GLUT4 compartments observed by TIRF microscopy are endosomes rather than GSVs. After insulin stimulation, however, Rab10-labeled GSVs begin to enter into the TIRF zone and fuse at the PM. Because they fuse efficiently with the PM upon entry into the TIRF zone, GSVs are only transiently observed in the TIRF zone before disappearing. This contrasts with peripheral endosomal GLUT4 compartments, which fuse with the PM less frequently. Therefore, GSVs comprise only a small percentage of the total GLUT4 compartments in the TIRF zone, even after insulin stimulation. Consequently, GLUT4 vesicle density in the TIRF zone should not be taken as a direct measurement of the delivery of GSVs to the cell periphery.

We are only at the very beginning of understanding the intricacies of GSV formation, translocation and fusion at the PM. Although our studies revealed Rab10 serves as a key component in mobilizing GSVs so that they reach the cell periphery in response to insulin stimulation, further studies are needed to determine what molecules in addition to Rab10 participate in subsequent steps, such as GSV docking and fusion at the PM. The IRAP-pHluorin labeling strategy for monitoring GSV dynamics described in our study should be helpful in revealing these molecular machineries, which include those for anchoring GSVs at the PM and mediating their final fusion (Fig. 1).

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
9. Stenmark H. Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol 2009; 10:513-25; PMID:19693039; http://dx.doi.org/10.1038/nrm2728.

10. Rowland AF, Faxaderley DJ, James DE. Mapping insulin/GLUT4 cyclicity. Traffic 2011; 12:672-81; PMID:21401839; http://dx.doi.org/10.1111/j.1600-0854.2011.01178.x.

11. Bugiani CB, Klip A. Glucose transporter 4: cycling, compartments and controversies. EMBO Rep 2005; 6:1137-42; PMID:16319959; http://dx.doi.org/10.1038/sjembr.7400584.

12. Kaddai V, Gonzalez T, Kesfai F, Grémeaux T, Bonnafous S, Gugenheim J, et al. Rab4b is a small GTPase involved in the control of the glucose transporter GLUT4 localization in adipocyte. PLoS ONE 2009; 4:e5257; PMID:19590752; http://dx.doi.org/10.1371/journal.pone.0005257.

13. Larance M, Ramg M, Stickli J, van Dam EM, Winata S, Wassing V, et al. Characterization of the role of the Rab GT-Pase-activating protein AS160 in insulin-regulated GLUT4 trafficking. J Biol Chem 2005; 280:37803-13; PMID:16154996; http://dx.doi.org/10.1074/jbc.M503897200.

14. Sun Y, Bilan PJ, Liu Z, Klip A. Rab8A and Rab13 are activated by insulin and regulate GLUT4 translocation in muscle cells. Proc Natl Acad Sci USA 2010; 107:19909-14; PMID:21041651; http://dx.doi.org/10.1073/pnas.100952107.

15. Sano H, Roach WG, Pecck GR, Fukuda M, Lienhard GE. Rab10 in insulin-stimulated GLUT4 translocation. J Biol Chem 2008; 283:411-89-95; PMID:18076383; http://dx.doi.org/10.1074/jbc.M702071318.

16. Sano H, Eguez L, Teruel MN, Fukuda M, Chuang TD, Chavez JA, et al. Rab10, a target of the AS160 Rab GAP, is required for insulin-stimulated GLUT4 trafficking in adipocytes. J Biol Chem 2008; 283:411-89-95; PMID:18076383; http://dx.doi.org/10.1074/jbc.M703897200.

17. Jiang L, Fan J, Bai L, Wang Y, Chen Y, Yang L, et al. Direct quantification of fusion rate reveals a distal role for AS160 in insulin-stimulated fusion of GLUT4 storage vesicles. J Biol Chem 2008; 283:8568-16; PMID:18063571; http://dx.doi.org/10.1074/jbc.M708688200.

18. Miesenböck G, De Angelis DA, Rothman JE. Activation of RabA is required for insulin-stimulated Glu4 trafficking to the plasma membrane via the exocyst and the motor protein Myo1c. Dev Cell 2007; 13:391-404; PMID:17756582; http://dx.doi.org/10.1016/j.devcel.2007.07.007.

19. Olson AL, Knight JB, Pessin JE. Syntaxin 4, VAMP2, and/or VAMP3/cellubrevin are functional target membrane and vesicle SNAP receptors for insulin-stimulated GLUT4 translocation in adipocytes. Mol Cell Biol 1997; 17:2425-35; PMID:911311.