Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Biochemical and Biophysical Research Communications 335 (2005) 1199–1210
BBRC
www.elsevier.com/locate/ybbrc

SNARE-mediated trafficking of $\alpha_5\beta_1$ integrin is required for spreading in CHO cells ☆

Michael Skalski, Marc G. Coppolino *

Department of Molecular and Cellular Biology, University of Guleph, Guelph, Ont., Canada N1G 2W1

Received 21 July 2005
Available online 15 August 2005

Abstract

In this study, the role of SNARE-mediated membrane traffic in regulating integrin localization was examined and the requirement for SNARE function in cellular spreading was quantitatively assessed. Membrane traffic was inhibited with the VAMP-specific catalytic light chain from tetanus toxin (TeTx-LC), a dominant-negative form (E329Q) of *N*-ethylmaleimide-sensitive fusion protein (NSF), and brefeldin A (BfA). Inhibition of membrane traffic with either E329Q-NSF or TeTx-LC, but not BfA, significantly inhibited spreading of CHO cells on fibronectin. Spreading was rescued in TeTx-LC-expressing cells by co-transfection with a TeTx-resistant cellubrevin/VAMP3. E329Q-NSF, a general inhibitor of SNARE function, was a more potent inhibitor of cell spreading than TeTx-LC, suggesting that tetanus toxin-insensitive SNAREs contribute to adhesion. It was found that E329Q-NSF prevented trafficking of $\alpha_5\beta_1$ integrins from a central Rab11-containing compartment to sites of protrusion during cell adhesion, while TeTx-LC delayed this trafficking. These results are consistent with a model of cellular adhesion that implicates SNARE function as an important component of integrin trafficking during the process of cell spreading.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Adhesion; Integrin; Membrane traffic; NSF; VAMP3

The process of integrin-mediated cell adhesion requires the precise regulation of integrin localization and activity, reorganization of the actin cytoskeleton, and localized membrane remodeling. Engagement of ECM proteins via cell surface integrin receptors causes a conformational change in and clustering of the integrins, which in turn triggers the association of signaling proteins, such as focal adhesion kinase (FAK), and scaffolding proteins, such as paxillin, vinculin, α-actinin, and talin, at the cytoplasmic domains of integrin subunits [1,2]. The association of signaling and scaffolding proteins with integrins, in "focal complexes," leads to activation of signaling pathways that induce the actin-propelled formation of Rac1-dependent lamellipodia and/or Cdc42-dependent filipodia. As the cell spreads, a shift in signaling occurs away from Rac1/Cdc42 signaling, leading to heightened RhoA signaling; as this occurs, integrins and scaffold proteins in focal complexes are forced into larger adhesive structures (focal adhesions) via myosin-based contraction. Spreading stops once a balance is achieved between actin-driven edge propulsion and forces generated by both myosin contraction and membrane tension [3].

☆ *Abbreviations:* Arf, ADP-ribosylation factor; BfA, brefeldin A; NSF, *N*-ethylmaleimide-sensitive fusion protein; CHO, Chinese hamster ovary; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; EGF, epidermal growth factor; FAK, focal adhesion kinase; FBS, fetal bovine serum; FN, fibronectin; GEF, guanine nucleotide exchange factor; GFP, green fluorescent protein; PDGF, platelet-derived growth factor; SNARE, soluble NSF attachment protein receptor; TMR-Tfn, tetramethylrhodamine-labeled transferrin; TeTx-LC, tetanus toxin light chain; VAMP, vesicle associated membrane protein.

* Corresponding author. Fax: +1 519 837 1802.
E-mail address: mcoppoli@uoguelph.ca (M.G. Coppolino).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.07.195

Integrin-mediated cell spreading is dependent on the deployment of integrins to the cell surface, particularly at sites where new cell–ECM contacts are to be formed. Several recent studies have determined that intracellular trafficking of integrins is required for spreading and migration. These studies have shown a requirement for Arf6 [4], Rab4 [5,6], Rab11 [5], Rab5 [7], Rab8 [8], and dynamin [9] GTPases in cell motility. These GTPases are part of multi-step membrane trafficking pathways, where Arf-family proteins and dynamin control budding at donor membranes, and Rab GTPases and their effectors control tethering and docking at target membranes [10,11]. It is via a series of steps regulated in this manner that integrins have been shown to traffic through an endocytic-recycling pathway, from the early endosome to the recycling endosome and then to the cell surface. For example, EGF- and PMA-induced cell spreading has been found to involve exocytosis of β_1 integrins from the recycling endosome to the leading edge in a pathway dependent on Arf6 and Rab11 [12]. Integrins have also been observed to traffic from early endosomes directly to the plasma membrane via a Rab4-dependent pathway. This pathway was found to enhance adhesion and spreading, but this 'rapid-cycling' was specific for $\alpha_v\beta_3$ integrin under PDGF stimulation; $\alpha_5\beta_1$ integrin, and $\alpha_v\beta_3$ in non-stimulated cells, continued to traffic through the recycling endosome in a Rab11-dependent way [5]. Both studies suggest that recycling endosomes may contain a reservoir of integrins for use in attachment at the leading edge during spreading and/or migration.

In the trafficking of integrin receptors during adhesion, how the final stages of the pathways are regulated is not well understood. Recent studies from our laboratory [13] and other's [14] have implicated the function of SNARE proteins in cell adhesion and migration. Most known membrane traffic pathways culminate in SNARE (soluble NSF attachment protein receptor)-regulated membrane fusion events. Specifically, docking and membrane fusion are regulated by membrane bound SNAREs found on the vesicle (v-SNARE) and target (t-SNARE) membranes [15]. SNARE-mediated traffic is thus potentially involved in several steps during integrin trafficking, including steps that connect early endosomes with recycling endosomes and steps that target integrins to the plasma membrane. Although these trafficking pathways are likely to have significant effects upon cell–ECM interactions, the SNARE-mediated pathways that control integrin traffic during cellular spreading remain to be fully characterized.

In the present study, we quantitatively assessed the requirement for SNARE-mediated membrane traffic during integrin-mediated spreading in CHO cells and investigated the mechanism by which SNAREs contribute to this process. We report that inhibition of the v-SNARE cellubrevin/VAMP3, a known component of recycling endosomes, by expression of the catalytic light chain of tetanus toxin (TeTx-LC) reduced the extent of $\alpha_5\beta_1$-mediated cell spreading on fibronectin (FN). Cell adhesion was more potently impaired by expression of dominant negative NSF (E329Q-NSF), which acts as a general inhibitor of SNARE-mediated membrane traffic. Neither of these disruptions to SNARE function altered cell attachment to FN. Significantly, blocking SNARE function with either TeTx-LC or E329Q-NSF impaired the transport of $\alpha_5\beta_1$ integrin from a central Rab11-containing compartment to sites of lamellipodium protrusion during cell spreading. These results define the role of SNARE-mediated membrane traffic in the movement of integrins through endosomal recycling compartments, a process which is required for normal cellular adhesion.

Materials and methods

Materials. All chemicals were purchased from Sigma Chemical (St. Louis, MO) unless indicated otherwise. Goat polyclonal antibody against TeTx was obtained from Biodesign International (Saco, ME) and the mouse anti-NSF antibodies were purchased from Stressgen (San Diego, CA). The goat anti-FN receptor ($\alpha_5\beta_1$ integrin) and rabbit anti-NSF antibodies were from Chemicon (Temecula, CA). Antibodies against Rab11 were obtained from BD Biosciences (Mississauga, Ont.). Rabbit polyclonal anti-mannosidase II antibody was a kind gift from Dr. M. Farquhar (University of California at San Diego, CA). All secondary antibodies and rhodamine-labeled phalloidin were purchased from Invitrogen (Mississauga, Ont.).

The generation of the pcDNA3.1-wtNSF and pcDNA3.1-E329Q-NSF plasmids and their characterization are described elsewhere [16]. The pcDNA3.1-TeTx-LC, peGFP-VAMP3, and peGFP-vwVAMP3 were kind gifts from Dr. W.S. Trimble (Hospital for Sick Children, Toronto).

Cell culture and transfection. Chinese hamster ovary (CHO)-K1 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (Sigma, Oakville, Ont.), or 5% FBS and 5% FetalClone II (VWR International, Mississauga) at 37 °C and 5% CO_2. The CHO-K1 cells stably expressing E329Q-NSF are described elsewhere [13] and were cultured in DMEM with 10% tetracycline-free FBS, hygromycin and G418 (BD Biosciences, Mississauga, Ont.). Cells were transfected with the various plasmid constructs using either FuGene6 Transfection Reagent (Roche Applied Science, Laval, QC) following the manufacturer's suggested protocol or with $CaPO_4$:DNA complex co-incubation. DNA complexes were incubated on the cells for 10 h when transfecting with NSF constructs or 24 h when transfecting with TeTx-LC. In experiments using BfA, cells were pretreated for 3 h with 5 μg/mL BfA and BfA was maintained at this concentration throughout the experiments.

Cell attachment and spreading assays. All attachment and spreading assays were done on glass coverslips coated with FN. To coat, individual coverslips were first cleansed with 0.1 M NaOH, washed with PBS, and then incubated in 20 μg/mL FN in PBS for 3 h. Before use, the coverslips were washed with PBS to remove excess FN. Treated cells were placed onto coated coverslips for 0.5 h (attachment assays) or 1.5 h (spreading assays). After the allotted time, the coverslips were gently washed and the samples were fixed with 4% paraformaldehyde in PBS. Attachment was assessed by taking digitized images using a 10× lens and counting attached cells in washed and unwashed samples. At least 50 transfected cells were imaged per condition for each experiment. Spreading was quantified by capturing digitized images

Download English Version:

https://daneshyari.com/en/article/10768466

Download Persian Version:

https://daneshyari.com/article/10768466

Daneshyari.com