Biochemical and Biophysical Research Communications 432 (2013) 242–247

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dissect Kif5b in nuclear positioning during myogenesis: The light chain binding domain and the autoinhibitory peptide are both indispensable

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ARTICLE INFO

Article history:
Received 31 January 2013
Available online 10 February 2013

Keywords:
Kif5b
Myonuclear positioning
KLC binding domain
Autoinhibitory peptide
Nesprin 4

ABSTRACT

The microtubule motor kinesin-1 is responsible for the nuclear positioning during myogenesis. Here we show that the coiled-coil stalk/tail domain containing the kinesin light chain (KLC) binding sites targets to the perinuclear region like endogenous Kif5b, while the globular tail domain cannot. To investigate which fragments of kinesin heavy chain (Kif5b) is responsible for the myonuclear positioning, we transfect Kif5b expression constructs into Kif5b deficient myoblasts and test their ability to rescue the myonuclear phenotype. We find that the KLC binding domain and the autoinhibitory peptide in the globular tail region are both indispensable for the nuclear membrane localization of Kif5b and the kinesin-1-mediated myonuclear positioning. These results suggest that while the KLC binding domain may directly targets Kif5b to the myonuclear membrane, the autoinhibitory peptide may play an indirect role in regulating the kinesin-1-mediated myonuclear positioning.

1. Introduction

Kinesin-1, the first discovered microtubule-based kinesin motor, consists of two heavy chains (KHCs) and two light chains (KLCs). The two KHCs form a homodimer, and each heavy chain associates with a light chain. In most non-neuronal mammalian cells, a ubiquitous heavy chain isoform Kif5b expressed along with two light chain isoforms KLC1 and KLC2, while in skeletal muscles, KLC2 is known to be the dominant light chain isoform [1]. KHC contains a head domain composed of a microtubule binding site and an ATP-binding/hydrolysis pocket. The following stalk/tail domain is important for interacting with KLC and other holoenzyme subunits or cargos [2–4]. KLCs contain a highly conserved N-terminal heptad repeat (HR) domain that is responsible for binding to KHC, and a following six modular imperfect repeats of 34 amino acids called tetratrico peptide repeats (TPR), which is known to bind to diverse cargos [5].

During myogenesis, the expression of Kif5b is up-regulated, implying significant function for this motor protein [6]. In wild type muscle fibers, the myonuclei redistribute along the elongating myotubes with proper distance between every two of them, and finally move to the periphery of the mature muscle fibers beneath the sarcolemma. However, the nuclei centrally aggregated in Khc/Kif5b deficient *drosophila* and cultured mammalian myotubes [7], as well as in *Kif5b* conditionally knockout mouse muscle fibers [6], which demonstrated an important function of kinesin-1 in myonuclear positioning.

It has been demonstrated that Ensconsin/MAP7 acts as a linker between Khc/Kif5b and the microtubule cytoskeleton in myogenesis. Kif5b motor domain fused with microtubule-binding domain of MAP7 rescues the centrally aggregated nuclei in MAP7 deficient myotubes [7]. But how Kif5b recruits to myonuclear membrane is less studied. KASH domain family members localized at the outer nuclear membranes and SUN domain family members localized at the inner nuclear membranes bridges the nuclear lamina to the cytoskeleton system [8]. For the recruitment of Kif5b to the nuclear membrane, a KASH protein UNC-83 serves as the adaptor for kinesin-1 in *Caenorhabditis elegants* [9]. In mammalian cells, one of the KASH family members, nesprin 2, associates with Kif5b in developing neuronal tissues [10]; and this interaction is mediated by KLC1 in several cell lines tested [11]. In skeletal muscles development, nesprin 1–3 are expressed [12,13]. Nesprin 1 and 2 are important for myonuclear envelope integrity [14]. Nesprin 1 links the nucleus to the actin cytoskeleton and is important for nuclear positioning [15], while nesprin 3 functions in linking the nucleus to the intermediate filaments [13]. Another KASH family member, nesprin 4, has not been studied in skeletal muscles. Nesprin 4 coimmunoprecipitates KHC in several epithelial cell lines. Yeast-two hybrid

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http://dx.doi.org/10.1016/j.bbrc.2013.02.006

analysis revealed KLCs 1, 2, 3, and 4 to be nesprin 4 binding partners [16].

In our experiment, we found that both endogenous Kif5b and the transfected Kif5b full-length tail strongly localize at the outer nuclear membrane in differentiating myoblasts. Functional analysis demonstrated that in addition to the KLC binding domain, the C-terminal globular tail domain, especially the autoinhibitory peptide, is indispensable for the Kif5b-mediated myonuclear positioning. The coiled-coil stalk/tail domain of Kif5b is necessary for the myonuclear membrane targeting, while the globular tail domain cannot target to nuclear membranes alone, suggesting other regulatory mechanisms involved.

2. Materials and methods

2.1. DNA constructs

HA-tagged and GST-tagged Kif5b constructs were described previously [6]. For Flag-tagged Kif5bs, Kif5b fragments were subcloned with a 3× Flag tag at N-terminus into pcDNA3.1. Mouse KLC2 and nesprin 4 cDNA was amplified by RT-PCR from differentiated wild type myoblast cells and sequenced correct. For Flag-tagged KLC2 constructs, KLC2 1–187 aa or 146–619 aa was subcloned with a 3×Flag tag at N-terminus into pcDNA3.1 to generate pKLC2-HR or pKLC2-TPR, respectively. For HA-tagged nesprin 4, full length nesprin 4 was subcloned with an HA tag at C-terminus into pcDNA3. For His-tagged nesprin 4, nesprin 4 was subcloned into pET28a to generate pET-nesprin 4.

2.2. Antibodies

Kif5b antibody was generated in rabbits against synthesized peptide: FDKEKANLEAFTADKDIA, and purified IgG was used in this study [17]. KLC antibody 63–90 was kindly provided by Dr. S.T. Brady (University of Illinois at Chicago, USA). Other antibodies were as follows: anti-lamin A/C, anti-His and anti-GST were all from Santa Cruz. FITC-Phalloidin (Sigma) was used for F-actin labeling.

2.3. Primary culture of myoblast cells

The primary myoblast cells from wild type ($kif5b^{+/fl}$) and Kif5b conditionally knockout mice ($Pax2\text{-}Cre: Kif5b^{-/fl}$) were isolated as described [6]. The myoblast cells were cultured in DMEM growth medium supplemented with 15% FBS and 2.5 ng/ml bFGF. Differentiation was promoted by changing medium to DMEM supplemented with 3% horse serum [18].

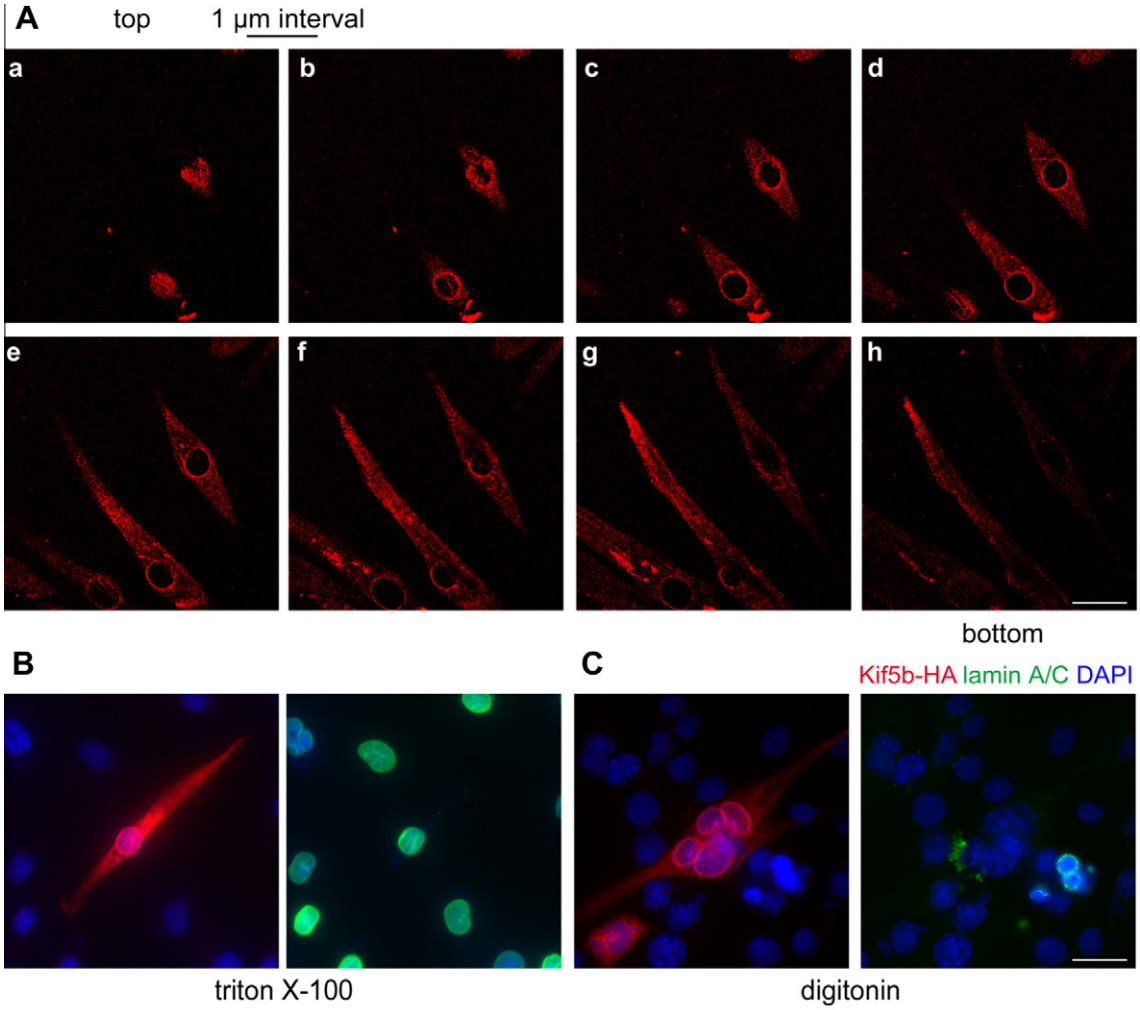

Fig. 1. Kif5b localizes at the outer nuclear membranes. (A) Wild type myoblast cells differentiated for 2 days were immunostained against endogenous Kif5b. The cells were scanned in Z-stack at 1 μm interval. Note that Kif5b formed a ring-like pattern at the nuclear membrane region (b–g). (B and C) Myoblast cells were permeabilized with 0.2% Triton X-100/PBS (B) or 30 μg/ml digitonin (C) before immunostaining. Kif5b staining at the nuclear membrane region was observed with only plasma membrane permeabilization (C), while lamin A/C as a nuclear protein control could only be stained with nuclear membrane permeabilization (B). Scale bar: 20 μm.