

A small part of myosin IIB takes on a big role in cell polarity

Aidan M. Fenix and Dylan T. Burnette

Vanderbilt University School of Medicine, Nashville, TN 37232

A migrating cell must establish front-to-back polarity in order to move. In this issue, Juanes-Garcia et al. (2015, *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201407059>) report that a short serine-rich motif in nonmuscle myosin IIB is required to establish the cell's rear. This motif represents a new paradigm for what determines directional cell migration.

Directed cell movement is instrumental for organismal development, immune responses, and the progression of diseases, such as cancer (Gardel et al., 2010). To achieve directed movement, an individual cell must establish front-to-back polarity, where there is coordinated protrusion of its front and retraction of its back (Fig. 1 A; Vicente-Manzanares et al., 2007). How polymerizing actin filaments drive protrusion of the front is understood in exquisite detail (Pollard and Borisy, 2003; Pollard, 2007). The mechanisms defining how actin filament contraction defines the back of the cell (Yam et al., 2007) have been more difficult to elucidate. Contraction of actin filaments in crawling cells is driven by nonmuscle myosin II (NMII; Vicente-Manzanares et al., 2009). NMII has three isoforms, NMII-A, NMII-B, and NMII-C, all of which can bind and contract actin filaments to generate force. Importantly, NMII-A and NMII-B have different cellular localizations, which could drive their functions (Kolega, 1998; Vicente-Manzanares et al., 2007). NMII-A localizes primarily to the front, protrusive edge and is required for adhesion maturation. In contrast, NMII-B localizes behind NMII-A, primarily to large and stable actin stress fibers in the middle and back of the cell (Kolega, 1998; Vicente-Manzanares et al., 2007). NMII-B is required for front-to-back polarity, as cells lacking NMII-B lose large stress fibers and fail to define their rear (Vicente-Manzanares et al., 2007). The major question of what drives the polarized localization of NMII-B is unknown. In this issue, Juanes-Garcia et al. report that a short serine-rich motif in NMII-B is responsible for both its localization and the establishment of front-to-back cellular polarity.

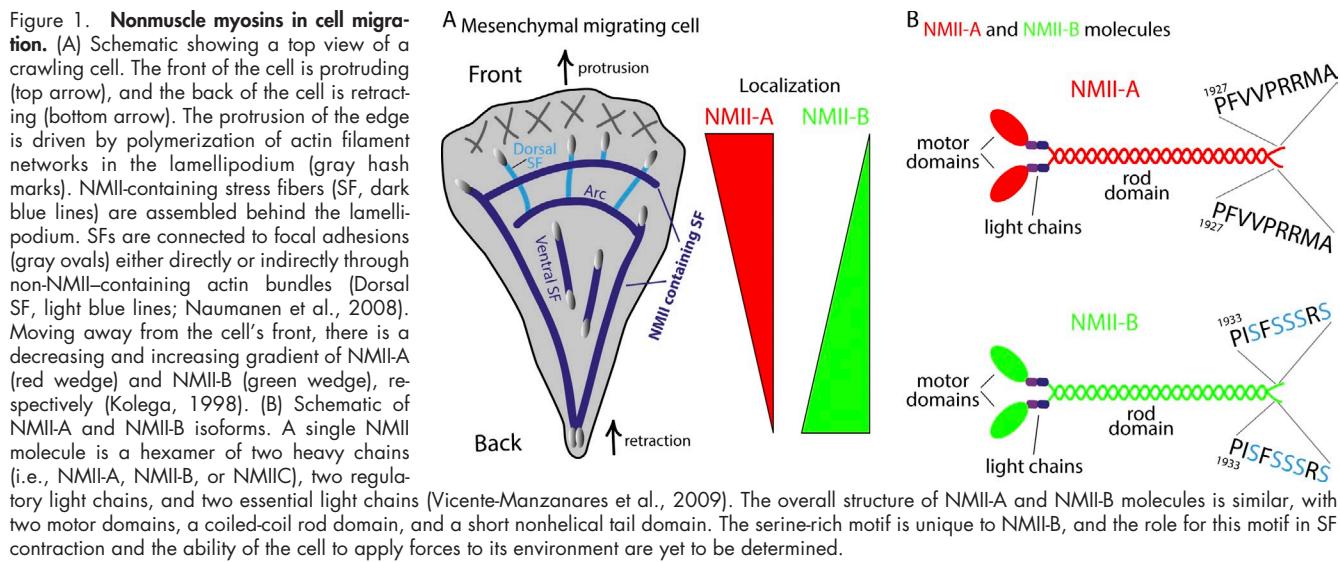
Though NMII-A and NMII-B are genetically and structurally very similar, Juanes-Garcia et al. (2015) identified a serine-rich sequence (SFSSRS) in the C terminus of NMII-B (Fig. 1 B). The authors effectively used cells depleted of NMII-B

(Vicente-Manzanares et al., 2007) to test the role of this serine motif in front-to-back polarity. Although expression of wild-type NMII-B rescued front-to-back polarity, expressing NMII-B lacking the serine motif did not. Interestingly, simply inserting the serine-rich motif from NMII-B into NMII-A (NMII-A5S) conferred the ability to rescue front-to-back polarity. In addition, NMII-A5S did not localize to the front of the cell or play a role in adhesion maturation like wild-type NMII-A. Mass spectrometry analysis revealed three of the residues in the serine motif of NMII-B were phosphorylated in cells, and one of these, serine 1935, was found to be crucial for the wild-type kinetics and localization of NMII-B. A phosphomimetic point mutation, S1935D, failed to rescue front-to-back polarity in NMII-B-depleted cells. In contrast, expression of the nonphosphorylatable mutant, S1935A, localized normally to large actin stress fibers and did rescue front-to-back polarity.

To provide further evidence that serine 1935 is a regulatory element of front-to-back polarity, Juanes-Garcia et al. (2015) investigated the role of PKC, which acts upstream of NMII in cell polarization (Gomes et al., 2005; Even-Faitelson and Ravid, 2006), in NMII-B-generated stable actin bundles. Cells expressing constitutively active PKC produced isotropic protrusions at the perimeter of the cell, while also failing to produce large, stable NMII-B decorated actin bundles. This isotropic protrusive phenotype was blocked when nonphosphorylatable NMII-B S1935A was expressed in cells but not with wild type or S1935D. Thus, PKC was implicated as the likely upstream regulator of NMII-B activity, which negatively regulates stable actin stress fiber formation by phosphorylating NMII-B at serine 1935. Taken together, the data presented strongly suggest that a small regulatory motif on NMII-B controls cellular front-to-back polarity in migrating cells.

The findings presented in this issue by Juanes-Garcia et al. (2015) shine a bright spotlight on a family of motors that has already taken "center stage" in cellular research (Vicente-Manzanares et al., 2009). Some exciting new questions as to how NMII-B functions in the establishment of asymmetric cellular shape and function can now be addressed, including but clearly not limited to: How does the unique enzymatic activity of NMII-B's motor domain synergize with the serine motif to

Correspondence to Dylan T. Burnette: dylan.burnette@vanderbilt.edu



drive front-to-back polarity (Billington et al., 2013)? What are the structural and dynamic implications for homo- and/or hetero-NMII filament formation (Ricketson et al., 2010; Beach et al., 2014; Shutova et al., 2014)? Does the NMII-B serine motif play a role in the establishment of more complex 3D cellular shapes? Is the serine motif required for directional cell migration through physiological environments?

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