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Perspective

Migration of Leukocytes is Controlled by Actin Flow Direction

Branchini Huta*

Department of Chemistry, Connecticut College, New London, USA

Introduction

The integrin lymphocyte function-associated antigen-1 (LFA-1, L2) is involved in a variety of sticky contacts, including antigen recognition, vascular emigration, and leukocyte movement within tissues. The extended-open conformation of integrin ectodomains binds ligand with 1,000-fold higher affinity than the bent-closed and extended-closed conformations. The binding of LFA-1 to ICAM ligands by the I domain in the integrin head is conveyed to the actin cytoskeleton through the subunit leg, trans membrane, and cytoplasmic domains via adaptors like talins and kindlins that bind particular locations in the -subunit cytoplasmic domain.

Measurements of traction force on substrates, as well as more detailed measurements of force within ligands and cytoskeletal components, have suggested that integrin's transmit force between extracellular ligands and the actin cytoskeleton, as discussed. In the 1 pN-6 pN range, forces on the cytoplasmic domain of the LFA-1 2-subunit have been quantified and linked to ligand binding and the cytoskeleton.

Tensile force applied via integrin's has the potential to straighten and align the domains in the force-bearing pathway in the direction of force exertion. Actin retrograde flow, which is generated by actin filament extension over the membrane at the cell front, is a good contender for the source of this force. If such alignment is seen, it can be used to distinguish between different integrin activation models.

According to certain models, binding the cytoskeletal adaptor protein talin to the integrin-subunit cytoplasmic domain is sufficient to activate the extracellular domain's high affinity for ligand. Other models have proposed that tensile force stabilizes the high-affinity, extended-open integrin conformation because of its increased length along the tensile force-bearing direction compared to the other two integrin conformations, as supported by Steered Molecular Dynamics (SMD) and measurements in migrating cells. The thermodynamic demonstration that tensile force is necessary to allow ultrasensitive modulation of integrin adhesiveness was recently made using measurements of the intrinsic affinity and free energy of the three conformational states of integrin.

Actin Flow Direction

The thermodynamic calculations demonstrate that there is a mechanism in the three conformational states of integrin's that activates integrin adhesiveness when the integrin contacts the actin cytoskeleton and an extracellular ligand that resists cytoskeleton-applied force. As a result, the same intracellular effectors that control actin dynamics can also control cell adhesion, providing traction for cellular chemo taxis and migration. Furthermore, directed migration is an important part of immune cell activity, and activation of integrin's would provide a directional sensing mechanism.

Structure biology and thermodynamic studies of integrin's in solution and on the surface of intact cells back up the traction force concept of integrin activation. In this study, we used an orthogonal technique that is extremely functionally relevant to evaluate the traction force model: fluorescence imaging of integrin's as they create traction for cell migration in living cells.

The cytoskeletal force model of integrin activation predicts that the tensile force exerted by integrin's between the actin cytoskeleton and extracellular ligands as they function in cell migration causes them to assume a specific orientation and tilt on the cell surface in relation to the direction of actin retrograde flow pulling on the integrin. For migratory fibroblasts and epithelial cells, actin flow is known to be spatially aligned. Actin is also known to flow centripetally in lymphocyte-mediated immune synapses; additionally, integrin LFA-1 is known to move centripetally but at a slower rate than actin, as evidenced by the movement of its ligand ICAM-1 in planar bilayers on the other side of the immune synapse.

Our observations of integrin orientation on cell surfaces in this paper also allow us to link integrin crystal structures on the length scale with microscopic measurements on integrin-bearing cells on the micron scale. Many sectors of biological research have long sought to integrate measurements at such various length scales. Despite the fact that integrin's, like other membrane proteins, are free to spin in the plane of the membrane, tensile force causes an integrin to orient in the same direction as the pulling force.

In cartoons, integrin's are shown projecting with their leg-like domains parallel to the plasma membrane; however, resting integrin's are free to tilt, and force could cause the integrin to tilt away from the membrane normal. Despite the abundance of membrane protein ectodomain structures, little is known about ectodomain orientation on cell surfaces. We leverage earlier integrin structural studies to position these structures in a reference frame that corresponds to the plasma membrane of a moving lymphocyte in this study. We employ crystal structures for the domain of LFA-1 linked to ICAMs, the LFA-1 headpiece, and two states of the bent ectodomain of the LFA-1 (L2) cousin, X2, in addition

***Corresponding Author**: *Huta B, Department of Chemistry, Connecticut College, New London, USA, E-mail:* branchinihuta@gmail.com

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to broad structural knowledge on several integrin families. The bent-closed, extended-closed, and extended-open conformations of the L2 and X2 ectodomains are also shown using negative stain EM class averages.

These structures, along with those of Green Fluorescent Protein (GFP), guided the creation of restricted integrin-GFP fusions and the use of Rosetta to predict the orientation of GFP and its fluorescence excitation/emission transition dipole relative to the integrin. The orientation of the transition dipole respect to the direction of actin flow can be measured using two different types of fluorescence microscopes. The spatially ordered organization of LFA-1 in the protrusive lamellipodial area is reliant on the movement vector of the underlying actin cytoskeletal framework and is caused by integrin-ligand interaction in combination with cytoskeletal force. The findings confirm the cytoskeletal force hypothesis of integrin activation by demonstrating that actin flow from the leading edge mandates a specific molecular orientation on the cell surface of LFA-1.