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Game of Zones: how actin-binding proteins organize muscle contraction

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ABSTRACT

Locomotion of *C. elegans* requires coordinated, efficient transmission of forces generated on the molecular scale by myosin and actin filaments in myocytes to dense bodies and the hypodermis and cuticle enveloping body wall muscles. The complex organization of the acto-myosin scaffold with its accessory proteins provides a fine-tuned machinery regulated by effectors that guarantees that sarcomere units undergo controlled, reversible cycles of contraction and relaxation. Actin filaments in sarcomeres dynamically undergo polymerization and depolymerization. In a recent study, the actin-binding protein DBN-1, the *C. elegans* ortholog of human drebrin and drebrin-like proteins, was discovered to stabilize actin in muscle cells. DBN-1 reversibly changes location between actin filaments and myosin-rich regions during muscle contraction. Mutations in DBN-1 result in mislocalization of other actin-binding proteins. Here we discuss implications of this finding for the regulation of sarcomere actin stability and the organization of other actin-binding proteins.

Text

In striated muscle, assembly, organization, and maintenance of contractile sarcomeres are essential for efficient force transmission and normal locomotion. Sarcomeres harbor the acto-myosin apparatus consisting of actin filaments, bipolar myosin filaments, and a host of effector proteins decorating actin filaments with diverse functions. Effectors, among other roles, maintain the integrity and stability of actin filaments by modulating actin polymerization dynamics and serve as linkers for cell-membrane attachment. In *C-elegans* body wall muscle cells, actin filaments are anchored with their barbed ends to dense bodies (analogous to mammalian Z-discs) and with the pointed end pointing to the myosin filaments residing at the so-called M-line (M-band).

In our recent study,[1] we found that drebrin-like protein DBN-1, a member of the neuronal actin-binding protein drebrin, is a novel sarcomere constituent involved in supporting muscle contraction. DBN-1 can bind actin filaments and shows actin bundling properties *in vitro*. Endogenous expression of fluorescent constructs in body-wall muscles shows DBN-1 in a striated pattern characteristic of a bona fide sarcomere component. When directly observing muscle contraction in living worms at higher magnification, surprisingly DBN-1 reversibly switches between actin regions and M-lines during muscle contraction cycles: in the relaxed state, DBN-1 is located at the M-line, while in the contracted state it relocates to actin filaments (I-bands). In both relaxed and contracted states, DBN-1 is also present at dense bodies. This reversible and fast swapping of positions is remarkable for an actin binding protein. It is not fully understood what causes DBN-1 to colocalize with myosin at the M-line.

When DBN-1 is either mutated or knocked-down, other actin-binding proteins in the sarcomere are affected and mislocalize within the actomyosin lattice. One example is α-actinin which loses its compact appearance at dense bodies during contraction cycles. Therefore we speculate that DBN-1 might serve as a scaffold or "usher" for other actin binding proteins. The absence of functional DBN-1 also has consequences for actin integrity itself: actin filaments appear diffuse and disorganized suggesting a role for DBN-1 in

actin stabilization and bundling. Here we give a brief overview of the role of the novel player DBN-1 in sarcomere dynamics and in the organization of other actin-binding proteins.

Protein re-arrangements inside the contractile apparatus

Structural analysis has demonstrated that contraction and relaxation of a sarcomere require conformational changes in the transverse lattice of actin at dense bodies (Z-discs) and myosin filaments respectively to accommodate the axial shortening of the acto-myosin lattice. At the onset of contraction, Ca^{2+}-induced conformational changes i.e. translocation of troponin and tropomyosin make actin filaments accessible for myosin binding. Actin filaments change from an orthogonal to a spatial basket-weave form, while the myosin lattice can undergo reversible displacements due to shear forces between neighboring myosins (reviewed in[4]). It appears that binding to actin filaments is always highly competitive between myosin, actin stabilizing and actin depolymerizing proteins. Besides other cytoskeleton regulators, actin depends on kettin, KETN-1 (functionally similar to mammalian nebulin) for the stability of filaments and linking actin to dense bodies (reviewed in [11]). Figure 1C highlights the roles of some selected actin-binding proteins in actin filament organization and stability. Actin filaments can be divided into proximal and distal zones relative to the dense bodies. Dense bodies themselves are essential for the generation of contractile force by the muscles as they anchor actin filaments through integrin/ankyrin/PDZ complexes to the muscle cell membrane. Proximal and
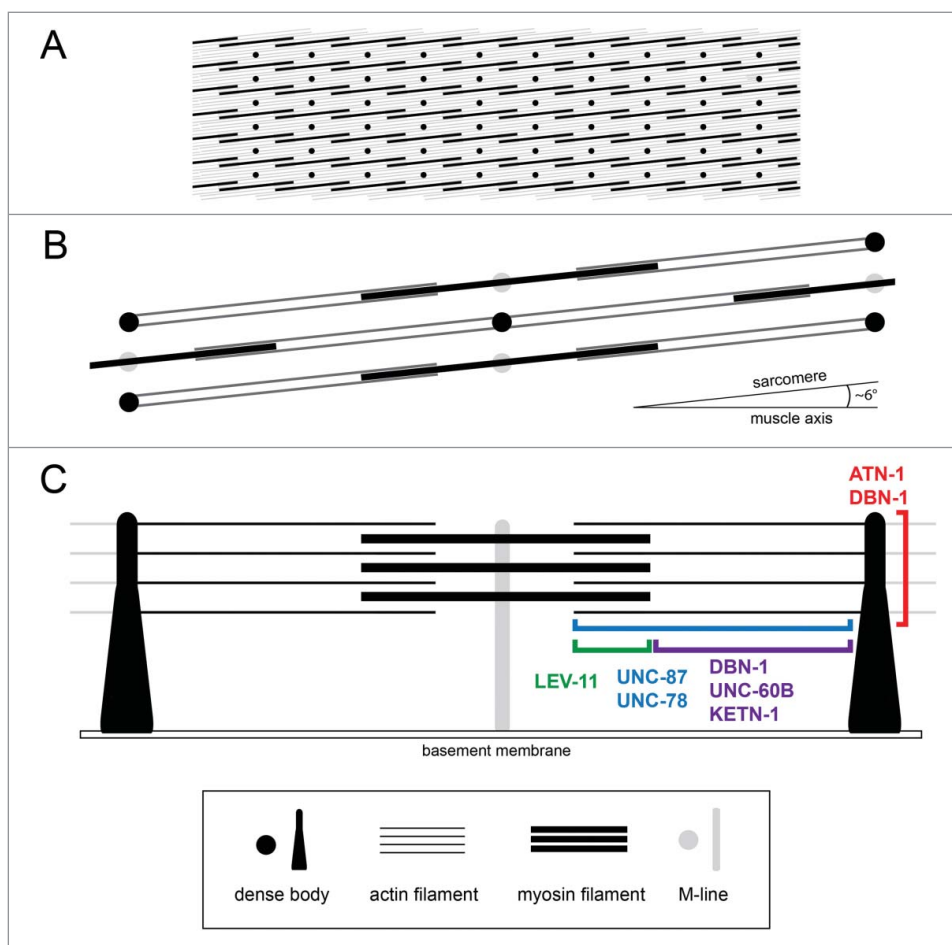

Figure 1. Actin zones and actin-binding proteins in the contracted sarcomere. A) A schematic overview of actin (thin lines), myosin (thick lines) and dense bodies (black dots) at low magnification. B) Top view onto sarcomere at higher magnification. Actin filaments are attached to dense bodies, myosin filament is attached to M-lines (gray dots). C) Side view onto sarcomere at higher magnification. DBN-1 is colocalizing with dense bodies and the proximal zone of actin filaments (red and purple area). Other actin binding proteins ATN-1/α-actinin (red), LEV-11/ tropomyosin (green), UNC-87/calponin-like protein, UNC-78 (blue), and UNC-60B/ADF- cofilin, and KETN-1/kettin (purple) are shown at their respective actin binding zones.

distal regions of actin can be discerned by the inherent decoration with different actin binding proteins: on the proximal end, close to dense bodies, we observe ATN-1/α-actinin, UNC-78, KeTN-1/kettin, UNC-60B/ADF cofilin, and DBN-1 whereas LEV-11/tropomyosin is residing on the distal end. Other proteins show no preferred binding zone such as UNC-78 and UNC-87/calponin-like-protein which are found distributed along the entire filament length. In the contracted state of the sarcomere, DBN-1 translocates to proximal actin zones associated with dense bodies. In the vicinity of dense bodies, DBN-1 might serve as actin stabilizer and transversal spacer, a hypothesis supported by *in vitro* AFM and sedimentation analysis showing actin bundling activity.[1]

How mechanical forces are generated and transmitted through cytoskeletal filaments is well known, but the accompanying dynamic rearrangements of proteins across compartments are much less well understood. DBN-1 is not the only sarcomeric protein to localize dynamically between dense bodies /Z-discs, cytosol, or M-lines. The actin stabilizer calponin-like protein UNC-87 binds directly to actin and myosin *in vitro*, regulating contractility.[12] In this case it is not clear, though, whether it does so in a competitive fashion *in vivo*. In intensively exercised muscle, myotilin, a binding partner of α-actinin at Z-discs, translocates from Z-discs to actin filaments and M-lines.[2] The functions of such inter-compartment translocations are so far elusive, but appear to be associated with mechano-transduction and intracellular signaling.[5]

Functions at dense bodies and M-line

The myosin filament lattice and actin filaments sarcomeres require firm attachment to the muscle cell membrane in order to transmit contractile forces to the cuticle. Two requirements, maintenance of filament length and of membrane attachment complexes (dense bodies) are paramount to achieve synchronous, reversible, and effective sarcomere contraction for locomotion. In contrast to clear locomotion defects seen as a consequence of mutations in the acto-myosin lattice in *C. elegans*, only few null mutants of dense-body and M-line proteins show obvious defects classified as uncoordinated.[9] An explanation might be the functional redundancy in the formation of coordinating complexes or scaffolds thus compensating for loss-of-function deficits. Alternatively, possible phenotypes might be too subtle to examine in standard locomotion assays. One

example is PKN-1,[14] a kinase both localized to dense bodies and M-lines, that exhibits a mild patterning defect of α-actinin in *pnk-1* null mutants. More drastically, however, a hyper-bending unc-phenotype is observed under heat-shock controlled overexpression of PKN-1, resulting in "loopy" locomotion. Other actin binding proteins such as α-actinin crosslink actin filaments by bundling and thereby alter actin dynamics and stabilize filaments. Remarkably, the *C. elegans* gene for α-actinin (atn-1) is not essential, but null mutants show sarcomeric abnormalities.[8] One explanation is a redundant safety network of actin binding proteins that substitute ATN-1 function as actin attachment and bundling factor to dense bodies. While DBN-1 would fulfill the bundling requirement, currently there is no evidence for a cytosolic anchor function to dense bodies. However it will be worth testing whether DBN-1 is functionally associated with an integrin-anchoring complex comprising LIM-domain proteins.[6] It is tempting to speculate that newly identified proteins at the dense body and M-line are not rigid scaffolds but modulators of force transmission recruiting signaling factors. In the future, subtle locomotion phenotypes demand more intricate methods measuring transmission by optical force, mechanical AFM probing of worm stiffness[13] and worm body bending properties (eg. sensors[9,16]) to better characterize the extensive sarcomere protein repertoire.

Actin dynamics in the muscle

The cytoskeleton, in contrast to the rigid bone skeleton of animals, is typically in a highly dynamic steady state, where components are continuously exchanged, filaments grow and shrink by polymerization and disassembly, while overall structures, including filament lengths appear uniform and constant. Such steady-state filament dynamics need to be tightly regulated to guarantee efficient muscle contraction. In the nematode muscle, control of actin filament length is performed by ADF cofilin/UNC-60B that destabilizes filaments and promotes actin depolymerization in the sarcomere, either alone or in cooperation with other destabilizing factors. Such an additional factor is CAS-1, cyclase-associated protein 1, which has been described as a sarcomere actin-assembly regulator. CAS-1 cooperates with UNC-60B to shorten actin filaments.[10] Conversely, tropomyosin has been shown to counteract cofilin activity in *in vitro* experiments. Tropomyosin isoforms differentially modulate cofilin depolymerizing

activity, presumably through allosteric inhibition.[15] With respect to actin polymerization and integrity, DBN-1 appears to be required for filament stability in the contracted state.[1] Upon contraction in *dbn-1* mutants, I-bands lose their compact line pattern and exhibit a diffuse, frayed actin staining which is reversible upon relaxation. Whether the diffuse appearance is accompanied by actin depolymerization is still unknown. It is tempting to speculate that accumulation of DBN-1 at dense bodies and in proximal actin zones protects filaments from depolymerization. The field is just at the beginning of drawing a comprehensive map of the actin-binding interactome. With further research and a better understanding of the control of actin dynamics in muscle, it will be possible to eventually understand the complex signaling processes that organize the functional steady-state of contractile muscle.

A functional cloud

Considering the fact that a large and complex group of actin binding and bundling proteins dynamically cooperate in the confined compartment of the sarcomere, it is appealing to invoke the concept of a "functional cloud" in trying to describe and understand how such an association of dynamic players self-organizes to control muscle activity. To achieve rapid contraction of the acto-myosin lattice across the whole muscle, spatio-temporal coordination of actin binding and release of filament stabilizers and myosin itself is crucial. A similar concept of a non-membrane-bounded transient organelle has been put forward to describe centrosome assembly and the pericentriolar material (PCM) organization at the microtubule organizing center.[7] The recruitment and organization of PCM proteins is described in a model where centrioles either serve as template structure for stepwise assembly or alternatively, a stochastic self-organized assembly based on reaction diffusion properties of proteins with similar functions. Transferring this concept to muscle, one advantage of such a stochastic assembly process is that the self-organization of actin-binding proteins can switch between different dynamic states, according to need, depending on contraction state. This hypothesis will be difficult to test, but support comes from the finding that the pericentriolar cloud also regulates actin organization.[3] Could muscle cells harbor structures to scaffold actin-binders that are functionally similar to the centrosomal centrin?

The task ahead is the characterization of the interdependences within the large and growing group of sarcomere proteins. Whether they are loosely organized in a functional cloud or organized by a yet to be described scaffold complex will be the subject of future studies. The study of the system in *C. elegans* will be both advantageous and limited. The nematode is viable without many modulators non-essential for forward locomotion, so it is a stable model system to look for subtle effects. It will then be essential to search for conserved features in higher mammals.

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