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Protein structure, function, and regulation in biological movement

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The production and regulation of physical movement by biochemical interaction among cellular proteins form a natural subject for a Highlight Issue of *Archives of Biochemistry and Biophysics*. However, a challenge of the seemingly straightforward task of editing an issue on contractile proteins is the coverage of the vast area of biological contractility. Contractile protein studies were traditionally focused on muscle. In the three centuries of scientific investigation since Leeuwenhoek and Croone set the stage for the cellular structure of striated muscle, knowledge produced from myofilament protein studies has led to extremely detailed understanding of muscle function and advances in the broad field of cell motility that allow us to understand all biological movements. With the enthusiasm of our outstanding contributing authors, this Highlight Issue of *ABB* consists of a broad spectrum of original research works that characterize contractile, cytoskeleton, and regulatory proteins for their molecular structure-function relationship in biochemical activity, force production, cell motility, cytokinesis, organ function, and pathogenesis.

To understand the structure and function of myosin motor, the laboratory of Doug Root investigated myosin structure and activity by CY3 fluorescent labeling of the 50-kDa cleft of subfragment-1. Fluorescence polarization indicates that this site becomes more mobile upon actin binding, supporting a location near the actomyosin interface. Molecular mechanics and stochastic dynamics simulations suggest that this site is sensitive to forced cleft opening and closure. To investigate the function of the myosin light chain 1Sa isoform (MLC1Sa), Peter Reiser's laboratory studied the heterogeneity in MLC1 isoform expression in adult pig diaphragm muscle for the relationship to contractile properties. By measuring shortening velocity and force generation in single fibers, they found that shortening velocity is inversely related to the level of MLC1Sa and that this isoform is associated with greater force generation.

To better understand the regulation of actomyosin interaction in striated muscle, Jack Rall and colleagues investigated the influences of Ca^{2+} concentration and dissociation rate from troponin C (TnC) on the kinetics of contraction. The results showed that the influence of $[Ca^{2+}]$ on kinetics of contraction is fiber type dependent and the maximum kinetics of contraction in fast-twitch fibers is dominated by kinetics of cross-bridge cycling over kinetics of Ca^{2+} exchange with TnC. To study the functional significance of troponin T isoforms, Murali Chandra's laboratory studied the alternatively spliced COOH-terminal variable region of fast skeletal muscle troponin T (TnT). In reconstituted fast skeletal muscle fibers, α-fsTnT resulted in myofilament Ca^{2+} sensitivity greater than that of β-fsTnT without changes in maximal tension, ATPase activity, or tension cost. In another troponin study, Wen-Ji Dong's laboratory examined the structural kinetics of the inhibitory region of troponin I (TnI) in regulated thin filament by site-specific labeling and FRET analysis. The results showed that troponin-

tropomyosin interaction affects the cooperativity and kinetics of the structural transition in TnI. While protein kinase A (PKA) phosphorylation of cardiac TnI decreased the Ca^{2+} sensitivity and accelerated the structural transition rate of the inhibitory region, strongly bound S1 significantly increased the Ca^{2+} sensitivity and slowed the kinetics of structural transition, suggesting a feedback mechanism for the fine-tuning of cardiac function by β-adrenergic stimulation. To study troponin structure and function in an integrated physiological system, X.P. Huang and associates investigated a cardiac TnI mutation (R192H) related to restrictive cardiomyopathy in a transgenic mouse model. The main functional alteration detected in the cardiac TnI193His mice by ultrasound cardiac imaging was impaired cardiac relaxation manifested by a decreased left ventricular end diastolic dimension and an increased end diastolic dimension in atria. The cardiac ejection fraction was significantly decreased in cardiac TnI193His mice with advanced disease, indicating a combined contribution of genetic conditions and postnatal adaptation to the development of the cardiac TnI mutation-caused cardiomyopathy.

Skeletal and cardiac muscle sarcomere also contains non-contractile filaments represented by the giant protein titin, which contributes to the passive property of muscle. The laboratory of Marion Greaser reports a study on the PEVK region of titin. Using synthetic and expressed protein fragments and circular dichroism, they demonstrated that both the positively charged PPAK modules and the negatively charged polyE repeats had similar disordered secondary structures. Gel permeation chromatography showed that both PPAK and polyE peptides had Stokes radii much larger than expected from their molecular mass. The properties of both the PPAK and polyE type peptides as intrinsically disordered structures help in elucidating the contribution of the titin PEVK region to the elasticity of muscle. The giant titin molecule consists of multiple repeats of modular structure including the Ig-like modules. Gary Kargacin and colleagues report a study on telokin, a protein that is identical in sequence to the COOH-terminal portion of myosin light chain kinase and contains an Ig-like domain. The use of specific monoclonal antibodies, immunofluorescence microscopy, and image reconstruction demonstrated the presence of telokin in cardiac myoctes at the intercalated disc. Western blots of isolated intercalated discs revealed a 23-kDa protein that co-migrates with purified telokin. The possible function of telokin in the intercalated discs extends the role of Ig-like domains in muscle structure and function.

To investigate the regulation of actomyosin activity in non-muscle-cell motility, the laboratory of Jim Lin studied the contractile ring and the cell cortex that generate force to divide the cell symmetrically during cytokinesis. Force-expression of misregulated constructs of tropomyosin and caldesmon in cells showed a positive correlation between actomyosin ATPase activity and the speed of cell division. Their results also suggest a model in which Ca^{2+}/calmodulin and Cdk1 dynamically control caldesmon inhibition of tropomyosin-regulated actomyosin to regulate division speed and to suppress membrane blebs. In another study on the actin filament-based regulation of contractility and cell motility, Albert Wang's laboratory demonstrated that caldesmon binds cortactin, with possible significance in actin polymerization and depolymerization. Binding of cortactin partially alleviates the inhibitory effect of caldesmon on the actomyosin ATPase activity. This study contributes to the understanding of the function of caldesmon, an actin-binding protein present in nearly all mammalian cells.

Looking further into the role of cytoskeletal proteins in the regulation of cell migration and invasion, Alan Mak and colleagues report a study on cortactin, an F-actin-binding protein enriched in dynamic organelles such as podosomes, invadopodia, and lamellipodia. They found that p21-associated kinase (PAK) is able to phosphorylate cortactin predominantly at Ser_{113}, Ser_{150}, and Ser_{282} at the NH_2-terminal actin-binding repeats. Binding of cortactin to F-actin is significantly reduced by PAK phosphorylation, suggesting a role in the regulation of the dynamics of branched actin filaments. Also investigating phosphorylation regulation of

contractility, Mitsuo Ikebe's laboratory reports a study on ZIP kinase and its role in regulating myosin phosphorylation. They identified a novel ZIP kinase isoform that lacks the COOH-terminal non-kinase domain containing a leucine zipper. The leucine zipper-minus ZIP kinase binds to MYPT1 as well as to myosin and localizes at stress fibers similar to those of the leucine zipper-plus isoform, indicating that the leucine zipper is not critical to these bindings and cellular localization. This intriguing finding urges new investigations on ZIP kinase structure-function relationships. To understand the phosphorylation regulation of cardiac muscle contractility and cytoskeletal function, Meredith Bond and colleagues studied the targeting of PKA by A-kinase anchoring proteins (AKAPs) that contribute to the specificity of PKA signaling pathways. To identify novel AKAPs that target PKA to the cytoskeleton or myofilaments, the intermediate filament protein, synemin, was identified as a putative PKA regulatory subunit type II-binding protein with increases in failing human hearts. The results suggest that synemin provides temporal and spatial targeting of PKA cardiac myocytes.

To determine the relationship between tension and biochemical regulation in muscle, the laboratory of Henk Granzier studied the effects of passive muscle stretch on the phosphorylation of myosin light chain 2v (MLC2v) in isolated rat heart preparations. They found changes in MLC2v phosphorylation in response to increased diastolic pressure with increased sensitivity toward the epicardium. This regulation may affect actomyosin interaction and play a role in the Frank-Starling mechanism of the heart. Compared with what is known from muscle studies, little is known about how mechanical forces of non-muscle cells are generated and regulated. To explore this vast area, Yu-li Wang and co-workers studied traction forces exerted by adherent cells on the substrate. By treating fibroblasts with agents that affect the myosin II-dependent contractile mechanism, they demonstrate that inhibition of the Rho-dependent kinase causes strong inhibition, while inhibition of the myosin light chain kinase has no detectable effect on traction forces. Their results suggest that these pathways play non-redundant roles in regulation of myosin II-dependent traction forces, even though both are known to affect the phosphorylation state of the regulatory light chain.

With the rapid progresses in structural biology, development of integrated physiological model systems, and genomic and proteomic approaches, we have arrived at an exciting stage of contractile protein research. Although this special issue of *ABB* represents only a very small fraction of the current research in the field, we hope it will serve as an invitation to all interested investigators to join the multidisciplinary workforce to explore the exciting world of biological contractility and motility. With a few years of collective effort, we hope to be able to present our readers with another collection of frontier research on contractile proteins.

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