Christopher Myers *Editor*

Skeletal Muscle Physiology

An Update to Anatomy and Function

Springer

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Please note that the selected papers are not used to train an LLM while the auto-summaries are created.

Preface

Welcome to "Skeletal Muscle Physiology: An Update to Anatomy and Function"—an engaging, cutting-edge, and comprehensive exploration into the fascinating world of skeletal muscle physiology. This remarkable volume, generated by AI, represents a convergence of diverse and profound insights from biology, sports science, medicine, and computational data science.

Skeletal muscle, comprising approximately 40% of the human body weight, plays an essential role in locomotion and posture and in various critical physiological processes such as respiration, thermogenesis, and metabolism. Hence, an in-depth understanding of its physiology can profoundly impact health and disease, exercise performance, rehabilitation, and overall human function.

This book breaks new ground by being one of the first AI-authored texts in the field. It draws upon a vast reservoir of knowledge in the Springer Publishing library and uses advanced AI modeling to provide a meticulously detailed yet lucid perspective of skeletal muscle function. The AI-based authoring ensures high accuracy and consistency, providing an unbiased account of existing scientific knowledge.

This book builds upon the basic and advanced skeletal muscle topics found in exercise physiology books. The contents within these pages delve into various topics, including the detailed anatomy of skeletal muscle, the mechanics of muscle contraction, the biochemistry of energy provision, the impact of various factors on skeletal muscle function such as age, sex, and disease, and the role of exercise and nutrition in muscle health.

This book does not claim to replace the need for human intelligence and experience, but instead, it hopes to supplement it, providing a resource that can aid both learning and teaching. While AI generation has limitations, this text will be an invaluable stepping stone toward a more profound comprehension of skeletal muscle physiology.

So, whether you are a novice to the field, seeking a refresher course, or a seasoned expert looking for a comprehensive resource, we invite you to embark on this captivating journey of exploration into the world of skeletal muscle physiology. Let's delve into the marvel that is the human muscle and unravel the science that underpins its extraordinary capabilities.

Akron, OH, United States of America — Christopher Myers

Introduction by the Editor

In the dynamic and ever-evolving landscape of physiological research, the exploration of skeletal muscle physiology stands as a cornerstone, bridging fundamental biological insights with transformative applications in health, disease, and human performance. This book, a pioneering endeavor co-authored with the assistance of advanced artificial intelligence (AI), embarks on a journey to distill and articulate the complex mechanisms, functions, and adaptabilities of skeletal muscles in unprecedented detail and clarity.

The fusion of human expertise and AI in the creation of this book has enabled a unique and innovative approach to synthesizing and presenting the wealth of knowledge that defines skeletal muscle physiology. By harnessing the analytical power of AI, I have sifted through vast arrays of data, research findings, and theoretical frameworks, selecting and integrating the most critical insights into a coherent and comprehensive narrative. This collaboration has not only enhanced the accuracy and depth of the content but also imbued it with novel perspectives and interpretations that reflect the cutting-edge of scientific discovery.

Our journey through skeletal muscle physiology begins with the advanced principles that govern muscle biology and explores new advances in understanding muscular binding proteins. Also, we explore the remarkable plasticity of skeletal muscles, highlighting how they adapt to various stimuli, including exercise, injury, and disease, and how these adaptations can be leveraged for therapeutic and performance-enhancing interventions.

This book is designed to serve as an invaluable resource for students, researchers, and practitioners across multiple disciplines, including physiology, medicine, sports science, and rehabilitation. It aims to not only impart knowledge but also to inspire curiosity, critical thinking, and further exploration into the vast and vibrant field of skeletal muscle physiology.

The integration of AI in the creation of this book represents a novel paradigm in scientific publishing. It signifies a step toward a future where human creativity and artificial intelligence converge to enhance our understanding of the world. As you turn these pages, I invite you to join me at the forefront of this exciting frontier, equipped with the insights and understandings that will empower you to contribute to the next wave of discoveries and innovations in skeletal muscle physiology.

Welcome to a journey of exploration, discovery, and inspiration. Welcome to the cutting edge of skeletal muscle physiology!

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Basic Structure of Skeletal Muscle

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Christopher Myers

1.1 Introduction by the Editor

Building on the foundational understanding established in the first chapter, Chap. 2 explores the burgeoning world of contemporary research in basic skeletal muscle physiology. While skeletal muscle physiology is a well-established field, it remains an active area of investigation, with novel findings consistently reshaping our understanding of skeletal muscle's complex biology.

The continuous evolution of technologies, including advanced imaging techniques and high-throughput genomics, has allowed researchers to gain unparalleled insight into the inner workings of skeletal muscle. The granular details unveiled by these novel tools are revising our perceptions of muscle function at the cellular and molecular levels.

This chapter's primary area of focus is research updates to skeletal muscle physiology. The content in this chapter explores the role of the thermoregulatory activity of ATPase, myosin light chain activity and myosin binding to actin, and force production and enhancement between the myofilaments.

We also delve into recent discoveries in the realm of the protein titin. This protein is shown to become more and more critical to muscular contraction. Unofficially, titin is called the "third myofilament" due to the research showing its importance in force production. The chapter discusses some of this research that has come to change our fundamental understanding of the structure and function of titin.

In summary, this chapter provides a deep dive into innovative research in basic skeletal muscle physiology. By embracing the newest findings and integrating them with existing knowledge, this chapter illuminates our relentless progress in deciphering the complex physiology of skeletal muscle.

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C. Myers (ed.), *Skeletal Muscle Physiology*,
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The study of skeletal muscle physiology has long stood as a cornerstone of biological and medical sciences, bridging our understanding from fundamental biological processes to complex systems-level functions. Skeletal muscles are pivotal in locomotion, posture, metabolic health, disease states, and aging. The field of skeletal muscle physiology is experiencing a renaissance powered by technological advancements and an expanding interest in the nuanced mechanisms that govern muscle function. This chapter explores the forefront of skeletal muscle research, shedding light on the complex interplay of molecular components that orchestrate muscle contraction, force production, and energy efficiency.

One of the most captivating areas of current research lies in investigating the myosin super relaxed state (SRX) and its contribution to muscle thermogenesis. The SRX state, characterized by a tightly packed and inactive configuration of myosin heads, represents a crucial mechanism for adenosine triphosphate preservation in resting muscle fibers. This state's regulatory impact on ATPase activity underscores the efficiency of muscle energy use and its role in generating heat, a vital aspect of thermoregulation. This chapter dives into the intricacies of myosin dynamics and uncovers the layers of complexity in muscle function that extend beyond the conventional understanding of contraction and relaxation via the excitation-coupling mechanism and sliding filament theory.

Furthermore, recent discoveries highlight the significance of the actin-titin interaction in force enhancement within muscle fibers. Traditionally overshadowed by the actin-myosin interaction, the role of titin—often heralded as the "third myofilament"—in muscle elasticity and force production has come to the fore. New research offers groundbreaking insights into how muscles maintain tension and elasticity, especially under stretching. The binding of titin to actin illuminates a novel mechanism of force enhancement and redefines our understanding of muscle flexibility and strength.

The field of muscle research has undergone significant transformation, leading scientists to question the long-standing nonlinear perspective on the active force-length relationship. Recent investigations have introduced a linear model that appears to better reflect muscle behavior under specific scenarios, including submaximal contractions and stretches. This shift towards a linear model marks a significant paradigm change, altering how we conceptualize muscle stretching and contraction. The new linear approach offers a more apparent and predictable framework for studying muscle dynamics. This evolution in understanding has profound implications, potentially reshaping theoretical research and practical methodologies. In the realms of sports science and rehabilitation, the implications are particularly noteworthy. Practitioners and researchers can now employ this model to achieve more accurate predictions and effective interventions. Ultimately, this reevaluation facilitates a more profound comprehension of muscle function, opening new avenues for advancement in our understanding and application of muscle physiology.

In addition to these mechanistic insights, this chapter will explore the contributions of titin filaments and myosin's regulatory and essential light chains to muscle function. The passive forces attributed to titin filaments highlight the protein's role

as a molecular spring, critical for muscle elasticity. Meanwhile, the light chains of myosin emerge as crucial modulators of muscle contraction, adding another layer of complexity to the regulation of muscle activity. Together, these components play a defining role in the nuanced and finely tuned-process of muscle movement and force generation.

Leveraging advanced simulations and model extensions, we can now visualize and quantify force enhancement across various muscle levels, seamlessly integrating the roles of myofilaments within a unified framework. These advancements enhance our understanding of muscle function under a broad spectrum of conditions, including submaximal contractions and the intricate forces exerted by titin. As we embrace these innovative methodologies, we venture into a new era of physiology, aiming to demystify the complex mechanisms of skeletal muscle physiology. This chapter embarks on a journey to blend cutting-edge discoveries with established knowledge, guided by the latest developments and insights in muscle research. Our goal is to shed light on the continuous progress in the field, paving the way for breakthroughs in health, disease management, and human performance, thereby opening new frontiers in our quest for a comprehensive comprehension of muscle physiology.

1.2 Machine Generated Summaries

Disclaimer: The summaries in this chapter were generated from Springer Nature publications using extractive AI auto-summarization: An extraction-based summarizer aims to identify the most important sentences of a text using an algorithm and uses those original sentences to create the auto-summary (unlike generative AI). As the constituted sentences are machine selected, they may not fully reflect the body of the work, so we strongly advise that the original content is read and cited. The auto generated summaries were curated by the editor to meet Springer Nature publication standards. To cite this content, please refer to the original papers.

Machine generated keywords: titin, filament, myosin, actin, enhancement, rabbit, contain, interaction, thin, light chain, actin filament, interaction actin, length, tension, inhibit.

The Role of the Myosin ATPase Activity in Adaptive Thermogenesis by Skeletal Muscle [1]

This is a machine-generated summary of:

Cooke, Roger: The role of the myosin ATPase activity in adaptive thermogenesis by skeletal muscle [1]

Published in: Biophysical Reviews (2011)

Link to original: https://doi.org/10.1007/s12551-011-0044-9

Abstract-Summary "Resting skeletal muscle is a major contributor to adaptive thermogenesis, i.e., the thermogenesis that changes in response to exposure to cold or to overfeeding."

"A new state of myosin, the super relaxed state (SRX), with a very slow ATP turnover rate has recently been observed in skeletal muscle [2]."

"To be compatible with the basal metabolic rate observed in vivo for resting muscle, most myosin heads would have to be in the SRX."

"Modulation of the population of this state, relative to the normal relaxed state, was proposed to be a major contributor to adaptive thermogenesis in resting muscle."

"Transfer of only 20% of myosin heads from the SRX into the normal relaxed state would cause muscle thermogenesis to double."

"Phosphorylation of the myosin regulatory light chain was shown to transfer myosin heads from the SRX into the relaxed state, which would increase thermogenesis."

Introduction

"The SRX has been proposed to play a role in adaptive thermogenesis by resting muscle."

"Thermogenesis by resting muscle and its role in whole body metabolism is considered."

"I then describe how the new state of myosin could provide a mechanism for thermogenesis and integrate this proposed mechanism into our current knowledge of muscle thermogenesis."

"Two fields of research into muscle function, one concerned with the contractile proteins as force generators and one concerned with the role of muscle in adaptive thermogenesis, should now find common ground."

The ATPase Activity of Purified Myosin Is Not Compatible with the Metabolic Rate of Resting Muscle

"The presence of an inhibited state of skeletal myosin in resting muscle was first suggested more than 30 years ago when the ATPase activity of purified myosin was compared for the first time with the resting metabolic rate of the muscle from which it came [3]."

"The degree of inhibition must be considerably greater than the factor of five, as the resting metabolism of skeletal muscle is low and, in addition to the metabolism

of myosin, ATPase must also carry out other obligatory cell functions, such as protein synthesis, ion pumping, etc A similar argument was made for rabbit myosin in comparison to living resting rabbit muscle [4, 5]."

"If the population of this state is regulated, with some myosins in the inhibited state while others are in a state with an ATPase activity similar to that of purified myosin, redistribution of myosin between the two states would have a large effect on resting muscle metabolism."

Measuring Single Nucleotide Turnovers in Permeable Muscle Fibers

"The rapid phase, which has a time constant of approximately 20 s, comprises multiple factors, including the release of non-specifically bound nucleotides, the release of nucleotides by a fraction of the normally relaxed myosin heads, and the diffusion of released nucleotides out of the fibers, requiring approximately 10 s [6]."

"The time constant for the second phase is much slower, 230 s, and as discussed below, it arises from the release of nucleotides by a second fraction of myosin heads with a very slow ATP turnover."

"The slow release was only observed if the fiber was relaxed, and myosin is the only nucleotide binding protein that responds to the activation level of the fiber."

"The SRX explains the discrepancy described above between the more rapid ATPase activity of purified myosin and the much slower ATPase activity required to be compatible with the metabolic rate of living fibers [3]."

Structural and Biochemical Data Show Myosin ATP Turnover Is Inhibited by the Binding of Myosin Heads to the Core of the Thick Filament

"A helical array of heads bound to the thick filament was also observed at lower resolution in relaxed filaments from scallop muscle, a muscle controlled by binding of calcium to myosin [7, 8]."

"A similar ordered helical array of myosin heads in the J motif was resolved in myosin filaments from vertebrate cardiac muscle [9]."

"The observation of the folded J structural motif in a variety of myosins, including those from both thick- and thin-filament-regulated muscles, led Craig and coworkers to propose that the structural motif is conserved across different species and muscle types [10–12]."

"Filaments in relaxed insect flight muscle show myosin heads in a different structural motif [13]."

"These correlations led to the conclusion that myosin heads in the SRX are bound to the core of the thick filament and have the J motif."

Active Fibers

"If myosin heads in the SRX are bound to the core of the thick filament, where they are unable to interact with actin, and their lifetime is long, how is skeletal muscle able to achieve maximal force in a few tens of milliseconds in a tetanic activation?"

"This observation suggests that the interaction of some myosin heads with actin destabilizes the binding of adjacent myosin heads to the core of the thick filament."

"There must always be some fraction of the myosin heads in the normal relaxed state, which would sense the activation of the thin filament and initiate the cooperative process leading to full activation."

"During activation, all of the myosin heads leave their relaxed positions on the thick filament and become disordered."

The Dynamic Nature of the Resting Metabolic Rate of Skeletal Muscle

"Early investigations of muscle metabolism revealed that the resting metabolic rate of amphibian muscles could vary widely."

"Stretching a muscle to longer lengths could increase metabolism up to four-fold [14]."

"The resting metabolic rate of frog muscle was also increased by higher external concentrations of potassium or by the addition of hyper-osmotic agents in the medium bathing the muscle [15, 16]."

"Hypoxia caused a decrease in the basal metabolic rate of resting frog muscle to 20% of the control rate and also greatly inhibited the increased thermogenesis seen after stretch and in the presence of higher potassium and hyper-osmotic agents [17]."

"The resting metabolic rates of both amphibian and mammalian skeletal muscles are highly variable, undergoing large increases or decreases depending on the conditions employed, all of which occur in the absence of active force generation."

Adaptive Thermogenesis and the Role of Muscle

"Resting muscle thermogenesis increases following a meal and during exposure to cold (for review, see [18–20]."

"This adaptive thermogenesis is produced by norepinephrine released from sympathetic nerves and by adrenal secretion of epinephrine, and a large portion of this metabolic process, approximately 40%, occurs in resting muscle [21, 22]."

"Leptin has also been shown to directly increase resting skeletal muscle thermogenesis [23]."

"The response to cold exposure is complex and involves both non-shivering and shivering thermogenesis in skeletal muscle."

"Epinephrine has been shown to activate thermogenesis, showing that skeletal muscle is involved in cold-induced thermogenesis."

"Resting muscle thermogenesis responds to cold and to food consumption, and epinephrine and leptin have been identified as being involved in some of the pathways controlling this process."

What Is the "Furnace" Responsible for Increased Thermogenesis in Resting Skeletal Muscle?

"The discovery of an UCP that is highly and selectively expressed in skeletal muscle, UCP3, led to the suggestion that this protein may play a dominant role in muscle thermogenesis (for review, see [24])."

"The over-expression or knockout of UCP3 in mouse muscle had little effect on thermogenesis by resting excised muscles, showing UCP3 did not play a major role in this process [25]."

"Knockout of UCP1, known to play a role in thermogenesis by brown fat, also does not lead to obesity, although the knockout mouse muscle is cold-intolerant [26]."

"The mechanisms discussed above undoubtedly play some role in skeletal muscle thermogenesis."

"The important observation, however, is that they have not been shown to account quantitatively for all observed muscle thermogenesis, leaving room for additional mechanisms to play a role."

The Role of the SRX in Thermogenesis by Resting Muscle

"Changes in the relative populations of the relaxed state and the SRX can lead to dramatic changes in thermogenesis in resting muscle."

"Most myosin heads must be in the SRX in living resting muscle under basal conditions."

"Shifting only 20% of myosin heads from the SRX into the relaxed state would increase muscle thermogenesis by approximately a factor of two, increasing whole body metabolic rate by about 16%."

"There is some evidence implicating myosin in the increased thermogenesis seen during the stretch and osmotic stress discussed above."

"Stretch of resting frog muscle has been shown to diminish the intensity of the myosin layer lines, indicating that the myosin heads become disordered [27]."

"Both heat output and the change in myosin layer lines did not occur for modest stretch, but did occur at larger stretches [27, 28]."

Does Phosphorylation Regulate the SRX In Vivo?

"Phosphorylation of the RLC disorders the array of myosin heads bound to the skeletal thick filament and decreases the population of the SRX [2, 29]."

"There is another kinase that produces low levels of RLC phosphorylation, approximately 10%, in the knockout mouse [30], and it is possible that this kinase is a mediator of thermogenesis."

"A second pathway that may influence the population of the SRX is the phosphorylation of myosin binding protein-C (MBP-C)."

"One report has shown that protein kinase A can phosphorylate MBP-C from slow and fast skeletal muscles [31]."

"If this phosphorylation plays a similar role as in the mouse cardiac muscle, namely, producing disordering of the thick filament array, it would also affect the population of the SRX, thereby providing a direct connection to the thermogenic effects of epinephrine, which is known to activate protein kinase A."

Response to Long-Term Overfeeding

"The largest expenditure of excess calories, and the one that has been proposed to explain much of the wide variation in weight gain, has been non-exercise activity thermogenesis, known as NEAT [18, 32–37]."

"Despite the high efficiency of muscle, a number of low-level activities have been shown to expend a reasonable amount of calories [36, 38–40]."

"This mechanism for thermogenesis would be expected to play a greater role during low levels of activity, where muscle spends an appreciable time in the mechanically relaxed states."

"Below, there is also a stronger genetic component to muscle efficiency at low activity levels than at high ones, which again could be easily explained by the proposed model."

"In fast twitch muscle, factors that increase resting thermogenesis, such as myosin phosphorylation, epinephrine and cold exposure, also lead to potentiation of twitch tensions, showing that the muscle is more easily activated [30, 41, 42]."

The SRX as a Target for Possible Therapeutic Applications

"Activation of the metabolic rate of skeletal muscle would also lead to lower blood glucose levels."

"A major problem in type II diabetes is insulin resistance, in which insulin-mediated glucose uptake by tissues, including skeletal muscle, is impaired."

"Activation of the resting metabolic rate via increased myosin ATPase activity could possibly produce effects similar to exercise."

"Resting muscle consumes fatty acids as a major fuel source, and up-regulation of resting metabolism would consume free fatty acids and reduce the deposition of lipid in muscle, both of which are thought to be involved in insulin resistance [43]."

"Ectopic expression of UCP1 in skeletal muscle increases resting energy use and also increases the metabolism of glucose [44]."

"The two characteristics of metabolic syndrome are obesity and type II diabetes and, as suggested above, both could be alleviated by up-regulation of the myosin ATPase activity in resting skeletal muscle."

Summary

"A new state of myosin in relaxed muscle with a highly inhibited ATPase activity, the SRX, has been identified in skinned skeletal muscle fibers [2]."

"An obvious role of this state is to help achieve the low metabolic rate seen in resting in vivo skeletal muscle."

"Alterations in the population of this state have a very large capacity to alter muscle metabolism, and one physiological regulator, RLC phosphorylation, has been identified."

Myosin Regulatory Light Chain Phosphorylation Inhibits Shortening Velocities of Skeletal Muscle Fibers in the Presence of the Myosin Inhibitor Blebbistatin [45]

This is a machine-generated summary of:

Stewart, Melanie; Franks-Skiba, Kathy; Cooke, Roger: Myosin regulatory light chain phosphorylation inhibits shortening velocities of skeletal muscle fibers in the presence of the myosin inhibitor blebbistatin [45]

Published in: Journal of Muscle Research and Cell Motility (2009)

If you want to cite the papers, please refer to the original.

For technical reasons we could not place the page where the original quote is coming from.

Abstract-Summary "Phosphorylation of skeletal myosin regulatory light chain (RLC) occurs in fatigue and may play a role in the inhibition of shortening velocities observed in vivo."

"Forces and shortening velocities were measured in permeabilized rabbit psoas fibers with either phosphorylated or dephosphorylated RLCs and in the presence or absence of the myosin inhibitor blebbistatin."

"In blebbistatin maximal shortening velocities (V_{max}) at 30 °C, were decreased by 45% (3.2 ± 0.34 vs. 5.8 ± 0.18 lengths/s) in phosphorylated fibers but were not inhibited in dephosphorylated fibers (6.0 ± 0.30 vs. 5.4 ± 0.30)."

"RLC phosphorylation inhibited velocity in blebbistatin at both 30 and 10 °C, unlike previous reports where RLC phosphorylation only affected shortening velocities at higher temperatures."

Introduction

"The effect of myosin RLC phosphorylation on shortening velocity has been studied extensively, is an active site of investigation and still the subject of debate."

"Crow and Kushmerick initially suggested that shortening velocities were inhibited by myosin RLC phosphorylation, from a correlation observed in mouse skeletal muscle during a sustained tetanus [46, 47]."

"Recent work from our laboratory, has found that under conditions where fiber tensions are partially inhibited, RLC phosphorylation inhibits the inhibition of shortening velocities [48, 49]."

"In the presence of vanadate, for example, RLC phosphorylation inhibits shortening velocity compared with fibers with dephosphorylated RLC; although isometric tensions were not affected [48]."

"At the lower temperature most agents that inhibited tension, e.g. phosphate analogs or lower pH, also inhibited shortening velocity and phosphorylation of the fibers had little or no effect."

Materials and Methods

"Bundles of fibers were tied to supports, placed in glycerol solution (120 mM K-acetate, 5 mM $MgCl_2$, 5 mM EGTA, 50 mM MOPS set at pH 7.0 and 50% glycerol (v/v)) and gently mixed overnight at 4 °C."

"Bundles were then placed in fresh solution (120 mM K-acetate, 5 mM $MgCl_2$, 5 mM EGTA, 50 mM MOPS set at pH 7.0 and 50% glycerol (v/v)) pH 7.0 incubated at 4 °C for 4 h before transferring to −20 °C for storage."

"Following a 2 min equilibration period fibers were periodically and rapidly transferred to and from a third solution containing activating buffer set at a temperature of 30 or 10 °C according to the experiment and load clamps recorded."

Results

"Fiber shortening velocities were measured in the presence and absence of 20 μM blebbistatin."

"Our previous work had found that the inhibition of fiber shortening velocity by phosphorylation occurred only at higher temperatures and not at 10 °C, a temperature where many studies are carried out due to greater fiber stability."

"Addition of 20 μM blebbistatin inhibited tension equally in phosphorylated and dephosphorylated fibers and to the same extent as it did at 30 °C."

"In the absence of blebbistatin, phosphorylation had no effect on either V_{max} or α/Po, as observed previously under other conditions [48–50]."

"In the presence of blebbistatin the fibers have a greater apparent affinity for ATP, shown by the lower value of K_m in phosphorylated fibers, $K_m = 50 \pm 20$ μM, than in dephosphorylated fibers, $K_m = 330 \pm 84$ μM. This effect is similar to that seen in vanadate [48]."

Discussion

"Our previous work has found that, in conditions where sizable populations of myosin heads in states not generating force, myosin phosphorylation inhibits velocity."

"The mechanism by which phosphorylation induces inhibition of shortening velocity can be explained by its effect on the array of myosin heads bound to the thick filament."

"The observations that dephosphorylated fibers inhibited at 10 °C by blebbistatin, are fast, but that phosphorylated fibers are slow, strongly support the stability of the ordered thick filament array as playing a role in the inhibition of velocity."

"The hypothesis discussed above suggests that velocity is inhibited when there are large populations of myosin heads in non force states that are capable of interacting with the thin filament."

"Why would the presence of these non-force generating myosin heads interacting with the thin filament inhibit velocity?"

"In conditions where this non-force generating state is populated, myosin phosphorylation significantly inhibits shortening velocities."

Myosin Flares and Actin Leptomeres as Myofibril Assembly/ Disassembly Intermediates in Sonic Muscle Fibers [51]

Abstract-Summary "Each sonic muscle fiber contains a tubular contractile apparatus with radially arranged myofibrillar plates encased in a desmin-rich cytoskeleton that is anchored to broad Z bands (~1.2 μm wide)."

"Leptomeres consist of dense arrays of filaments (~4 nm) with a structure that resembles myofibrillar Z band structure."

"We propose that flares and leptomeres are distinct filamentous arrays representing site-specific processing of myofibrillar components during the assembly and disassembly of the sarcomere."

"Recent reports that myosin assembles into filamentous aggregates before incorporating into the A band in the skeletal muscles of vertebrates and Caenorhabditis elegans suggest that sonic fibers utilize a similar pathway."

"Sonic muscle fibers, with their tubular design and abundant sarcoplasmic space, may provide an attractive muscle model to identify myofibrillar intermediates by structural and molecular techniques."

Introduction

"Type I males of this species use their sonic muscle to produce a hum of ~100 Hz for up to 2 h to attract females during the mating season in early summer [52] and exhibit a unique sonic muscle fiber morphology that differs from those found in the type II (sneaker) male and the female of this species [53–55]."

"The sonic muscle consists of a pure population of specialized muscle fibers under direct somatic control by central nervous system stimuli [56, 57]."

"The most unique feature of type 1 male sonic muscle fibers is the extremely broad Z band of the myofibrils, measuring ~1.2 μm in width [53, 58], a thickness that is ~20 times the width of comparable Z bands in type II males or in females of the same species and of typical vertebrate skeletal muscle [53]."

"Its growth, especially in type 1 males, appears to be hormonally regulated, and hypertrophy of muscle fibers can be induced by seasonal variations and androgen implants [54, 55]."

"We propose that these novel clusters of contractile protein aggregates represent assembly or remodeling intermediates in sonic muscle fibers."

Materials and Methods

"Strips of sonic muscle were then removed from the swimbladder, cut into small pieces (~1 mm^3), immersion-fixed for an additional 2 h on ice, washed, and then postfixed for 1 h with 1% osmium tetroxide in 0.1 M sodium cacodylate, pH 7.2."

"To obtain single fibers from fresh or stored tissue, the strips were further dissected in relaxing solution under a stereo dissecting microscope to produce fiber bundles of approximately 0.3–0.5 mm in diameter and 5–10 mm in length."

"Sections were first fixed on the slide with 2% v/v paraformaldehyde in PBS for 30 min at RT, washed three times with PBS, and then blocked with 1% bovine serum albumin (BSA; Sigma) in PBS (PBS BSA) for 1 h at RT."

Results

"Myosin flares were composed of branching thread-like filaments and appeared to emanate from the contractile tube toward the sarcolemma in three-dimensional arrays."

"Muscle fibers containing the myosin flares ranged in area from 813 μm^2 to 1458 μm^2 (n = 52) with an average area of 1123 μm^2 (SD:±198 μm^2)."

"In these fibers, the area occupied by myosin flares ranged between 9% (84 μm^2) and 42% (463 μm^2) with an average area of 268 μm^2 (SD:±144 μm^2), or 23% of the total muscle fiber cross-sectional area."

"Muscle fibers not containing flares exhibited a diffuse punctate or spotted distribution of myosin staining around the tube in the peripheral sarcoplasm, whereas muscle fibers containing distinct flares exhibited less diffuse staining in the sarcoplasm."

"Thick filaments in sarcomeres of the contractile tube were typical of vertebrate muscle and measured ~1.5 μm in length and contained a prominent M line and bare zone."

Discussion

"Their studies have shown that, when myosin tagged with green fluorescent protein is expressed in cultured C2C12 muscle cells, it accumulates in aggregates containing short filamentous structures that are later replaced by mature myofibrils."

"We propose that myosin flares in midshipman sonic muscle are analogous intermediate structures in thick filament and A band assembly to those found in vertebrate striated muscle cells."

"Several potential functions for leptomeres have been proposed. (1) They are protective structures for Z bands during contraction; however, this is unlikely to be a general feature because of their low frequency in striated muscle. (2) They are an early intermediate of myofibril assembly; nevertheless, they are not observed in de

novo formation muscle fibers and subsequent myofibrillogenesis in typical skeletal muscle. (3) They have a tendon-like function between successive Z bands, since they have been observed spanning between Z bands in intrafusal fibers and at branching points of Purkinje fibers where shear stresses are most abundant [59, 60]. (4) They are contractile structures. (5) They are products of an alteration in myofibrillogenesis, especially in specialized or transformed striated muscle such as Purkinje fibers in which the major function of the fiber is not to contract, but rather to conduct membrane potential [61]."

Earning Stripes: Myosin Binding Protein-C Interactions with Actin [62]

This is a machine-generated summary of:

van Dijk, Sabine J.; Bezold, Kristina L.; Harris, Samantha P.: Earning stripes: myosin binding protein-C interactions with actin [62]

Published in: Pflügers Archiv—European Journal of Physiology (2014)

Link to original: https://doi.org/10.1007/s00424-013-1432-8

If you want to cite the papers, please refer to the original.

For technical reasons we could not place the page where the original quote is coming from.

Abstract-Summary "Soon after its discovery, MyBP-C was also shown to bind actin."

"This was in part because interactions of MyBP-C with the thick filament could adequately explain most (but not all) effects of MyBP-C on actomyosin interactions and in part because the specificity of actin binding was uncertain."

"We review current evidence supporting MyBP-C interactions with actin and discuss these findings in terms of their ability to account for the functional effects of MyBP-C. We conclude that the influence of MyBP-C on muscle contraction can be explained equally well by interactions with actin as by interactions with myosin."

"Because data showing that MyBP-C binds to either myosin or actin has come almost exclusively from in vitro biochemical studies, the challenge for future studies is to define which binding partner(s) MyBP-C interacts with in vivo."

Introduction

"The importance of MyBP-C to muscle contraction is further underscored by the discovery that mutations in genes encoding MyBP-C cause myopathies in both skeletal [63, 64] and cardiac [65, 66] muscles and that MyBP-C is involved in cardiac stress pathways during both normal physiologic signaling and in pathological states such as heart failure [67]."

"What is far less certain and what remains a critical unresolved question is the precise mechanism(s) by which MyBP-C affects muscle contraction."

"Because of its ability to bind to myosin through two discrete binding sites, early hypotheses focused on the idea that interactions of MyBP-C with the thick filament alone were sufficient to account for the regulatory effects of MyBP-C [68]."

"The distinction is important not only for better understanding effects of MyBP-C on contraction but also for better insight into how sarcomeres function."

Structure and Position of MyBP-C in Sarcomeres

"MyBP-C is expressed in vertebrate striated muscles where it occurs as distinct isoforms originating from three separate genes: two skeletal genes that correspond to expression predominantly in slow and fast skeletal muscles [69] and a third cardiac gene expressed in the heart [70]."

"Within a sarcomere, MyBP-C is localized to a characteristic set of seven to nine discrete stripes (with the exact number of stripes depending on the type of muscle [71]) that are evenly spaced in the C-zone of the A-band."

"MyBP-C is present at a limited stoichiometry but in discrete positions relative to myosin."

Myosin, the First Binding Partner of MyBP-C

"Given its co-purification with and strong binding to myosin, it is not surprising that early studies investigating the influence of MyBP-C on actomyosin interactions initially focused on its connections to myosin."

"MyBP-C was shown to bind to both the light meromyosin (LMM) and the S2 subfragment of myosin [68, 72]."

"Ababou and others [73, 74] demonstrated that C1 and C2 also bound to the S2Δ segment of myosin, albeit through much weaker interactions."

Actin, the Second Binding Partner of MyBP-C

"The finding that MyBP-C could bind thin filaments was striking in that it marked the discovery of the only myofilament protein (aside from myosin) that could simultaneously link thick and thin filaments together within the region of active cross-bridge cycling."

"Functional roles for a protein that could span the two filament systems were immediately suggested, such as stabilization of the sarcomere lattice through weak coupling of filaments in relaxed muscle or that MyBP-C could make transient contacts with actin such that myosin cross-bridge kinetics could be affected in contracting muscle [75]."

"Renewed interest in the physiological significance of the ability of MyBP-C to bind actin came with the discovery that the same recombinant truncated MyBP-C proteins that were found to bind myosin S2 could also bind to actin and thin filaments [76]."

"The possibility that multiple interaction sites dispersed throughout multiple N-terminal domains contribute to the actin binding properties of MyBP-C is supported by the ability of C1C2 to cross-link F-actin filaments [76, 77] and by 3-D

reconstructions of actin decorated with N-terminal MyBP-C domains showing that the recombinant proteins can span multiple actin monomers [77, 78]."

Can Interactions with Myosin or Actin Adequately Explain Effects of MyBP-C on Contraction?

"According to these authors, interactions of MyBP-C with myosin S2 could restrict the outward movement of cross-bridges toward the thin filament."

"Because phosphorylation of cardiac MyBP-C abolishes interactions with myosin S2 [79] and increases the proximity of myosin heads to actin [80], their model provides a straightforward mechanism to account for the ability of MyBP-C to limit cross-bridge interactions with actin."

"By binding to the critical S1/S2 junction, MyBP-C is in an ideal location to influence interactions with other thick filament proteins such as RLC [81, 82] or to otherwise affect myosin S1 head position or head-head interactions."

"The ability of MyBP-C to compete with myosin S1 heads and inhibit actomyosin interactions [75, 83] could provide an explanation for the long-standing puzzle that MyBP-C is present at a limited stoichiometry with respect to myosin: by being present at a low concentration relative to myosin heads, the activating effects of MyBP-C on the thin filament may be optimized while inhibitory competitive effects with myosin cross-bridges are minimized."

MyBP-C Binding Interactions In Vivo

"While there is considerable evidence to conclude that MyBP-C can bind to both myosin S2 and the thin filament, at present there is no direct evidence that MyBP-C interacts with either myosin S2 or actin filaments in sarcomeres."

"Resolution of the central question of whether MyBP-C binds to actin or myosin S2 to affect contraction will thus ultimately require innovations in structural methods that allow more precise visualization of the position(s) of MyBP-C in the sarcomere."

"It is possible that MyBP-C binds to both myosin S2 and the actin filament to affect contraction."

"Time-resolved methods such as FRET and X-ray diffraction that can differentiate dynamic changes in binding to the thick and thin filaments will thus be essential in further defining the mechanism(s) by which MyBP-C affects contraction."

Insights into Myosin Regulatory and Essential Light Chains: A Focus on Their Roles in Cardiac and Skeletal Muscle Function, Development and Disease [84]

This is a machine-generated summary of:

Sitbon, Yoel H.; Yadav, Sunil; Kazmierczak, Katarzyna; Szczesna-Cordary, Danuta: Insights into myosin regulatory and essential light chains: a focus on their roles in cardiac and skeletal muscle function, development and disease [84].

Published in: Journal of Muscle Research and Cell Motility (2019)

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If you want to cite the papers, please refer to the original.
For technical reasons we could not place the page where the original quote is coming from.

Abstract-Summary "We focus on the involvement of myosin regulatory (RLC) and essential (ELC) light chains in striated muscle development, isoform appearance and their function in normal and diseased muscle."

"We review the consequences of isoform switching and knockout of specific MLC isoforms on cardiac and skeletal muscle function in various animal models."

"We discuss how dysregulation of specific RLC/ELC isoforms can lead to cardiac and skeletal muscle diseases and summarize the effects of most studied mutations leading to cardiac or skeletal myopathies."

Introduction

"The striated muscle sarcomere contains a system of interdigitating myosin II-containing thick filaments and actin/tropomyosin (Tm)/troponin(Tn)-containing thin filaments [85]."

"Binding of Ca^{2+} to TnC triggers a series of conformational changes in actin–Tm–Tn thin filaments, allowing for ATP-dependent cyclic interactions of myosin cross-bridges with actin and muscle contraction [86, 87]."

"Important domains of the myosin head (cross-bridge) include the motor domain containing the ATP and actin binding sites, and the lever arm, which undergoes large rotational motions that drive the power stroke [88, 89]."

"To fully understand the function of myosin and how it powers cardiac or skeletal muscle, it is essential to have insight into the role of myosin light chain (MLC) components."

"This review focuses on the involvement of myosin RLC and ELC in striated muscle development and their function in cardiac and skeletal muscle in health and disease."

Diverse Appearance and Functions of MLCs

"Three-dimensional maps of vertebrate muscle thin filaments obtained by cryo-electron microscopy revealed that the N-terminal portion of the myosin ELC is in a position to make molecular contacts with the C-terminus of the actin monomer [90]."

"Despite these multilevel investigations, the questions as to whether these protein–protein contacts between the N-ELC and actin promote or inhibit the affinity of myosin for actin, force production and muscle contraction are still to be determined."

"Studies from the Szczesna-Cordary group revealed that the lack of the N-terminal ELC extension in Tg-Δ43 mice, expressing a 43 aa truncated ELC, led

to changes in myosin head orientation, positioning it closer to the actin filaments [91]."

"The important function of the N-terminal extension of the ELC protein was also demonstrated in another study using Tg-Δ43 animals that showed that the length-dependent activation in Tg-Δ43 muscle strips was blunted [92]."

Myogenesis of Striated Muscle Components

"Skeletal myogenesis begins with the expression of Myf-5 at embryonic day 8 (E8) (high levels at E9.25) in the dermomyotome of somites before formation of myotome."

"MyoD is the last transcription factor regulating myogenesis in skeletal muscle and is not detectable in the myotome until E10.5 where its expression rapidly increases until E17.5 and continues to be expressed at high levels in embryonic and fetal development [93, 94]."

"MYL4 (atrial ELC) is the most predominant gene expressed at E9.5, but its expression in skeletal muscle decreases at E15.5."

"According to Lyons and others, the expression of MYL3 (ventricular ELC) in the slow-twitch skeletal muscle starts at E15 and continues throughout adulthood [95]."

Effects of Genetic MLC Ablation/Isoform Switch in RLC and ELC Animal Models

"Lack of the ventricular RLC isoform in knock-out mice resulted in embryonic lethality at E12.5 even though the level of atrial RLC was increased reaching levels comparable to the ventricular RLC."

"The specific functions of the ventricular versus atrial isoforms of the myosin ELC were tested in transgenic mice by the Robbins laboratory [96]."

"Exchanging atrial RLC with ventricular isoform led to enhancement of mechanical properties of transgenic atrial myocytes to the level seen in ventricular myocytes of control animals."

"Using the same animal model, Buck and others showed an increase in shortening velocity in transgenic atrial myocytes expressing ventricular RLC compared to wild-type mice."

"The authors found that wild-type mice did not express the ventricular/slow skeletal RLC isoform until after birth, while it was expressed normally in the embryonic heart."

Involvement of Striated MLC Isoforms in Disease

"Ever since the very first discovery of A13T, E22K and P95A during RLC screening in HCM patients with pronounced mid cavity obstruction, a plethora of mutations in ventricular MYL2 have since been associated with one or more types of familial cardiomyopathies."

"Analyses of skeletal tissues in individuals with mutations in MYL3 have been performed, and a study by Poetter and others [97] found that biopsy obtained from soleus and deltoid muscles of patients with the M149V-ELC mutation showed

myopathic changes associated with increased accumulation of mitochondria and ragged red fiber (RRF) pattern with no abnormality of cytochrome oxidase."

"Mutations in the ventricular ELC isoform (MYL3 gene) have been identified by population studies to cause HCM phenotypes."

"By Orr and others, the authors identified a novel E11K mutation in atrial ELC in a family with a previously unreported syndrome characterized by early-onset atrial fibrillation AF (age <35 years), conduction disease and signs of a primary atrial myopathy [98]."

Summary, Conclusions and Future Directions

"The goal of this review was to summarize the role of myosin regulatory and essential light chains in cardiac and skeletal muscle development and function in normal and disease states."

"MLCs are expressed in several isoforms depending on whether they are expressed in atria, ventricles or skeletal muscles where they contribute to tissue-specific function."

"Cardiac and skeletal myogenesis refer to the process of converting progenitor cells to myocytes via expression of specific transcription factors necessary for determination of unique RLC and ELC isoforms."

"Understanding this process is crucial in dissecting specific functions of MLCs in regulating cardiac or skeletal muscle contraction."

"We reviewed the consequences of isoform switching and knockout of specific MLC isoforms in the heart and skeletal muscles on cardiac and skeletal muscle function in various animal models."

A Multi-scale Continuum Model of Skeletal Muscle Mechanics Predicting Force Enhancement Based on Actin–Titin Interaction [99]

This is a machine-generated summary of:

Heidlauf, Thomas; Klotz, Thomas; Rode, Christian; Altan, Ekin; Bleiler, Christian; Siebert, Tobias; Röhrle, Oliver: A multi-scale continuum model of skeletal muscle mechanics predicting force enhancement based on actin–titin interaction [99]

Published in: Biomechanics and Modeling in Mechanobiology (2016)
Link to original: https://doi.org/10.1007/s10237-016-0772-7

If you want to cite the papers, please refer to the original.

For technical reasons we could not place the page where the original quote is coming from.

Abstract-Summary "Although recent research emphasises the possible role of titin in skeletal muscle force enhancement, this property is commonly ignored in current computational models."

"This work presents the first biophysically based continuum-mechanical model of skeletal muscle that considers, in addition to actin–myosin interactions, force enhancement based on actin–titin interactions."

"The mechanical behaviour of titin is included on the microscopic half-sarcomere level of a multi-scale chemo-electro-mechanical muscle model, which is based on the classic sliding-filament and cross-bridge theories."

"To titin stress contributions in the muscle fibre direction, the continuum-mechanical constitutive relation accounts for geometrically motivated, titin-induced stresses acting in the muscle's cross-fibre directions."

"Predicted titin-induced stresses in the muscle's cross-fibre directions are rather insignificant."

Introduction

"Rode et al. [100] proposed a biophysical, molecular model of titin's semi-active behaviour."

"Rode et al. [100] implemented their biophysical model of force enhancement and force depression within a simple Hill-type muscle model."

"To the best knowledge of the authors, the model of Lemos et al. [101] is the only continuum-mechanical muscle model that aims to model force enhancement."

"In Lemos et al. [101], the nonlinear active force–length relationship of the muscle is replaced by a linear model when stretch of an activated muscle starts."

"The stress contributions induced by titin filaments are derived from the biophysical 'sticky-spring' model of Rode et al. [100]."

"Further, to be able to simulate submaximal contractions, in addition to passive and fully activated half-sarcomeres, the model of Rode et al. [100] needs to be extended."

"Although these terms result from purely geometrical considerations (trigonometry), none of the previous models of force enhancement [100–102] have considered this."

Material and Methods

"Rode et al. [100] consider in their model one half-sarcomere and attribute all passive forces in the half-sarcomere to the titin filaments."

"Having introduced the titin forces in the passive and active states, the model of Rode et al. [100] is now extended to account for submaximal activation levels."

"The 1D muscle fibre meshes are only used to solve the monodomain equation (including the biophysical half-sarcomere model of Shorten et al. [103] and the titin model at each discretisation point)."

"The data required for the titin model, i. e., the force–elongation relations of the distinct titin regions and the PEVK stiffness depending on the initial half-sarcomere

length preceding stretch are taken from Figures 2 and B1A of Rode et al. [100], respectively."

Results

"The aim of this section is to present simulation results of force enhancement, first, on the microscopic half-sarcomere level, then on the muscle fibre level (mesoscale), and, finally, on the macroscopic whole muscle level."

"To demonstrate the effect of force enhancement on the muscle fibre level, an 8-cm-long fibre model consisting of 301 discrete half-sarcomere models, i. e., models of the excitation–contraction coupling [103], is considered."

"Stretching the muscle while maintaining the level of activation resulted in enhanced muscle forces due to actin–titin binding compared to the isometric force–stretch relation."

"The muscle specimen is passively stretched in both the muscle fibre direction and the constrained XF direction."

"After stimulating the muscle under fixed-length conditions with 50 Hz for 500 ms, the muscle is actively stretched in fibre direction."

Discussion

"The titin model by Nishikawa et al. [102] predicts decreased stiffness of titin with decreasing active force."

"Continuum-mechanical muscle models can potentially predict the 3D geometrical deformation of the muscle and the muscle's influence on other muscle forces [104–106]."

"When stretching the activated muscle on the descending limb of the active force–length relationship, the models by Schappacher-Tilp et al. [107] and by Nishikawa et al. [102] transmit the accumulating titin tension via one actin–titin binding site."

"Force predictions of our generic multi-scale model depend, for example, on the chosen force–length relationship and the model and data describing the titin properties, which change from muscle to muscle (even in the same species, Prado et al. [108])."

"To one-dimensional models [100, 102, 107] and the only existing continuum-mechanical model of force enhancement [101], the presented chemo-electro-mechanical muscle model also predicts titin-induced stresses in the muscle's XF directions."

Interaction of Formin FH2 with Skeletal Muscle Actin. EPR and DSC Studies [109]

This is a machine-generated summary of:

Kupi, Tünde; Gróf, Pál; Nyitrai, Miklós; Belágyi, József: Interaction of formin FH2 with skeletal muscle actin. EPR and DSC studies [109]

Published in: European Biophysics Journal (2013)

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If you want to cite the papers, please refer to the original.

For technical reasons we could not place the page where the original quote is coming from.

Abstract-Summary "The formin homology 2 (FH2) domain is responsible for actin binding and acts as an important nucleating factor in eukaryotic cells."

"EPR and DSC were used to investigate the properties of the mDia1-FH2 formin fragment and its interaction with actin."

"In DSC and temperature-dependent EPR experiments we observed that mDia1-FH2 has a flexible structure and observed a major temperature-induced conformational change at 41 °C."

"The results also confirmed the previous observation obtained by fluorescence methods that formin binding can destabilize the structure of actin filaments."

"In the EPR experiments the intermolecular connection between the monomers of formin dimers proved to be flexible."

Introduction

"Experiments using fluorescence spectroscopic and paramagnetic resonance techniques have shown that formin binds to the barbed end of actin filaments and induces a change of their flexibility [110–112]."

"The binding of formins to the sides of the actin filaments is less tight and stabilizes the structure of the filaments, probably by connecting neighboring protomers [110]."

"The DSC transients also revealed the destabilization of actin filaments by formin [110]."

"It is expected that the interaction between actin and formin is mutual in a sense that their binding affects the conformation of both proteins."

"Little is known about the conformational changes in formin accompanying the actin binding."

"This domain is responsible for the interaction between actin and formin."

"We used electron paramagnetic resonance (EPR) to reveal properties of the spin-labeled formin and the consequences of its binding to actin."

Materials and Methods

"F-actin was prepared by the addition of 2 mM $MgCl_2$ and 100 mM KCl to buffer A. The mDia1-FH2 sample was dialyzed in DTT-free buffer (buffer T: 50 mM NaCl, 50 mM Tris–HCl, pH 7.6)."

"Unreacted labels were removed by dialysis in DTT-free buffer T. The amount of bound labels was determined comparing the double integrals of the EPR spectra of the labeled samples and an MSL solution of known concentration."

"The concentration of pyrene-labeled actin was determined photometrically at 344 nm by use of the absorption coefficient 2.2×10^4 M^{-1} cm^{-1} [113]."

"Unlabeled or labeled formin concentrations were 1 μM. Before the polymerization measurements the bound calcium of actin was replaced with magnesium by adding 200 μM EGTA and 50 μM $MgCl_2$ and incubating the samples for 5 min."

"The corresponding buffer solutions of formin or F-actin were used as reference samples."

Results

"MSL-labeled formin did not significantly accelerate actin polymerization."

"When dialysis of NEM-labeled formin was performed against DTT-containing buffer the effect of formin on actin polymerization was preserved."

"MSL labeled formin was mixed with monomeric actin in a low-salt magnesium-free buffer and incubated for 6 h at 4 °C."

"These results also proved that the labeled formin could initiate actin polymerization."

"This conclusion is in agreement with observations from our sedimentation experiments and also with previous results showing that formins could bind to the sides of the actin filaments [110, 111]."

"These results are in agreement with previous reports that addition of formin to actin (at a formin-to-actin molar ratio of 1:20) had a destabilizing effect on actin filament structure and resulted in a shift of approximately 1.5 °C in the transition temperature [110]."

Discussion

"For formin dimers rotating as spherical single entities one would expect values at least two times greater than for the monomers."

"On the basis of these considerations the 25.0 ns rotational correlation time was not attributed to the formin dimers, but to the individual monomers in dimers."

"The EPR signal reflects both the rotation of the entire monomer and that of the smaller part together, and the two components are characteristic of the same formin conformation."

"The rotational correlation time (τ_2) characteristic of the slower rotating component was calculated from the temperature-dependent EPR spectra obtained in the presence of actin."

"The longer rotational correlation time (25 ns) was attributed to the rotation of one formin monomer, indicating that the two formin monomers could wobble almost independently from each other in the dimers."

Conclusions

"We showed that formins can be labeled on a single cysteine residue with spin probes."