Positioning nuclei within the cytoplasm of striated muscle fiber
Cooperation between microtubules and KASH proteins

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Striated muscles contain a tightly ordered cytoplasm in which the shape and size of the nuclei are comparable and nuclear distribution is uniform. These features were recently shown to be essential for muscle function. In an attempt to elucidate mechanisms regulating the position and shape of myonuclei, we analyzed the function of the two KASH proteins that are uniquely present in the Drosophila genome, MSP-300 and Klarsicht, both expressed in striated muscles. We demonstrated that both KASH proteins cooperate to construct a unique ring composed of MSP-300 protein that surrounds and attached to the nuclear envelope. The MSP-300 nuclear ring structure recruits and associates with a network of polarized astral microtubules that enables the dynamic movement and uniform spacing between the nuclei in each muscle fiber.

Introduction

The shape of the nucleus and its positioning within the cytoplasm have been implicated as important factors in controlling chromosomal architecture and transcriptional regulation. Striated fly muscles exhibit extremely ordered cytoplasm, in which the myonuclei are evenly spaced along the entire muscle fiber, distributed at the fiber periphery, close to the sarcolemma and separated from the acto-myosin compartment. This cellular architecture is disrupted in various human myopathies, including Emery-Dreifuss muscular dystrophy (EDMD) and arthrogryposis (in which embryonic movement is arrested) suggesting a link between nuclear positioning and muscle function. Importantly, recent evidence in the model organism, Drosophila, demonstrated a direct link between myonuclei positioning and proper muscle function, emphasizing the physiological significance of correct myonuclear positioning in proper movement of the organism.7,9 Striated muscle fibers are multinucleated cells of variable size, morphology and physiology (e.g., fast or slow muscles),10-12 but in each of the distinct muscle types, the nuclei, as well as other cytoplasmic organelles are evenly distributed along the entire cytoplasm, suggesting a mechanism capable of sensing muscle dimensions, which is coupled to the cellular machinery capable of moving and/or anchoring organelles within the cell. Surprisingly, information regarding such mechanisms is extremely limited, possibly due to the inability to follow the dynamics of organelle positioning within contracting muscles in live organisms.

The involvement of KASH-SUN proteins in the regulation of nuclear positioning has been described in various cell types.13-17 SUNs are inner nuclear membrane proteins, with their SUN domain located at their C-terminal end. This domain is inserted into the inner nuclear membrane and was recently shown to form trimers within the inner nuclear membrane.18,19 The N-terminus of SUN proteins associates with LaminA/C, which forms part of the nuclear matrix, associated with chromosomal organization. KASH proteins are nuclear envelope associated proteins, which bind to a variety of cytoskeletal elements through their variable N-terminal domains. Their
C-terminal KASH domain potentially forms trimers that directly associate with the SUN trimers at the perinuclear space. Distinct KASH proteins can associate with microtubules (MT) via interaction with MT motor proteins or with intermediate filaments and/or with the actin cytoskeleton. Because KASH proteins are often co-expressed in more than one tissue, it is difficult to reveal their specific functional contribution to a given cell type. For example, Nesprin1 and Nesprin2 are both expressed in muscles as well as in neurons and their single or double knock down in mice induces a complex phenotype and early lethality (double KO); however, their specific contribution for the establishment of muscle fiber structure and function has been unclear.

To reveal the involvement of KASH proteins in the establishment of muscle fiber architecture as well as in muscle function, we analyzed the specific contribution of each KASH protein and the combination of both in Drosophila larval striated muscles. The Drosophila genome contains only two KASH proteins, MSP-300 and Klarsicht (Klar), both are expressed in muscles. Below, I describe our most significant findings and present a molecular model for the specific activity of KASH proteins in promoting organelle positioning in muscles and explain its physiological significance. This model will be discussed in view of recent studies suggesting novel molecular players in organelle positioning in muscles.

**Nuclear Positioning, but Not Number Changes During the Growth and Differentiation of Striated Muscles**

In Drosophila, multinucleated myotubes are formed by fusion of a fixed number of myoblasts to a muscle founder cell, the myotubes subsequently migrate and attach to a specific tendon cell. Muscle striation takes place at a later developmental stage after the myotube has established its myotendinous junctions with tendon cells at both muscle ends. Whereas the number of nuclei in each muscle type is fixed for a given muscle, the subcellular localization of the nuclei changes at each of these stages (see Fig. 1). Accordingly, the regulation of nuclear positioning differs at each developmental stage. This was shown by demonstrating the differential phenotypes of klar and msp-300 homozygous mutant embryos. Whereas Klar is essential for myonuclear positioning in the non-striated myotubes, MSP-300 is dispensable. Conversely, in fully differentiated striated muscles, both MSP-300 and Klar are essential and cooperate in the induction of nuclear positioning. Thus, myonuclear positioning is a dynamic process, which continuously accommodates nuclear distribution to the shape, dimensions and differentiation state of muscle fibers.

**Klar and MSP-300 Cooperate to Maintain Myonuclear Positioning in Striated Muscles**

In Drosophila, the embryonic somatic muscles grow about 40-fold to accommodate the large size of the third instar larvae; however, the number of the myonuclei does not change. Both klar null mutants and msp-300 null mutants, in which the KASH and a large portion of the spectrin repeats are deleted, exhibit a muscle phenotype in which myonuclei are aggregated leading to significantly slower larval movement, consistent with the idea that myonuclear positioning is essential for muscle function (Fig. 2B and C). The autonomous requirement of each of these proteins in muscles was proven by a muscle-specific rescue (in the case of Klar) or by the muscle specific expression of MSP-300.

To differentiate between parallel vs. cooperative function of both Klar and MSP-300 proteins, the phenotype of double-heterozygous larvae carrying a single mutant allele for each of these genes was analyzed. Single heterozygous larvae for either klar or msp-300 mutant alleles exhibit wild type muscles in which the nuclei are distributed in a wild type pattern and larval locomotion is normal (data not shown). However, double heterozygous klar/+; msp-300/+ larvae exhibit a significant disrupt of myonuclear positioning (Fig. 2D), thus they interact genetically. The genetic interaction between the two KASH proteins suggest functional cooperation between these proteins in promoting proper myonuclear positioning. Further evidence for such cooperation between Klar and MSP-300 was deduced from aberrant subcellular distribution of MSP-300 in klar mutant muscles. In wild type muscles, MSP-300 forms a specialized ring surrounding each nucleus and is connected to the Z-discs (Fig. 3A). In klar, or in msp-300 mutant muscles, the MSP-300 nuclear ring dissociates from the nuclei but is still detectable (Fig. 3B and C), whereas in the double mutant klar;msp-300 the...
The MSP-300 Nuclear Ring Bridges Between Polarized Astral Microtubule Network Surrounding Each Nucleus

Subsequent analysis revealed the contribution of the MSP-300 nuclear ring to the attachment of a specialized network of polarized MT to the nuclear envelope. Microtubule organization is a dynamic process that is controlled in a spatial and temporal manner in every cell type. Developing muscles show a dynamic distribution of MT that changes at different developmental stages. For example, prior to the development of sarcomeric architecture, the MT are arranged with their plus ends facing the ends of the muscles close to the myotendinous junction.24 However, in fully striated larval muscles, the MT at the surface are arranged in an astral organization with their plus ends facing each nucleus and in association with the MSP-300 nuclear ring." (Fig. 4, upper panel). It is assumed that the minus ends are distal to the nuclear envelope, although this has
The muscle cytoplasm. Our phenotypic analysis demonstrated that this role is unique to MSP-300 and is not shared with Klar and MT. Furthermore, the spacing and positioning of other organelles such as mitochondria and ER are also severely affected only in msp-300 but not in klar mutant muscles. How MSP-300 mediates this activity is not clear. In contrast to Klar, which is uniquely detected only at the nuclear envelope, MSP-300 is also expressed along the Z-disks. Because both the nuclei and the mitochondria as well as ER structures are distributed on both sides of the Z-disks, we hypothesize that MSP-300 physically connects each organelle to the Z-disks, explaining the phenotype of msp-300 mutant larvae and placing MSP-300 as a key protein in promoting positioning of all muscle organelles.

Concluding Remarks and Open Questions

Nuclear positioning is a dynamic process, which is tightly coupled to a given cell type, its state of differentiation, morphology and overall size. The results obtained was demonstrated in keratinocytes it is possible that MSP-300 (homologous to Nesprin) associates with the Kinesin light chain, as well. The dynamic nature of nuclear translocation within muscles is manifested by the involvement of both opposing MT motors in this process but the mechanism whereby this translocation is temporally controlled is yet to be elucidated. A similar process of nuclear separation and their even distribution was described within the early syncytium Drosophila embryo. In this process, the involvement of astral MT surrounding each nucleus and attached to actin-related structures was demonstrated. Whether these proteins participate in nuclear positioning within the large striated muscle cell is, as yet, unknown.

Anchoring the Nuclei to the Acto-Myosin Compartment Is a Unique Function of MSP-300

To enable proper order of the organelles in muscle fibers, which continuously contract, it is essential to physically anchor the organelles to existing structures within the muscle cytoplasm. Our phenotypic analysis demonstrated that this role is unique to MSP-300 and is not shared with Klar and MT. Furthermore, the spacing and positioning of other organelles such as mitochondria and ER are also severely affected only in msp-300 but not in klar mutant muscles. How MSP-300 mediates this activity is not clear. In contrast to Klar, which is uniquely detected only at the nuclear envelope, MSP-300 is also expressed along the Z-discs. Because both the nuclei and the mitochondria as well as ER structures are distributed on both sides of the Z-discs, we hypothesize that MSP-300 physically connects each organelle to the Z-discs, explaining the phenotype of msp-300 mutant larvae and placing MSP-300 as a key protein in promoting positioning of all muscle organelles.

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so far suggest that KASH proteins that intimately interact with the nuclear envelope continuously sense and respond to the dynamic changes in organization and polarization of the MT as well as the actin cytoskeleton. These interactions enable the nucleus and other organelles to change their position in response to growth and differentiation. The next challenge in this field will be to elucidate the dynamic properties that enable the KASH proteins to respond to the changing cytosplasmic environment within the muscles.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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