

# Unconventional myosins muscle into myofibrils

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**“Myosin” is famous as a component of muscle fibrils, but the majority of myosin family members act elsewhere with roles unrelated to muscle contraction. The biological functions of a relatively new family of these unconventional myosins, myosins 18A and 18B, are poorly understood. New research from Horsthemke *et al.* describes a new isoform (Myo18A $\gamma$ ) that is essential for heart function and viability in mice. Their findings both support and contradict other work in the field and raise new questions about the roles of myosin 18 proteins *in vivo*.**

The name “myosin” (from the Greek *mys*, *myos* meaning muscle) derives from the fact that ~50% of skeletal muscle mass is composed of the proteins currently designated as myosin II family members. These proteins assemble into bipolar filaments arranged in repeating arrays that slide relative to actin filaments during muscle contraction, a process propelled by myosin’s F-actin-activated ATPase. Although myosin was originally assumed to be unique to muscle cells, smaller proteins with F-actin-activated ATPase activities were then identified in *Acanthamoeba* (1), and the field of unconventional myosins in nonmuscle cells was born. Fast forward 46 years, and there are now at least 30 classes of myosins, most of which function outside of muscle (2).

Myosin 18 (myosin XVIII) is a relatively new class of unconventional myosins. The first isoform, Myo18A $\alpha$ , was discovered as a differentially displayed cDNA in bone marrow cells that support hematopoiesis, suggesting that it might play a role in cellular architecture. This protein was named MysPDZ because of a PDZ domain preceding the predicted myosin ATPase domain (reviewed in Ref. 3). Subsequently, Myo18A $\alpha$  was reported to localize to the vicinity of the Golgi and to be present only in mature macrophages, whereas a differentially spliced protein (Myo18A $\beta$ ) lacking the PDZ domain was found in most hematopoietic cells. However, subsequent work found neither co-localization of Myo18A $\alpha$  with the Golgi nor effects on Golgi morphology after shRNA-mediated knockdown or CRISPR/Cas9-mediated deletion of Myo18A messages in cultured cells (4), and a separate study indicated that myosin 18A is required for zebrafish muscle integrity (5). Meanwhile, a pro-

tein (Myo18B) with a predicted ATPase domain and C terminus structurally similar to those in the Myo18A isoforms was cloned from muscle (6) and shown to be required for sarcomere assembly in muscle and for the stacking of myosin II into stress fibers (see Ref. 7 and references therein). These findings effectively bring the field of unconventional myosin-based motility back to its intellectual home base, the muscle. However, the true function of myosin 18A remains unclear.

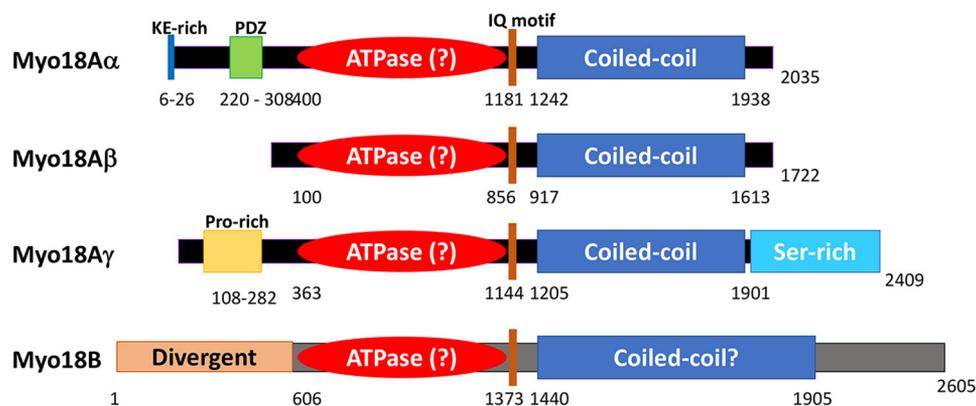
To explore this question, Horsthemke *et al.* (8) started by confirming that myosin 18A is required for muscle integrity in mice, complementing the zebrafish study (5). Specifically, they observed that homozygous deletion of myosin 18A in the whole embryo or in cardiac myocytes results in disorganization of muscle tissue in mutant embryo hearts at day 10.5 and embryonic lethality by about day 12.5. RNA sequence analyses of messages from mouse heart myocytes revealed that myosin 18A and 18B mRNA sequences together are surprisingly abundant, at ~10% of the abundance of Myh6, the major sarcomeric myosin II. Moreover, analyses of samples from mouse and human heart tissue reveal that the myosin 18A present in these muscles is a new splice form (Myo18A $\gamma$ ) that diverges at both its N and C termini from the previously characterized myosin 18 sequences (Fig. 1). The Myo18A $\gamma$ -specific, proline-rich N terminus is well-conserved among mammalian species; regions within the serine-rich C terminus also are found in corresponding sequences from chicken, frog, and zebrafish. The localization of EGFP-tagged Myo18A $\gamma$  to the myosin II-rich A-bands in cardiomyocytes suggests that, like Myo18A $\beta$  (9) and Myo18B (7), Myo18A $\gamma$  may co-assemble with myosin II filaments or bundles.

But what about myosin 18A’s other reported roles? Surprisingly, given the original report of Myo18A $\alpha$  in mature macrophages and subsequent studies in cultured cells (3), Horsthemke *et al.* find that Myo18A $\beta$  is the predominant isoform in macrophages. They also report that Golgi morphology is unaffected by the myeloid-restricted ablation of Myo18A messages and that Myo18A-deficient macrophages exhibit normal cell shapes, motility, and chemotaxis, in agreement with the work of Bruun *et al.* (4).

So where do these results leave us? Horsthemke *et al.* raise a host of questions about Myo18A that should keep this field busy for some time. First and foremost is the question of what specific roles Myo18A $\alpha$  and Myo18B play during sarcomere assembly and how they perform them. Deletion of either protein causes embryonic lethality due to cardiac defects. This is likely due to nonoverlapping functions because heterozygotes of each knockout strain survive and breed, but protein levels in

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**Figure 1. Comparative structures of the three characterized Myo18A isoforms and Myo18B.** Sequence conservation between Myo18B and corresponding regions in Myo18A is 40–45%. The divergent N and C termini of the Myo18A isoforms arise from differential splicing. Coiled-coil structure is predicted by the Eukaryotic Linear Motifs (ELM) program (elm.eu.org) (please note that the JBC is not responsible for the long-term archiving and maintenance of this site or any other third party hosted site) and has been demonstrated for Myo18A $\beta$  by EM (9). Myo18 $\gamma$  and Myo18B are each required for normal sarcomeric formation, cardiac development, and embryonic viability.

the heterozygotes have not been reported, and regulation of protein expression or degradation could theoretically obscure potential dosage effects. A second important question is whether Myo18A $\gamma$  is able to co-assemble with myosin II. The extensive coiled-coil domain in all Myo18A isoforms is followed in Myo18A $\gamma$  by a serine-rich region that might be either incompatible with myosin II co-assembly or limit bipolar filament formation to an even greater extent than in Myo18A $\beta$  (9), which has a much shorter C terminus (Fig. 1). Third, does the predicted myosin ATPase domain contain an actual myosin-like, F-actin-stimulated ATPase, and if so, is it important for function? Cell-based experiments are consistent with such activity *in vivo*, but it's possible that this ATPase activity comes from associated myosin II, and *in vitro* experiments have not been able to confirm the presence of an inherent ATPase (3). Fourth, do the novel Myo18A $\gamma$  N and C termini lead to new protein–protein interactions or serve as regulatory sites? Sequence analyses predict that the proline-rich N terminus should bind to class I and class II SH3 domains and that both the N terminus and the serine-rich C terminus are kinase substrates, leaving open a world of possibilities. Abundant interaction partners have been identified for the Myo18A $\alpha$  N terminus, but these interactors are not expected to be relevant for the cardiac isoform, given the predicted absence of their N-terminal binding sites. Paradoxically, it should be noted that overexpression of the Myo18A $\alpha$  PDZ domain in zebrafish recapitulates the knockdown phenotype (5), raising questions about whether Myo18A $\alpha$  is present in zebrafish heart or whether the PDZ domain interferes with cardiac function through an unknown mechanism. Finally, are the defects in the Myo18A and Myo18B mice limited to the sarcomeric apparatus? Staining for dystrophin and dystroglycan at the muscle membrane (sarcolemma) is reportedly disrupted in *myo18A*-deficient zebrafish muscle (5), inviting speculation about potential additional defects in gene expression, differentiation, or sarcomeric connections to the sarcolemma. Additional or alternative non-sarcomeric changes would be consistent with the reported

nuclear localization during myodifferentiation of Myo18B (6). Thus, as is typical for exciting research areas, answers to the initial questions about myosin 18 isoforms have led to a trove of additional questions of interest to researchers in muscle and nonmuscle motility.

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