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One Small Step for Muscle: A New Micropeptide Regulates Performance

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Micropeptides represent an emerging class of eukaryotic regulators that are easily missed in conventional genome annotation. Anderson et al. (2015) describe how a new tissue-specific micropeptide, myoregulin (MLN), interacts with the skeletal muscle calcium handling machinery to moderate contractile activity, representing a promising drug target for improving muscle performance.

From professional athletes at one extreme to patients with degenerative muscle diseases at the other, the effective function of our musculature is of critical importance. It is a growing issue in an increasingly elderly population, where the age-related decline in muscle function impedes quality of life and is emerging as a confounder of many age-related medical conditions. It is hardly surprising then that boosting the functional capacity of skeletal muscle is a current area of intense study. Of the many factors affecting muscle performance, calcium handling plays a central role in regulating contractile function. In striated muscle cells, the sarco/endoplasmic reticulum Ca-ATPase (SERCA) is a membrane protein responsible for the active removal of cytosolic calcium away from contractile proteins, sequestering it in the lumen of the sarcoplasmic reticulum (SR) to allow muscle relaxation (reviewed in Bassel-Duby and Olson, 2006). The SERCA protein family includes SERCA1, expressed in all skeletal muscle fibers, whereas SERCA2 predominates in heart and slow skeletal muscle fibers. The rate at which SERCA pumps calcium ions across the SR membrane has a direct impact on muscle performance and is controlled by the association with regulatory proteins phospholamban (PLN) and sarcolipin (SLN), both of which reduce the rate of calcium movement in heart and slow skeletal muscle fibers, modulating SERCA pump function. This has left fast muscle fibers, the most prevalent and powerful components of mammalian skeletal muscle, without a corresponding calcium handling control—until now.

While hunting for new skeletal muscle genes in an unbiased bioinformatics screen, Olson and colleagues (Anderson et al., 2015) stumbled upon a missing member of the SERCA regulatory protein family, which they call myoregulin (MLN). This 46-aa micropeptide had been missed for years, hidden in an uncharacterized vertebrate transcript annotated as a long noncoding RNA (lncRNA). The tiny 138-bp open reading frame (ORF) embedded in the third exon of the MLN transcript is heavily conserved between mouse and human and is expressed exclusively in skeletal muscle during embryogenesis and into adulthood. Like its related protein family members PLN and SLN, the MLN micropeptide comprises a single-pass transmembrane α helix that interacts specifically with SERCA. Elegant analyses in cultured skeletal muscle cells showed that MLN regulates SR calcium levels by inhibiting SERCA1 pump activity (Figure 1). The MLN gene is controlled in both fast and slow fibers by a core muscle transcription circuit, with functional binding sites in its promoter for MyoD and MEF2, both of which regulate skeletal myogenesis. When the group knocked out MLN gene function using TALEN technology in mice, the results were subtle but striking: the healthy animals were better performers in treadmill tests, running 55% longer distances than their wild-type littermates. Although no structural changes in muscle fiber size or composition were detected, skeletal myoblasts cultured from the MLN KO mice showed significantly increased SR calcium levels. Neither the expression of SERCA1 nor the ryanodine receptor, which pumps out calcium from the SR, was affected in MLN KO cells, nailing the function of MLN as the dominant regulator of SERCA1 activity in adult skeletal muscle and solving a longstanding mystery.

The discovery of a micropeptide embedded in a sea of untranslated lncRNA sequences is not novel: indeed, in hindsight the MLN structure is not surprising, given that both its cousins, PLN and SLN, also exist as micropeptides within larger transcripts (Fujii et al., 1987; Odermatt et al., 1997). So why did such an important regulator of muscle contractility go missing for so long, and what other hidden treasures lie undetected in the hundreds of lncRNA sequences encoded in our genomes? The oversight can be partly blamed on current annotation strategies, which are not yet sufficiently fine-tuned to discern small ORFs among the genomic noise and tend to relegate most lncRNA sequences to the non-coding pile. In fact, typical algorithms for identification of ORFs discard sequences less than 100 aa in length. It's obviously time for a second look at this rich source of potential regulators, which are now being identified with new methodologies (Bazzini et al., 2014). The authors (Anderson et al., 2015) attribute their success in discovering MLN to sequence conservation and the presence of an identifiable functional domain. Perhaps a multitude of these micropeptides waits to be discovered, controlling the activity of ubiquitous enzymes in exquisitely tissue-restricted patterns.

More intriguing questions remain. Beyond gene expression, does additional

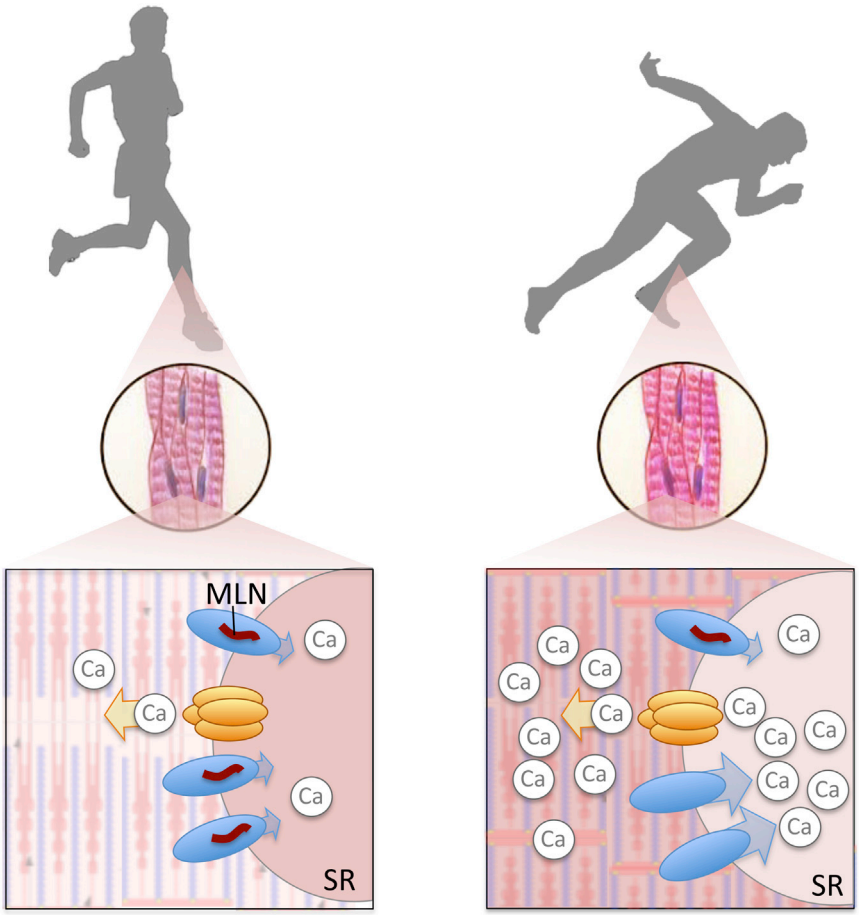

Figure 1. Enhancing Muscle Performance by Restricting MLN
In normal muscle cells (left), MLN (dark red) binds to SERCA (blue) in the membrane of the sarcoplasmic reticulum (SR) and restricts the import of calcium. In reduced MLN (right), SERCA pump activity is liberated to keep calcium high in the SR lumen, resulting in higher calcium availability and increased export by ryanodine receptors (orange) to increase contraction of working muscle cells and improve performance.

regulation of the MLN RNA and/or protein exert more subtle controls on muscle contraction? In the case of PLN, inhibition of calcium traffic is mediated by protein phosphorylation, which may also be a regulatory feature of MLN action. As in the actinin-3 polymorphism, which enhances elite power and sprint performance (Head et al., 2015), do some of our high-performance athletes harbor natural mutations in MLN that ensure better muscle function? It will be of interest to determine the role of MLN in muscle diseases specifically targeting SERCA function, such as Brody myopathy (Guglielmi et al., 2013). Being small and skeletal muscle-specific, MLN represents a particularly attractive target for drugs or small molecules to improve muscle contractility in the aged or in debilitating degenerative diseases such as amyotrophic lateral sclerosis or spinal muscular atrophy. Long-term side effects that could have been missed in the initial MLN KO mouse analysis might emerge to counter-indicate this therapeutic approach, but may not deter professional athletes more focused on the most immediate benefits of performance-enhancement regimes.

The first eukaryotic micropeptides were characterized in 2002, translated from polycistronic plant mRNA (Rohrig et al, 2002). In the last fifteen years, only a handful of additional micropeptides have been fully annotated (reviewed in Andrews and Rothnagel, 2014). Common features include the lack of an N-terminal signal sequence, although some micropeptides have cell-non-autonomous functions, suggesting potential translocation properties yet to be described. These findings are humbling: despite extraordinary technical advances in genomic data gathering and analysis, our understanding of the genetic circuitry encoded within our DNA is still remarkably patchy. We are taking small but important steps.

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