Mechanisms underlying myogenesis: complex and likely to become more so!

IN THIS ISSUE, Geiger and her colleagues (7) provide an excellent and comprehensive database of mRNA abundance and protein expression of myosin heavy chain (MHC) isoforms in the rat diaphragm during a period of rapid growth [i.e., postnatal days (P) 0, 14, 28, and 84 (adult)]. Although the authors postulated that changes in mRNA abundance would be followed by concomitant changes in MHC protein expression, this was not the case. For example, by P-28, the abundances of mRNAs for MHC_{Slow} and MHC_{2X} were largely unchanged, yet significant increments in protein expression of these isoforms were observed. Even expression of the neonatal isoform, MHC_{Neo} (interpreted by the investigators as being consistent with transcriptional control), is complex. Initially, the relative abundance of MHC_{Neo} mRNA increased significantly, whereas the relative expression of the protein decreased significantly. At P-28, the relative abundance of MHC_{Neo} mRNA was 34% with zero protein expression. Given the complex nature of myogenic regulation during development and growth and a multitude of factors impacting on the entire process of MHC protein synthesis and phenotypic expression, these results are not surprising. This editorial will provide a brief overview of these major complexities and hopefully stimulate questions and avenues for future research.

Abundance of a specific mRNA is commonly used as a “measure of transcription.” Steady-state levels of mRNA may be affected by several processes that impact on the primary transcript, such as splicing, capping, and polyadenylation, as well as regulatory influences on mRNA stability. Key transcription factors influencing gene promoter activity and important in the transcriptional control of myogenesis are MyoD, a basic helix-loop-helix (bHLH) myogenic regulatory factor (MRF), and related factors Myf5, myogenin, and MRF-4 (for excellent reviews, see Refs. 3 and 18). MyoD binding to E proteins, such as HEB, to form MyoD-E protein heterodimers capable of binding E boxes on muscle specific gene promoters, is important in myogenesis (4), with p38 MAPK exerting some regulatory influence on the heterodimer formation (15). Furthermore, myocyte enhancer factor 2 (MEF2), another transcription factor, acts in concert with MyoD to activate myogenesis. A number of factors [e.g., inhibitors of differentiation (Id) proteins] are repressors of myogenic transcription by impairing MRF-E protein heterodimer formation or interacting with MEF2 (e.g., Twist). In addition, a number of histone modifying factors [e.g., histone acetyltransferases (HAT) and histone deacetylases (HDAC)] and other chromatin-remodeling factors (e.g., SWI/SNF) can promote MyoD activator or repressor function. MyoD can also complex with the HAT-containing proteins, p300 and pCAF. Thus HDAC negatively regulates myogenesis, whereas SWI/SNF transcription factors facilitate binding of MyoD and MEF2 and activate transcription. The latter may also be influenced by p38 MAPK. Thus the MRFs together with MEF2 coordinate the activities of activators and repressors and interact with signaling proteins, chromatin-remodeling proteins, and transcription factors to achieve tight control of myogenesis. Generally, MyoD and Myf5 act to commit skeletal muscle specificity, whereas myogenin functions as a terminal differentiation factor, with MRF-4 capable of subserving both functions.

Various mechanisms have been proposed to account for the phenotypic expressions of MHCs. It has been proposed that early phenotypic differentiation may be driven by several gene families (e.g., Hox and Wnt) to regulate production of myogenic regulatory factors. During later stages, other factors such as hormonal influences, growth regulators, cytokines, innervation, transcription factors, and factors associated with activation history, add further regulatory control on phenotypic expression (21). It has been proposed that calcineurin (a calcium-calmodulin-activated serine/ threonine phosphatase), interacting with the transcription factors NFAT and MEF2, activates MHC_{Slow} gene promoters (18). This, however, is not the sole mechanism capable of promoting MHC_{Slow} expression (11). Furthermore, myoblast culture experiments reported that MyoD or Myf-5 overexpression activated the 2B promoter, whereas NFAT or activated calcineurin overexpression activated the 2A promoter. Specific factors influencing the 2X promoter were less defined (1). Alterations in muscle insulin-like growth factor (IGF)-I levels or thyroid deficiency or excess, are also known to alter phenotypic expression. Prenatal undernutrition has been reported to reduce the proportion of type II fibers of the diaphragm during early postnatal development, possibly secondary to reduced local muscle IGF-I associated with undernutrition (13, 17).

Translation is the process whereby the codons on a particular mRNA are translated to generate a string of amino acids in forming a specific protein. Translational efficiency relates the fractional synthesis rate of the protein to steady-state mRNA levels. Translation is composed of several stages subject to regulatory control. These include translation initiation [whereby Met-transfer (t) RNA and mRNA are recruited to a translationally competent ribosome], elongation (movement of the ribosome along mRNA with successive addition of amino acids), and termination (whereby the protein product is released). Several signal transduction pathways influence translation initiation. These include the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway. mTOR phosphorylates both the downstream repressor eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) to release eIF4E, as well as the 70-kDa ribosomal protein S6 kinase (p70S6K) to initiate and augment the process of translation initiation. Hormones such as insulin and IGF-I, as well as nutrients such as the amino acid leucine, may positively influence PI3K/Akt/mTOR signaling to enhance translation, whereas hormones such as corticosteroids and cytokines such as tumor necrosis factor (TNF)-α [likely acting via nuclear factor (NF)-κB] inhibit translation initiation (8, 10). Thus conditions that influence these factors impact on translation initiations of muscle proteins (e.g., 12). The same signaling pathway has been shown in myoblast cell lines to be important in myoblast differentiation (22). Thus a common signaling pathway may influence both transcriptional regulation of myogenesis as well as exerting an impact on differentiated myocytes. Diaphragm hypoplasia and reduced fiber size in the IGF-I knockout mouse likely reflects negative influences on this signaling pathway (6). It should be noted that amino acids might serve as signals for protein translation initiations independent of the PI3K/Akt/mTOR pathway (14). Finally, translation can also be enhanced by phosphorylation and inactivation of glycogen synthase kinase-3 (GSK-3) by Akt to diminish its inhibitory effect on another initiation factor, eIF2B.

Posttranslational protein modifications are important in producing a functional product. A key modification that bestows functional and regulating properties as well as structural integrity is
that of protein folding, which is assisted by molecular chaperones. A myriad of biochemical modifications can occur posttranslationally. An important and common example is that of phosphorylation, which endows functional activity to the protein. Others include nitration and S-nitrosylation, acetylation, glycosylation, etc. Proteins may also be translated as proproteins requiring enzymatic cleavage. Of note, proteolysis may also be considered a form of posttranslational modification. Following myofibrillar disassembly, proteolysis of muscle contractile proteins is mediated chiefly by the ubiquitin-proteasome system (16). Of interest, there are recent data indicating that Akt may link and bridge the synthesis and degradation arms of muscle protein turnover. Akt1 can phosphorylate and inhibit Foxo transcription factors, thus blocking their induction of muscle-specific ubiquitin E3 ligases, important in ubiquitin-mediated protein breakdown (20).

So where does the study of Geiger et al (7) fit within this complex regulation of MHC protein expression? The authors used mRNA abundance as a marker of transcription. From a purist’s viewpoint, mRNA abundance, while commonly used, is not a true measure of transcription. Other assays, such as nuclear run on, may be a more accurate determination. Furthermore, mRNA abundance may not distinguish differences in transcriptional activity from issues related to mRNA stability, etc. Nevertheless, this is a useful first approach. Apart from transcriptional regulatory influences during diaphragm development, the data reported in this paper clearly suggest that several different posttranscriptional regulatory systems impact to generate the final protein product. In addition, it should be emphasized that when evaluating postnatal diaphragm development, one is dealing in a way with a “moving target.” This is because major temporal changes in breathing pattern, chest mechanics, and lung growth ensue that impact significantly on the developing diaphragm, evolving mechanotransductive and other biochemical signals. With this in mind, it is likely that the IGF system plays an important role. Indeed, several spliced gene variants are expressed locally in muscle to exert autocrine/paracrine effects. This includes the constitutively expressed IGF-IEA (liver, muscle, other cells) and the “inducible” mechanogrowth factor (IGF-IEb in rats; IGF-IEc in humans). The latter could exert important effects along with the changing milieu of the diaphragm during postnatal development, as it can stimulate satellite cells; is stretch, exercise, and muscle injury responsive; and can promote muscle anabolism (8). Another confounder to consider (as highlighted by the authors) are states of rapid change. This can result in coexpression of MHC isoforms with the production of hybrid fibers, which further compounds analysis of regulatory mechanisms. The latter may in part result from a bias toward synthesis of mature MHC isoforms along with the programmed degradation of developmental isoforms during rapid growth. The concept of “functional gene groupings” in the molecular regulation of individual muscle fibers is thus an attractive one (19). The authors provide a solid database on which to inspire future research.

Why would data evaluating mechanisms underlying MHC expression during development and rapid growth of the rat diaphragm, as reported by Geiger and coworkers (7), be important? An important clinical implication for better understanding such mechanisms lies in the fact that during the process of muscle regeneration, MHC regulation and expression commonly mirror or recapitulate events during myogenesis, development, and growth (3, 5). It is, therefore, likely that diaphragm plasticity to various interventions or disease processes share, at least in part, common mechanisms.

REFERENCES


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