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REVIEW

Mechanisms of muscle gene regulation in the electric organ of *Sternopygus macrurus*

Robert Güth, Matthew Pinch and Graciela A. Unguez*

Department of Biology, New Mexico State University, Las Cruces, NM 88003, USA

*Author for correspondence (gunguez@nmsu.edu)

Summary

Animals perform a remarkable diversity of movements through the coordinated mechanical contraction of skeletal muscle. This capacity for a wide range of movements is due to the presence of muscle cells with a very plastic phenotype that display many different biochemical, physiological and morphological properties. What factors influence the maintenance and plasticity of differentiated muscle fibers is a fundamental question in muscle biology. We have exploited the remarkable potential of skeletal muscle cells of the gymnotiform electric fish *Sternopygus macrurus* to trans-differentiate into electrocytes, the non-contractile electrogenic cells of the electric organ (EO), to investigate the mechanisms that regulate the skeletal muscle phenotype. In *S. macrurus*, mature electrocytes possess a phenotype that is intermediate between muscle and non-muscle cells. How some genes coding for muscle-specific proteins are downregulated while others are maintained, and novel genes are upregulated, is an intriguing problem in the control of skeletal muscle and EO phenotype. To date, the intracellular and extracellular factors that generate and maintain distinct patterns of gene expression in muscle and EO have not been defined. Expression studies in *S. macrurus* have started to shed light on the role that transcriptional and post-transcriptional events play in regulating specific muscle protein systems and the muscle phenotype of the EO. In addition, these findings also represent an important step toward identifying mechanisms that affect the maintenance and plasticity of the muscle cell phenotype for the evolution of highly specialized non-contractile tissues.

Key words: electrocyte, muscle-derived cells, muscle regulatory factors, post-transcriptional regulation.

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Introduction

The wide range of contractile, biochemical and morphological properties among muscle fibers is a common feature of skeletal muscle in animal species (Burke et al., 1973; Burke et al., 1974; Talmadge et al., 1993). However, these phenotypic properties, once established, are not fixed as they can be altered in response to changes in functional demands such as exercise, mechanical load and electrical activation patterns (Liu et al., 2005; Schiaffino, 2010; Schiaffino et al., 2007). The molecular mechanisms that link external factors to changes in muscle-specific gene expression have been the object of intensive investigation. In spite of this, mechanisms by which external signals are converted into gene regulatory processes that affect muscle properties remain unknown. In some vertebrates, skeletal muscle fibers are known to exhibit an extreme phenotypic plasticity by losing their contractility during normal development to give rise to specialized cells that produce heat, as in the case of cells of heater organs in billfishes and one species of mackerel (Block, 1986; Block, 1994; Carey, 1982; Tullis and Block, 1997), emit light, as in the bioluminescent tissue in a scopelarchid fish (Johnston and Herring, 1985), or generate electricity, as in the case of electric organs (EOs) of electric fish (Bennett, 1971; Schwartz et al., 1975). The cells that make the heater organs, light organs and EOs are not only novel in their functional specializations but also unique in that they retain some phenotypic properties of their myogenic precursors. How the expression of genes coding for a select number of muscle-specific proteins is downregulated while that of others is maintained represents an intriguing problem in our current understanding of the regulation of the skeletal muscle program. The increased amenability of the gymnotiform electric fish *Sternopygus macrurus* to a variety of experimental manipulations and the availability of myogenic molecular markers have allowed us to study the role that molecular and cellular mechanisms play in regulating specific subsets of muscle proteins in mature electrocytes, the non-contractile electrogenic cells of the EO. Here we discuss findings from expression studies of distinct muscle genes at the transcript and protein levels in skeletal muscle fibers and electrocytes of *S. macrurus*. To date, these data suggest that research using EOs stands to yield important insights for our understanding of the plasticity of the skeletal muscle program and for expanding our current viewpoint of transcription-dependent myogenesis in vertebrate systems.

Muscle regulatory factors and their role in driving the vertebrate myogenic program

The discovery that muscle cell differentiation is linked to the expression of a complex group of regulators that derive from one of two major families of transcription factors was a significant step toward understanding how the skeletal muscle phenotype is regulated (Allen et al., 2001; Black and Olson, 1998; Chin et al., 1998; Gundersen, 2011; Rana et al., 2008; Schiaffino et al., 2007). These key myogenic transcription factors include the MyoD and MEF2 families (Bassel-Duby and Olson, 2006; Black and Olson, 1998; Weintraub et al., 1991; Wu et al., 2000). The myogenic

regulatory factors (MRFs) are a family of basic helix–loop–helix (bHLH) transcription factors, including MyoD, myogenin, MRF4 and Myf-5, that have been shown to play important roles in muscle cell commitment and differentiation (Arnold and Braun, 1996; Buckingham, 1992; Olson, 1990; Pownall et al., 2002; Weintraub et al., 1991). MRFs have been described as 'master' regulators of the muscle phenotype because of their potential to turn on the myogenic program following their forced expression in a variety of non-muscle cell types (Braun et al., 1989; Braun et al., 1990; Choi et al., 1990; Davis et al., 1987; Edmondson and Olson, 1989; Hopwood and Gurdon, 1990; Hopwood et al., 1991; Ishibashi et al., 2005; Kocaefe et al., 2005; Miner and Wold, 1990; Rhodes and Konieczny, 1989; Santerre et al., 1993; Weintraub et al., 1989; Weintraub et al., 1991). Gene knockout animals further demonstrate that MRFs are important for skeletal muscle development (Braun et al., 1992; Braun et al., 1994; Hasty et al., 1993; Nabeshima et al., 1993; Rudnicki et al., 1992; Rudnicki et al., 1993; Venuti et al., 1995). These genetic studies support the broadly accepted idea that expression of MRFs is essential for a cell to fully activate the skeletal muscle program.

However, other studies have shown that expression of MRFs alone is not enough to fully establish the myogenic program. For one, myogenic conversion of cells in culture requires permissive conditions such as the absence of growth factors (Braun et al., 1989; Brunetti and Goldfine, 1990; Davis et al., 1987; Edmondson and Olson, 1989). In addition, forced expression of MRFs failed to fully activate the muscle program in 3T3 fibroblast, CV1 kidney and hepatocyte cells in culture and in cells of mouse and *Xenopus* germ layers *in vivo* (Braun et al., 1990; Faerman et al., 1993; Hopwood and Gurdon, 1990; Hopwood et al., 1991; Russo et al., 1998; Schäfer et al., 1990). More importantly, some mature animal cells that express MRFs do not manifest the contractile muscle phenotype. Purkinje fibers of the cardiac conductive system express MyoD and myogenin, and contain some myofibrillar structures throughout their cytosol, but these are not functional (Takebayashi-Suzuki et al., 2001; Thornell and Eriksson, 1981). Mammalian myofibroblasts from liver, kidney and lung tissues express many skeletal muscle proteins including MRFs, yet they lack sarcomeric structures (Mayer and Leinwand, 1997; Rice and Leinwand, 2003; Walker et al., 2001). Myoid cells of the thymus also express MRFs, but their sarcomeric structures, if present, are disorganized (Drenckhahn et al., 1979; Grounds et al., 1992; Kornstein et al., 1995). MRF expression has also been reported in the muscle-derived cells of EOs in electric fishes. The EOs of the strongly electric elasmobranch fish *Torpedo californica* and *T. ocellata* contain transcripts for MyoD, MRF4 and Myf5 (Asher et al., 1994; Neville and Schmidt, 1992), but they lack myofibrillar structures and many sarcomeric proteins (Fox and Richardson, 1978; Fox and Richardson, 1979; Mate et al., 2011). These data provide strong evidence in support of a myogenic program that likely involves the expression of additional factors and signaling pathways that interact with MRFs.

The incomplete MRF-dependent myogenic program of electrocytes of *S. macrurus*

EOs are tissues specialized for the generation of electric fields that are used for navigation, communication and mating (Bennett, 1971). In all electric fish, with the exception of the Apteronotidae, mature EOs are composed of non-contractile electrocyte cells that derive from skeletal muscle fibers during development (Bass, 1986; Bennett, 1971). The origin of electrocytes from striated muscle fibers during development and tail regeneration in the adult has been well characterized in the South American gymnotiform *S. macrurus* (Kirschbaum and Schwassmann, 2008; Unguez and Zakon, 1998a). Ultrastructural studies show that electrocytes in *S. macrurus* are multinucleated like their muscle precursors but do not have sarcomeres or T-tubules (Fig. 1) (Unguez and Zakon, 1998a; Unguez and Zakon, 1998b). Consistent with results from ultrastructural studies, immunolabeling studies show that electrocytes express some muscle proteins including desmin, titin, contractile proteins α-actinin and α-actin, and endplate proteins dystrophin and acetylcholine receptors (AChRs), but do not express sarcomeric proteins like myosin heavy chains (MHCs), tropomyosin, and troponin-T (Figs 1, 2) (Cuellar et al., 2006; Kim et al., 2008; Patterson and Zakon, 1996; Unguez and Zakon, 1998a). As the nervous system plays a major role in the maintenance and plasticity of muscle fibers in adult vertebrates, it is important to note that the EO of *S. macrurus* is innervated by a population of spinal motoneurons that exerts activation patterns (continuous rate of 50–200 Hz) (Bennett, 1971; Mills et al., 1992) that are markedly different from those that activate muscle fibers (Bellemare et al., 1983; Coughlin and Rome, 1999). Characterization of skeletal muscle and EO properties in *S. macrurus* has helped establish this gymnotiform as an ideal experimental system to study the cellular mechanisms responsible for the phenotypic transformation of muscle fibers into electrocytes (Unguez and Zakon, 1998a; Unguez and Zakon, 2002) and the regulatory processes that allow electrocytes to downregulate some, but not all, components of the muscle program (Cuellar et al., 2006; Kim et al., 2004; Kim et al., 2008; Unguez and Zakon, 1998a; Unguez and Zakon, 1998b).

The wide phylogenetic distribution of the MyoD family across animal taxa (Atchley et al., 1994; Zhang et al., 1999) and

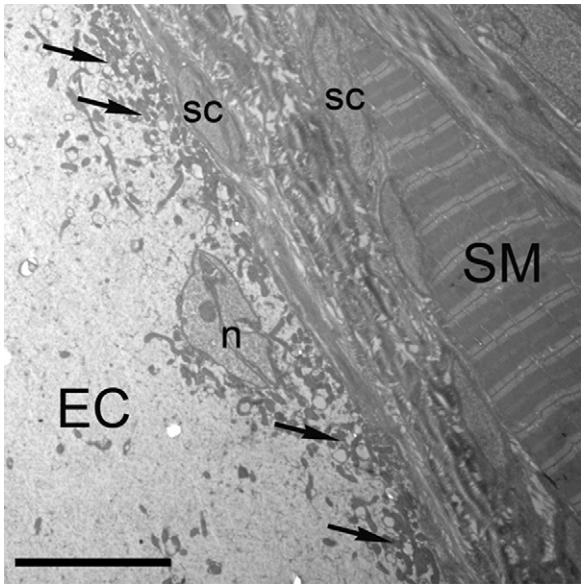

Fig. 1. Mature electrocytes of *Sternopygus macrurus* lack sarcomeric structures. Electron micrograph of a longitudinal section taken from a control tail showing part of a skeletal muscle fiber (SM) and an electrocyte (EC). The muscle fiber contains a regular array of sarcomeres. These structures are absent in the electrocyte. Mitochondria and vacuole-like structures that resemble sarcoplasmic reticula are found peripherally in electrocytes (arrows), as are nuclei (n). Note the presence of satellite cells (sc) associated with both the muscle fiber and electrocyte. Scale bar, 10 μm.

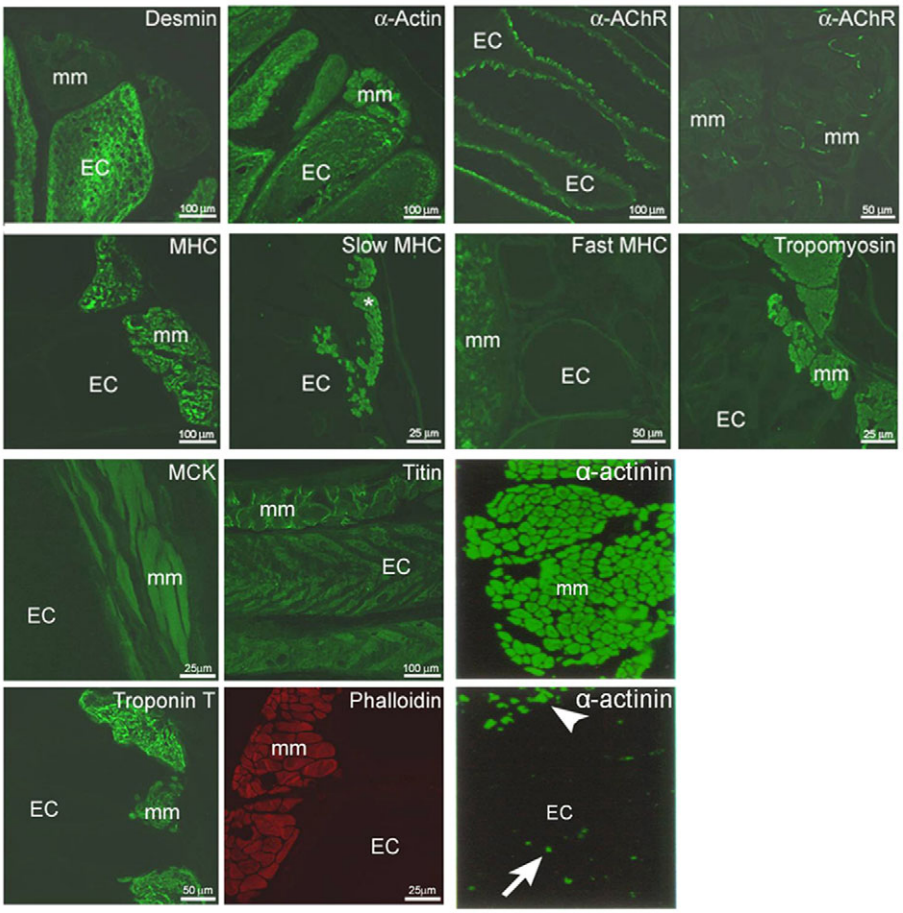

Fig. 2. Muscle protein expression in *S. macrurus* electric organ (EO). Immunolabeling of tail cryosections for various muscle proteins (green) reveals staining of electrocytes (EC, arrow in α-actinin panel) for desmin, α-actinin, α-acetylcholine receptor (AChR) and titin but not for myosin heavy chain (MHC), tropomyosin or troponin-T. Electrocytes were immunolabeled with anti-α-actin but not phalloidin, which stains filamentous actin (red). In contrast to electrocytes, skeletal muscle fibers (mm; * in the slow MHC panel; arrowhead in the α-actinin panel) stained for all muscle markers. The α-actinin image is reproduced from Kim et al. (Kim et al., 2004) (see their fig. 3) with permission from Springer Science+Business Media; all other images are reproduced from Cuellar et al. (Cuellar et al., 2006) with permission from *FASEB Journal*.

expression of some MRFs in the myogenically derived EO of *Torpedo* (Neville and Schmidt, 1992) suggested to us that the MRFs may also be pivotal to the proper development of *S. macrurus* muscle and EO. Specifically, we speculated that the expression of only some muscle genes in electrocytes is associated with an incomplete expression of the MRF genes. Cloning of *S. macrurus*-specific MRFs showed that the functional domains observed in mammalian MyoD, myogenin, MRF4 and Myf5 are conserved in *S. macrurus* MRFs (Kim et al., 2004; Kim et al., 2008). Expression analyses revealed that mature electrocytes transcribe all four MRF genes, with expression levels of myogenin, MRF4 and Myf5 transcripts being significantly higher than those detected in skeletal muscle fibers (Fig. 3A) (Kim et al., 2004; Kim et al., 2008). Immunolabeling studies using *S. macrurus*-specific antibodies against MyoD and myogenin confirmed that these MRFs are translated and localized in nuclei of muscle fibers and electrocytes (Fig. 3B) (Kim et al., 2009). Together, these data reveal that expression of multiple MRFs is not sufficient to induce the non-contractile electrocytes to fully express the striated muscle program. To our knowledge, the EO of *S. macrurus* is the first example of a non-muscle cell that expresses the full complement of MRFs and continues to retain an incomplete muscle phenotype.

Post-transcriptional regulation of the muscle program in the EO of *S. macrurus*

The predominant view to date has been that MRFs regulate the types of transcripts and proteins expressed in a developing muscle fiber (Bassel-Duby and Olson, 2006; Braun and Gautel, 2011; Schiaffino and Reggiani, 1996). Hence, to further investigate the transcriptional regulation of the electrocyte phenotype by MRFs in *S. macrurus*, we determined the expression profiles of target muscle genes with MRF binding sites, known to be activated by MRFs. These genes include sarcomeric MHC, troponin-T, tropomyosin and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) (Allen et al., 2001; Baker et al., 1998; Gaillard et al., 1998; Sehnert et al., 2002; Simonides et al., 1996; Takeda et al., 1992). In general, quantitative analysis showed similar levels of these muscle transcripts in EO and skeletal muscle, confirming previously published qualitative reports (Fig. 4) (Cuellar et al., 2006). Detection of transcripts for these contraction-associated genes in EO was unexpected given that protein expression studies using mammalian antibodies against tropomyosin, troponin-T, fast SERCA, and slow and fast isoforms of MHC failed to detect these proteins in mature electrocytes and EO lysate (Fig. 2, Fig. 4B) (Cuellar et al., 2006; Kim et al., 2004; Patterson and Zakon, 1996; Unguez and Zakon, 1998b).

While detection of these transcripts suggested that the MRF-dependent muscle activation in mammals is conserved in *S. macrurus* muscle fibers and electrocytes, the transcription of some muscle genes without apparent translation suggested that post-transcriptional events might be involved. In contrast to the above contraction-associated genes, expression profiles of metabolic genes and other muscle components such as α-actin, α-AChR and desmin show little evidence of post-transcriptional regulation as the relative abundance of transcripts and corresponding proteins or enzymatic activities are similar between skeletal muscle and EO (Fig. 4). In summary, these results suggest that maintenance of the mature EO phenotype involves complex multi-level gene

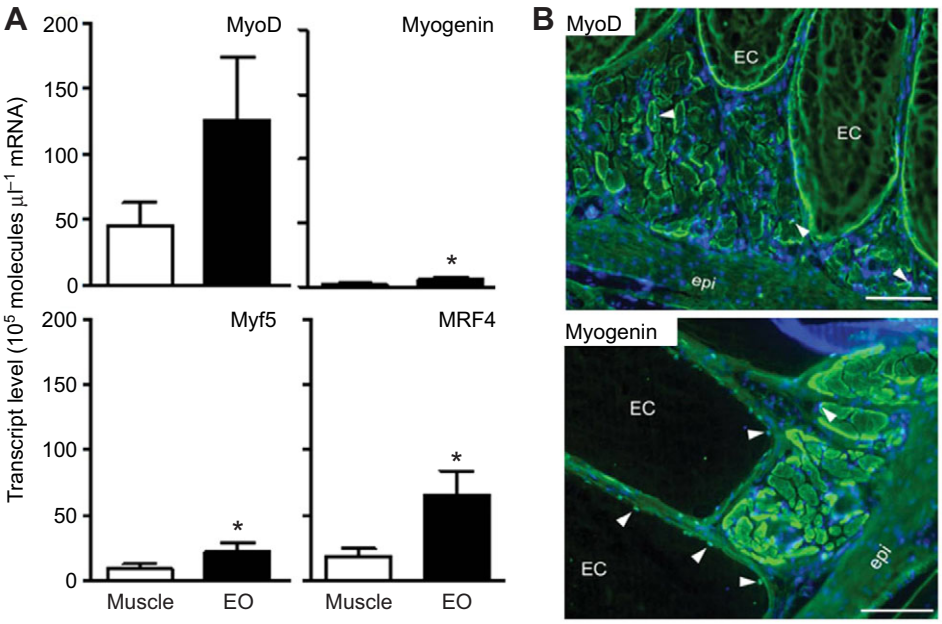

Fig. 3. Muscle regulatory factors (MRFs) are expressed in *S. macrurus* EO. (A) Transcripts of all four MRFs are detected in EO and skeletal muscle of *S. macrurus*. Quantitative RT-PCR shows significantly higher levels of myogenin, Myf5 and MRF4 transcripts (*$P \leq 0.05$) in EO than in skeletal muscle. Bars represent means + s.e.m. Reproduced with permission from Kim et al. (Kim et al., 2008). (B) MyoD and myogenin proteins are detected in the nuclei of muscle fibers and electrocytes (EC) by immunolabeling of adult tail cryosections with *S. macrurus*-specific antibodies (green). Nuclei were counterstained with Hoechst 33342 (blue). White arrowheads point to nuclei in electrocytes and muscle fibers that were double-labeled with antibody and Hoechst. epi, epidermis. Scale bars, 100 µm. Reproduced from Kim et al. (Kim et al., 2009) with permission from *International Journal of Developmental Biology*.

regulation of the vertebrate myogenic program and post-transcriptional regulation of distinct muscle genes associated with sarcomere expression.

Candidate pathways for post-transcriptional regulation of muscle genes in *S. macrurus*

The observation that mature electrocytes transcribe several muscle genes but do not appear to translate them implicates post-transcriptional events in the homeostasis of muscle-specific properties in electrocytes after differentiation. The involvement of post-transcriptional regulation has similarly been suggested to occur in skeletal muscle systems of some mammals, such as in the diaphragm and in hindlimb muscle of hibernating species (Geiger et al., 2003; Geiger et al., 2006; Rourke et al., 2006; Shanely et al., 2004; Yang et al., 1998). This provides an exciting opportunity to investigate transcriptional and post-transcriptional regulation of genes coding for a select number of muscle-specific proteins. Although little is known about the direct contribution of post-transcriptional events in the maintenance and plasticity of muscle differentiation, there are several reports documenting post-transcriptional control of the expression of specific muscle genes. For instance, targeting of select mRNAs for post-transcriptional regulation *via* their 3′ untranslated regions (3′UTRs) has been reported for MHC (Kiri and Goldspink, 2002; Vracar-Grabar and Russell, 2004) and a number of genes encoding synaptic proteins in muscle cells (Chakkalakal and Jasmin, 2003; Deschênes-Furry et al., 2005).

Translational control *via* transcript stability and translation initiation

A class of non-coding RNAs, the microRNAs (miRNAs), has emerged in recent years as a new layer of regulators of gene expression at the post-transcriptional level with crucial roles in muscle development and physiology. miRNAs are single-stranded, 21–23 nucleotide RNAs that generally inhibit translation or promote mRNA degradation by base pairing to complementary sequences, often within the 3′UTRs, of target mRNAs (Ambros, 2004; Bartel and Chen, 2004). Some miRNAs have been identified as muscle specific (Lagos-Quintana et al., 2002; Sempere et al., 2004; Small et al., 2010; van Rooij et al., 2007; van Rooij et al., 2009), and have been shown to play important roles in the proliferation, differentiation and fusion of myoblasts and in the regulation of myofiber properties (Anderson et al., 2006; Callis et al., 2009; Carè et al., 2007; Chen et al., 2006; Dong et al., 2010; Ikeda et al., 2009; Kim et al., 2006; McCarthy et al., 2009; Mishima et al., 2009; van Rooij et al., 2009). MRF expression has also been shown to be regulated by miRNAs, as in the case of Myf5 in the mouse neural tube (Daubas et al., 2009). At present, no experimental evidence of miRNA expression is available from any electric fish. However, preliminary data from *in situ* hybridization experiments show expression of miR-1 and miR-206 in skeletal muscle and EO of *S. macrurus* (G.A.U., unpublished). The fact that miRNAs and their translational repression through binding to 3′UTR sequences of target mRNAs are highly conserved across invertebrate and vertebrate species gives reason for an exploration of their function in regulating muscle gene expression in the muscle-to-electrocyte cell conversion in gymnotiforms.

Translationally silent mRNAs have been detected in cytoplasmic protein aggregates known as processing bodies, or P bodies (Kulkarni et al., 2010; Parker and Sheth, 2007; Shyu et al., 2008). While P body components have yet to be fully determined (Eulalio et al., 2007; Kulkarni et al., 2010; Parker and Sheth, 2007), preliminary work in our laboratory has verified the presence of the P body-associated protein components Dicer, RCK and HuR in the EO of *S. macrurus* (G.A.U., unpublished) (Bhattacharyya et al., 2006; Brennan and Steitz, 2001; Eulalio et al., 2007). These findings provide a stimulus to identify further markers of translational inhibition and to determine whether P bodies contribute to translational inhibition of muscle gene transcripts in EO and its muscle phenotype.

Besides miRNAs, additional mechanisms have been implicated in post-transcriptional regulation in muscle cells. Cytosolic calcium levels have been implicated in the modulation of sarcomeric protein levels without changing mRNA levels (Nikcevic et al., 2000). Also, evidence suggests that some aspects that distinguish different muscle fiber types are post-transcriptionally regulated, and this post-transcriptional regulation is dependent on the electrical activity patterns imposed on the muscle fiber (Hoover et al., 2002).

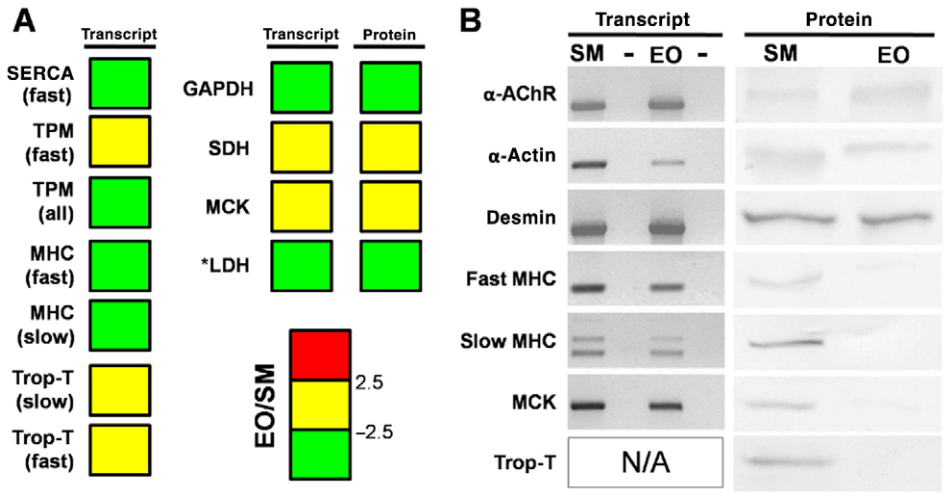

Fig. 4. Some muscle genes exhibit post-transcriptional regulation in *S. macrurus* myogenic tissues. (A) The abundance of contraction-associated and metabolic transcripts was determined in EO relative to skeletal muscle (SM) by quantitative RT-PCR. The expression of metabolic proteins was determined in EO relative to SM using western blotting. Coloration indicates a higher abundance in EO (>2.5 times, red) or in muscle (>2.5 times, green), or a similar abundance in the two tissues (<2.5 times difference between EO and SM, yellow). *Lactate dehydrogenase protein data represent an indirect measure of LDH enzymatic activity determined by assaying lactate concentration per gram of tissue. Data from R.G. and G.A.U. (unpublished). (B) Detection of muscle gene transcripts and corresponding proteins in *S. macrurus* SM and EO using RT-PCR and western blotting. 'No RT' lanes (–) demonstrate the absence of DNA contamination in samples without reverse transcriptase treatment. Adapted from Cuellar et al. (Cuellar et al., 2006) with permission from *FASEB Journal*. Muscle creatine kinase protein expression was undetectable in EO in B but detectable with increased protein loading in A. AChR, acetylcholine receptor; MHC, myosin heavy chain; MCK, muscle creatine kinase; Trop-T, troponin-T; TPM, tropomyosin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SDH, succinate dehydrogenase; LDH, lactate dehydrogenase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

The latter result is of great relevance to our model system as the electrical activation pattern of the EO is very different from that of muscle (Coughlin and Rome, 1999; Mills et al., 1992) and, interestingly, mature electrocytes re-express MHC and tropomyosin and form sarcomeres *de novo* after removal of neuronal input (Unguez and Zakon, 1998b). Whether miRNAs are involved in mediating the electrical activity-dependent inter-conversion between muscle cells and electrocytes is yet to be explored. If our future experiments reveal that neural input does regulate muscle gene expression differentially in these two myogenic cells *via* an miRNA-dependent mechanism of translational regulation, our data would be consistent with studies showing that miRNAs mediate gene expression in response to electrical activity in neurons and muscle cells (Wayman et al., 2008; Williams et al., 2009; Yang et al., 2007).

Post-transcriptional control of sarcomere formation

In *S. macrurus*, the formation of electrocytes from muscle fibers involves the disassembly of all sarcomeric structures. As previously cited, mature electrocytes do not form new sarcomeres even though they continue to express many sarcomeric components (Figs 1, 2) (Schwartz et al., 1975; Unguez and Zakon, 1998a). It is not known what factors and mechanisms contribute to sarcomere disassembly during electrocyte formation and to inhibition of sarcomere re-assembly in mature electrocytes. Data from mouse embryos and cultured myocytes have shown that perturbations in the expression of sarcomeric proteins can lead to disrupted sarcomere formation and their stability in striated muscle cells (Gotthardt et al., 2003; Gregorio et al., 1998; Nishii et al., 2008). Interestingly, changes in the expression of miRNAs such as miR-1, miR-133, miR-221 and miR-222 have also been implicated in the disruption of sarcomere organization (Ai et al., 2012; Cardinali et al., 2009; Mishima et al., 2009). By further characterizing the myogenic program in *S. macrurus* electrocytes, we have now opened up the possibility of carrying out a much needed mechanistic interrogation of the cellular and molecular muscle biology of *S. macrurus*. In summary, the elucidation of post-transcriptional and post-translational mechanisms in the formation and maintenance of the EO will not only have an impact on our understanding of events underlying the plasticity of skeletal muscle cells into non-contractile cells but also contribute to our general knowledge of events involved in the regulation of gene expression in other cell types.

Conclusions

That vertebrate skeletal muscle possesses a remarkable phenotypic plasticity is evident in its ability to completely transform into non-contractile specialized cells such as electrocytes of electric fish. The regulatory pathways that allow a differentiated electrocyte to downregulate some, but not all, components of the muscle program have yet to be identified. The recent advances addressing this question that have been reviewed here have resulted from the expansion of *S. macrurus* as a model system for cellular and molecular experimentation. We have determined that mature electrocytes in *S. macrurus* transcribe and translate all MRFs known to be essential in vertebrate myogenesis (Fig. 3). In fact, mature electrocytes transcribe many contraction-associated genes that are targets of MRFs. Yet, their corresponding protein product levels do not correlate with their transcript levels (Fig. 4). These findings were unexpected given the emphasis of transcriptional MRF-dependent myogenesis in our current understanding of muscle differentiation and maintenance. These observations lead to the conclusion that subsets of skeletal muscle genes contributing to sarcomeric structures are regulated in part by post-transcriptional mechanisms in the EO of *S. macrurus* (Fig. 5). However, it is currently unclear whether the mismatch of transcript and protein expression of sarcomeric muscle genes in

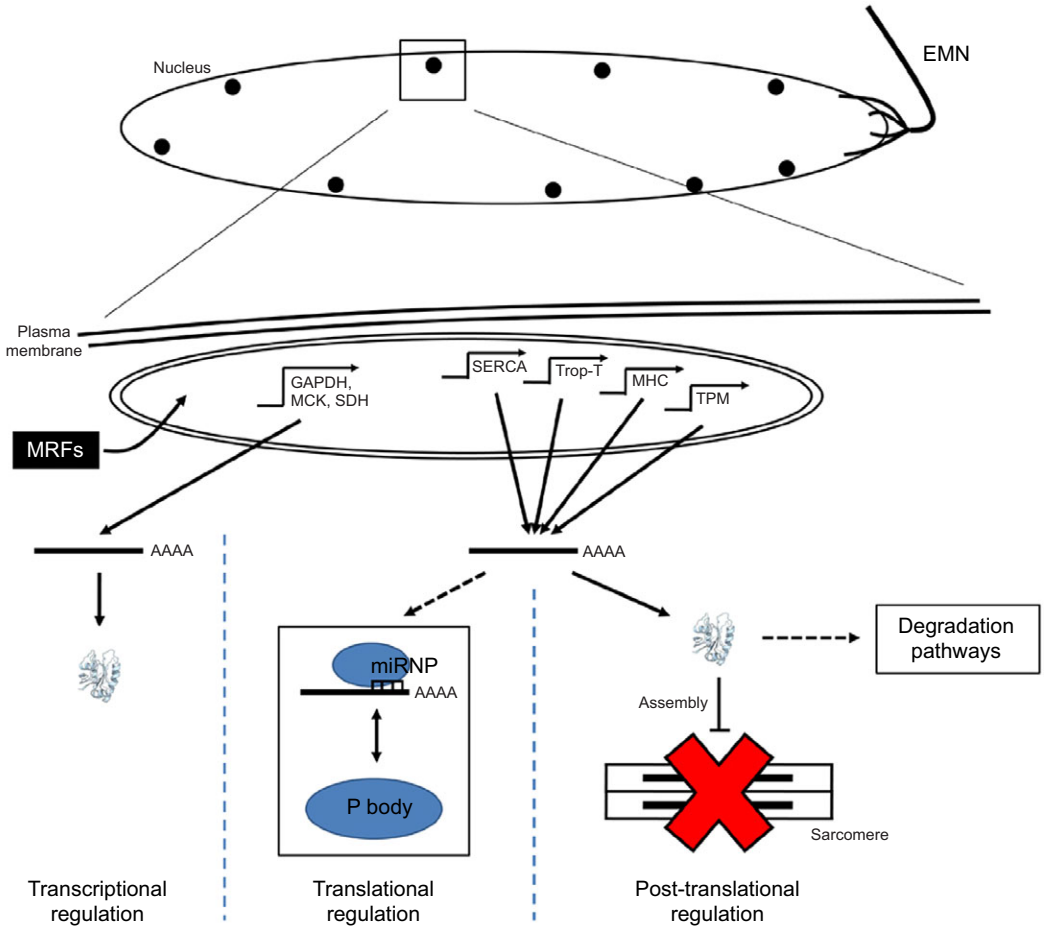

Fig. 5. Schematic diagram of regulation of the muscle program in *S. macrurus* electrocytes. Gene expression in *S. macrurus* electrocytes is regulated by a range of mechanisms as determined by quantitative analyses at the transcript and protein levels. Possible mechanisms include translational silencing of mRNAs *via* microRNA–nucleoprotein complexes (miRNP) and P body pathways, as well as post-translational regulation of protein stability and assembly into myofibrillar structures. The identity of upstream signals regulating these processes is currently unknown, but likely includes neural factors. EMN, electromotor neuron; MRFs, myogenic regulatory factors; all other abbreviations as given in Fig. 4.

EO is dependent on processes regulating translation or derived from post-translational events. Recent findings using skeletal muscle from vertebrates including fish have identified the involvement of different mechanisms that affect muscle protein composition including P-body-mediated mRNA storage (Kulkarni et al., 2010; Parker and Sheth, 2007; Shyu et al., 2008), miRNA-mediated transcription silencing (Lagos-Quintana et al., 2002; Mishima et al., 2009; Sempere et al., 2004; Small et al., 2010; van Rooij et al., 2008; van Rooij et al., 2009), changes in protein degradation rate (Beckmann and Spencer, 2008; Sandri, 2011), and stability of sarcomere structure (Boateng and Goldspink, 2008; Gregorio et al., 1998) (Fig. 5). Determining whether these processes are conserved in *S. macrurus* electrocytes and how electrocytes have exploited them to differentially suppress specific features of the muscle program provide exciting opportunities to further our understanding of the evolution of striated muscle into specialized electrogenic cells.

The fact that the suppression of select muscle gene expression in mature electrocytes is reversible and depends on a continuous, high-frequency electrical activation pattern – a process that is also implicated during the transformation of skeletal muscle fibers into electrocytes during tail regeneration (Unguez and Zakon, 2002) – also emphasizes the conserved regulation of vertebrate muscle properties by the nervous system. Such progress in our understanding of how electrocytes maintain a partial muscle phenotype has invigorated a more thorough dissection of the molecular and cellular mechanisms that can independently activate or suppress specific features of the skeletal muscle program. We believe these studies in *S. macrurus* will also provide important insights into the multiple molecular processes that regulate muscle gene expression in other vertebrates.

Future studies

The mature electrocyte phenotype is more than just a partially suppressed muscle because electrocytes also express some genes that muscle fibers do not, such as keratin and the Na(v)1.4a sodium channel (Patterson and Zakon, 1996; Unguez and Zakon, 1998b; Zakon et al., 2006). Identification of additional genes that are upregulated in electrocytes and not expressed in muscle fibers will provide a more comprehensive characterization of the electrocyte phenotype. It will be intriguing to test whether genes that are upregulated in electrocytes and not expressed in muscle fibers are regulated at the transcriptional and/or post-transcriptional level. These data will provide exciting insights into the molecular mechanisms that govern the suppression of muscle properties as well as the induction of electrogenic components.

Post-transcriptional regulation

Whether regulators of gene expression at the post-transcriptional level influence the muscle-to-electrocyte inter-conversion in electric fish is yet to be explored. Identification of the miRNA expression profile of skeletal muscle and EO in *S. macrurus* would be a logical first step. Emphasis would be placed on studying miRNAs with known functional roles in muscle phenotype regulation. myomiRs, a collection of miRNAs specifically expressed in muscle, have been shown to play roles in myogenesis, muscle fiber size, sarcomere organization and muscle gene expression (Ai et al., 2012; Bell et al., 2010; Callis et al., 2009; Liu et al., 2008; Mishima et al., 2009; Sokol and Ambros, 2005; van Rooij et al., 2007; van Rooij et al., 2009; Zhao et al., 2007). Electrocytes may contain a composition of myomiRs that is different from that in skeletal muscle. However, characterization of miRNAs expressed in electrocytes should also include those that have been shown to inhibit the skeletal muscle program (Sun et al., 2011).

Neural input

The reversibility of muscle gene expression in electrocytes that is dependent on input from the nervous system provides an exciting opportunity to investigate the neural regulation of genes coding for a select number of muscle-specific proteins. The current literature gives little insight into the activity-dependent coordination of subsets of muscle protein systems, i.e. sarcomeric, sarcolemmal and metabolic genes that more fully define the adult skeletal muscle phenotype. Further, that MRFs might not regulate changes in expression in all muscle gene warrants the identification of additional transcription factors and target genes that couple neuronal activity to remodeling of different muscle fiber properties. Other issues concerning how electrical activity patterns are converted into gene regulatory processes remain unknown. For example, it is unclear whether patterned nerve activity is coupled only to the processes that regulate gene transcription (Buonanno and Fields, 1999; Rana et al., 2009; Schiaffino et al., 1999; Schiaffino et al., 2007). Ultimately, understanding how neural input might be contributing to the regulation of gene expression in the EO of *S. macrurus* must also take into account non-electrical activity synaptic factors, or neurotrophic factors, as these are known to play important roles in not only the development and differentiation of myoblasts and muscle fibers but also the phenotypic properties of mature skeletal muscle in vertebrates (Sakuma and Yamaguchi, 2011). It is becoming increasingly clear that a complex network of transcriptional and post-transcriptional regulation is pivotal for determination and maintenance of the skeletal muscle phenotype. Research on how neural input modulates the myogenic phenotype of skeletal muscle through gene expression mechanisms at all levels can benefit from studies on vertebrates like *S. macrurus* wherein transcriptional regulation of subsets of muscle genes are independently activated or repressed and can be studied with high precision.

Comparative studies

EOs have evolved in independently at least six times and are formed *via* transformation of skeletal muscle in all families of electric fish except the Apteronotidae (Bass, 1986; Kirschbaum, 1983). Although electrocytes found in different families of electric fish share the same bio-electrogenic function, they exhibit diverse morphology, discharge patterns and myogenic phenotype (Bass, 1986; Bennett, 1971). For example, electrocytes of some African mormyriforms contain whole sarcomeric structures in the central regions of the cells (Bass et al., 1986; Schwartz et al., 1975) whereas whole sarcomeric structures are completely absent in gymnotiforms including *S. macrurus* (Bass, 1986; Bennett, 1971; Schwartz et al., 1975; Unguez and Zakon, 1998a; Unguez and Zakon, 1998b). While our studies in *S. macrurus* are uncovering the involvement of post-transcriptional events and input from the nervous system in the regulation of the muscle program in electrocytes, whether similar modes exist in other electric fish that have striated muscle cells transformed into electrocytes will need to be addressed with a comparative approach. Uncovering the differences in electrocyte formation between species could also help explain why EOs occur in some animals but not others.

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Author contributions

R.G., M.P. and G.A.U. wrote the manuscript. G.A.U. obtained the image used in Fig. 1. R.G. and M.P. obtained data used in Fig. 4A.

Competing interests

No competing interests declared.

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