Review

Regulation of myoglobin expression

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Summary

Myoglobin is a well-characterized, cytoplasmic hemoprotein that is expressed primarily in cardiomyocytes and oxidative skeletal muscle fibers. However, recent studies also suggest low-level myoglobin expression in various non-muscle tissues. Prior studies incorporating molecular, pharmacological, physiological and transgenic technologies have demonstrated that myoglobin is an essential oxygen-storage hemoprotein capable of facilitating oxygen transport and modulating nitric oxide homeostasis within cardiac and skeletal myocytes. Concomitant with these studies, scientific investigations into the transcriptional regulation of myoglobin expression have been undertaken. These studies have indicated that activation of key transcription factors (MEF2, NFAT and Sp1) and co-activators (PGC-1α) by locomotor activity, differential intracellular calcium fluxes and low intracellular oxygen tension collectively regulate myoglobin expression. Future studies focused on tissue-specific transcriptional regulatory pathways and post-translational modifications governing myoglobin expression will need to be undertaken. Finally, further studies investigating the modulation of myoglobin expression under various myopathic processes may identify myoglobin as a novel therapeutic target for the treatment of various cardiac and skeletal myopathies.

Key words: hypoxia, transcriptional regulation, calcineurin, myoglobin, NFAT, MEF2, PGC-1α, transgenic mice.

Introduction

Myoglobin is a monomeric hemoprotein that has captivated the interests of scientists and physicians for the past 60 years. It is a cytoplasmic hemoprotein that is expressed primarily within cells of striated muscle lineages of vertebrates (i.e. cardiomyocytes and oxidative skeletal muscle myofibers). Previous biophysical, cellular, molecular, pharmacological and physiological investigations have elucidated important findings regarding myoglobin's structure and functional role within cardiac and skeletal muscles of various vertebrate species (Flogel et al., 2004; Flogel et al., 2005; Flogel et al., 2001; Garry et al., 1998; Godecke et al., 1999; Godecke et al., 2003; Grange et al., 2001; Hendgen-Cotta et al., 2008; Kanatous et al., 1999; Kanatous et al., 2002; Kanatous et al., 2008; Kendrew, 1963; Kendrew et al., 1958; Kendrew et al., 1960; Kendrew et al., 1954; Mammen et al., 2003; Meeson et al., 2001; Noren et al., 2001; Noren et al., 2005; Takahashi et al., 2000; Wittenberg, 1965; Wittenberg, 1966; Wittenberg, 1970; Wittenberg, 2007; Wittenberg and Wittenberg, 1987; Wittenberg and Wittenberg, 2003; Wittenberg and Wittenberg, 2007; Wittenberg et al., 1975; Wittenberg et al., 1985). These studies have clearly demonstrated myoglobin's role as an oxygen (O_2) storage hemoprotein, facilitator of intracellular O_2 transport, and modulator of O_2 and nitric oxide (NO) homeostasis. As there have been several recent reviews that have focused on the functional roles of myoglobin, the current review will instead highlight and summarize studies undertaken to enhance our understanding of the mechanisms regulating myoglobin expression.

Myoglobin genomic organization and gene structure

Myoglobin is a member of the globin family, which includes cytoglobin, hemoglobin and neuroglobin. Myoglobin is phylogenetically most similar to cytoglobin, another monomeric hemoprotein that is ubiquitously expressed in all vertebrate tissues (Burmester et al., 2002; Singh et al., 2009; Trent and Hargrove, 2002). Myoglobin, cytoglobin and hemoglobin are believed to have shared a common ancestral globin more than 500 million years ago (Burmester et al., 2002). The genomic structure of the myoglobin gene has been extensively studied in a variety of species, including the Grey seal (*Halichoerus grypus*), human (*Homo sapiens*), mouse (*Mus musculus*) and sperm whale (*Physeter catodon*) (Blanchetot et al., 1986; Weller et al., 1986; Wittenberg and Wittenberg, 1989). These studies have demonstrated a considerable homology among various species in regards to the genomic structure of the myoglobin gene. The overall genomic structure of the myoglobin gene is relatively simple and is composed of only three exons and two introns. Among all the species studied, exon 2 encodes the heme-binding domain, which is responsible for the reversible binding to various ligands (i.e. O_2, NO, free radicals, etc.) (Blanchetot et al., 1986).

Tissue-restricted myoglobin expression within vertebrates

In vertebrates, myoglobin transcript is predominantly expressed in striated muscle lineages and is generally believed not to be expressed in non-muscle tissues nor in myogenic progenitor cells (Graber and Woodworth, 1986; Underwood and Williams, 1987). With that stated, there is recent evidence that myoglobin may be

expressed in smooth muscle cells, certain human epithelial cancers and in non-muscle tissues of certain species of fish (Cossins and Berenbrink, 2008; Cossins et al., 2009; Flonta et al., 2009; Fraser et al., 2006; Rayner et al., 2009; Roesner et al., 2006). Although the ectopic expression of myoglobin in these tissues is presumably regulated at the transcriptional level, the exact regulatory pathways governing myoglobin expression in these non-muscle tissues have not yet been determined.

In both humans and mice, an increase in myoglobin transcript level occurs during *in vitro* myoblast differentiation and during early myogenesis (Fig. 1A–C) (Garry et al., 1996; Ordway and Garry, 2004; Weller et al., 1986). During murine embryogenesis, myoglobin mRNA level is first detected at E9.5 within the myotomes of developing somites and continues to increase in a tissue-restricted pattern through the later stages of embryogenesis (Fig. 1C) (Mammen et al., 2006).

During the postnatal period there is a robust increase in both myoglobin transcript and protein levels within striated muscles, which parallels the increase in the expression of nuclear-encoded mitochondrial genes (i.e. cytochrome oxidase-1, β-hydroxyacyl CoA dehydrogenase, lactate dehydrogenase, malate dehydrogenase and phosphofructokinase) (Baldwin et al., 1978; Garry et al., 1996; Kelly et al., 1991; Kim et al., 1995; Yan et al., 2001). The co-expression of these sets of genes defines the oxidative fiber phenotype of fast and slow myofibers, which becomes evident within the first 10 days of postnatal life (Garry et al., 1996). Therefore, by adulthood there is considerable heterogeneity in myoglobin expression within mammalian striated myofibers (Garry et al., 1996; Peter et al., 1972; Underwood and Williams, 1987). Muscles designed for prolonged contractile activity (i.e. cardiac myocytes and slow-twitch, mitochondria-rich, oxidative skeletal muscle myofibers) have high myoglobin concentrations as compared with muscles adapted for short periods of contractile work (i.e. fast-twitch, glycolytic skeletal muscle myofibers). Finally, myoglobin protein concentration within adult mammalian striated muscle can be enhanced with increased contractile activity by electrical stimulation, exercise training or exposure to hypoxic conditions (Garry et al., 1996; Mammen et al., 2003; Pattengale and Holloszy, 1967; Rayner et al., 2009; Underwood and Williams, 1987).

Similar to skeletal muscle development during postnatal development, there exists a temporal–spatial expression pattern for myoglobin within the developing heart. Initially, myoglobin transcript is expressed within the developing ventricles and is absent in the atria (Garry et al., 2003). Within the ventricles, there is a gradient in myoglobin expression with more abundant expression in the subendocardium as compared with the subepicardium (Fig. 2A) (Parsons et al., 1993). However, during the later stages of embryogenesis this gradient within the ventricles is diminished, and in the adult heart there is uniform myoglobin expression in both the ventricles and the atria (Fig. 2B) (Garry et al., 2003; Parsons et al., 1993). Because the temporal–spatial pattern of transcript and protein expression for myoglobin are similar during embryogenesis, it is presumed that myoglobin expression within the developing heart is regulated at a transcriptional level; however, there is no study to definitively support this conclusion.

Network of regulatory motifs within the myoglobin promoter

The observation of differential expression pattern of myoglobin during myogenesis and in various adult striated muscle fiber types has prompted investigators to study the putative myoglobin promoter and the various transcription factors that are responsible for the regulation of myoglobin transcription. The early studies investigating the transcriptional regulation of the myoglobin gene were predominantly speared headed by R. S. Williams and his colleagues initially at Duke University and then at the University of Texas Southwestern Medical Center.

Utilizing standard *in vitro* transcriptional assays, it has been demonstrated that the 2 kb upstream fragment of the human myoglobin gene is able to drive transgene expression similar to that of the endogenous myoglobin gene (Devlin et al., 1989). Analysis of the 2 kb putative myoglobin promoter region has led to the identification of a 57 base pair (bp) cis-acting element, which is conserved in other mammals (i.e. mouse and seal) (Devlin et al., 1989). This positive control element is located between –261 and –205 from the transcription start site. Utilizing cultured skeletal myoblasts obtained from breast muscle from 11 to 12 day-old chick embryos, the authors demonstrated that a 167 bp fragment (–371 to –205) of the myoglobin promoter region containing the 57 bp cis-acting element enhanced reporter activity only in differentiated myotubes but not in undifferentiated myoblasts or fibroblasts. The authors concluded from this study that the 2 kb putative myoglobin promoter region contains a muscle-specific enhancer region that is essential in regulating myoglobin transcription during skeletal myocyte differentiation.

In order to verify the significance of this putative 2 kb myoglobin promoter region in an *in vivo* animal model, a transgenic promoter–reporter mouse was engineered by cloning the 2 kb putative human myoglobin promoter region into a LacZ-reporter
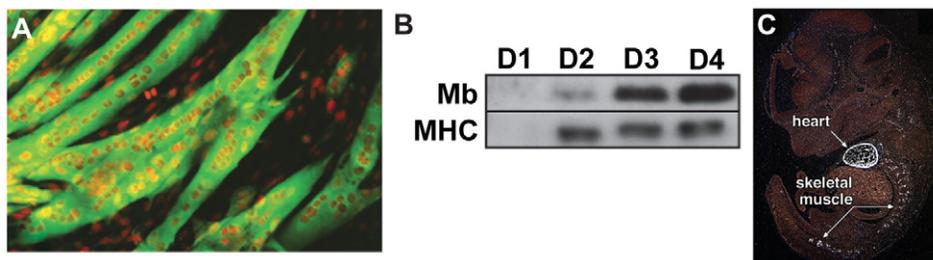

Fig. 1. Differential expression pattern of myoglobin during myogenesis. (A) Immunohistochemical staining indicates myoglobin (green) is localized to the cytosol and is robustly expressed within fully differentiated C2C12 myotubes. Propidium iodide (red) is used to identify and demarcate the nuclei. (B) Western blot analysis reveals a significant increase in myoglobin and myosin heavy chain protein levels during C2C12 cell differentiation. The number of days the C2C12 myoblasts were exposed to differentiating media are identified as D1, D2, D3 and D4. (C) *In situ* hybridization of a mouse E12 embryo reveals marked expression of myoglobin transcript levels in the developing heart and myotomes of the developing somites (Mb, myoglobin; MHC, myosin heavy chain). Modified from Ordway and Garry, and Mammen et al. (Ordway and Garry, 2004; Mammen et al., 2006).
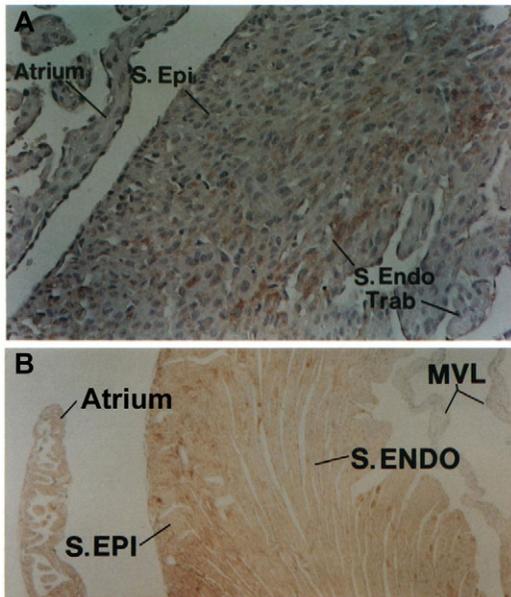
Fig. 2. Gradient in endogenous myoglobin expression during murine cardiogenesis. Immunohistochemical staining of myoglobin expression (brown) in the developing mouse heart at E18.5 (A) and in the adult murine heart (B). During cardiogenesis no staining was noted in the atrium, while in the ventricle there was greater myoglobin expression in the subendocardium as compared with the subepicardium. In the adult heart, there was uniform expression of myoglobin in both the atrium and ventricle but no expression on the mitral valve leaflet (MVL, mitral valve leaflet; S. Endo, subendocardium; S. Epi, subepicardium; Trab, ventricular trabeculae) (modified from Parsons et al., 1993).

construct that was then injected into fertilized murine oocytes (Parsons et al., 1993). This mouse model demonstrated that the 2 kb putative human myoglobin promoter region is able to direct muscle-specific (skeletal and cardiac muscles) and developmentally regulated β-galactosidase expression in a pattern similar to endogenous myoglobin expression (Figs 3 and 4). Therefore, this study suggests that the 2 kb putative human myoglobin promoter region contains the necessary transcriptional control elements to regulate myoglobin transcription, at least under resting, normoxic conditions.

In a subsequent study utilizing *in vitro* transcriptional assays and transgenic mice, a CCAC-box motif, located within the cis-acting element, and a downstream evolutionary conserved A/T motif have been established as key elements required for the transcriptional activation of myoglobin within cardiac myocytes of the intact heart

(Bassel-Duby et al., 1993). These motifs are evolutionarily conserved among a variety of specifies (human, mouse and seal). Further studies have established a collaborative interaction between the transcription factors MEF-2, which recognizes the A/T motif, and Sp1, which is one transcription factor recognizing the CCAC-box motif, and it is this collaborative interaction that participates in the regulation of myoglobin transcription in a muscle-specific manner (Grayson et al., 1998; Grayson et al., 1995). However, it should be noted here that Sp1 is not the only transcription factor believed to recognize the CCAC-box motif. Data by the Williams laboratory suggest that the myocyte nuclear factor (MNF), a member of the winged-helix family of transcription factors, also recognizes this DNA-binding motif and serves a role in myogenesis and muscle regeneration (Bassel-Duby et al., 1994; Garry et al., 2000; Garry et al., 1997; Hawke et al., 2003; Meeson et al., 2007; Shi et al., 2010; Yang et al., 1997; Yang et al., 2000). However, to date there are no detailed studies investigating the role of MNF in modulating myoglobin transcriptional activity. Finally, *in vitro* studies investigating the role of a TATA sequence within the myoglobin promoter have indicated that the transcriptional regulation of the myoglobin gene is sensitive to mutations within this specific sequence (Wefald et al., 1990). Furthermore, the synergistic effects of various transcription factors binding to the CCAC-box and A/T motifs is dependent on the intact muscle-specific TATA sequence found within the myoglobin promoter (Grayson et al., 1995).

In addition to the importance of the CCAC-box, A/T and TATA motifs, two evolutionarily conserved NFAT response elements (NRE) are also noted within the 2 kb putative human myoglobin promoter region. Elegant studies were undertaken that demonstrated a synergistic interaction between the transcription factors NFAT and MEF-2 in regulating myoglobin and other muscle-specific gene expression patterns (Chin et al., 1998). Because transcriptional activation of NFAT and MEF-2 is mediated by calcineurin, a calcium–calmodulin activated phosphatase, this study supported the role of a combinatorial interaction between the calcineurin/NFAT and calcineurin/MEF-2 pathways in regulating muscle-specific myoglobin expression (Chen et al., 2007; Chin et al., 1998; Molkentin et al., 1998; Olson and Williams, 2000; Schaeffer et al., 2004). Studies also undertaken in a fibroblast cell line deficient in these muscle-specific transcription factors failed to reveal calcineurin-dependent activation of myoglobin, further supporting the role of the calcineurin pathway in regulating myoglobin transcription. Finally, two conserved E-box motifs flanking the A/T motif in the proximal portion of the putative myoglobin promoter region have been identified to negatively regulate myoglobin expression and thus provide another level of regulation to the tightly controlled expression pattern of myoglobin
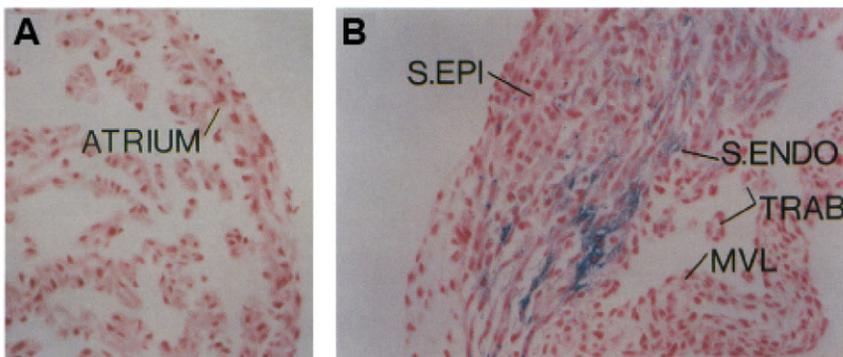

Fig. 3. Differential myoglobin promoter–reporter activity in the embryonic murine heart. β-galactosidase staining (blue) of a transgenic myoglobin promoter–LacZ reporter mouse recapitulated endogenous myoglobin expression within the developing mouse heart (E18.5). There was no staining in the atrium (A) or on the mitral valve leaflet. In the ventricle (B), there was greater staining in the subendocardium as compared with the subepicardium (MVL, mitral valve leaflet; S. Endo, subendocardium; S. Epi, subepicardium; Trab, ventricular trabeculae) (modified from Parsons et al., 1993).
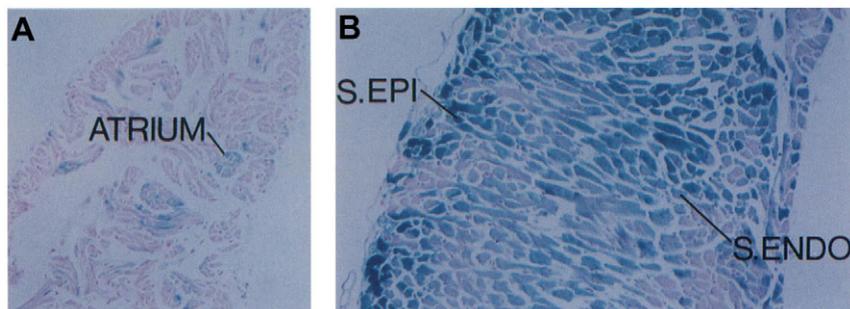
Fig. 4. Uniform myoglobin promoter–reporter activity in the adult murine heart. β-galactosidase staining (blue) of a transgenic myoglobin promoter–LacZ reporter mouse reveals uniform staining within the atrium (A) and ventricle (B). The β-galactosidase staining recapitulates the endogenous myoglobin expression within the adult murine heart (S. Endo, subendocardium; S. Epi, subepicardium) (modified from Parsons et al., 1993).

(Yan et al., 2001). Although the two E-box motifs have been identified as important regulatory elements the exact transcription factor(s) that bind to this motif are unknown at this time.

Finally, a recent study by Lin et al. has demonstrated that the transcriptional co-activator, peroxisome-proliferator-activated receptor-γ co-activator-1 (PGC-1α), also serves a significant role in modulating myoglobin gene transcription (Lin et al., 2002). In this elegant study by the Spiegelman laboratory, the investigators engineered a transgenic mouse overexpressing PGC-1α and demonstrated that this transcriptional co-activator regulates the expression of type I skeletal muscle fibers by regulating the expression (at both the transcript and protein levels) of various type I muscle fiber specific proteins (i.e. troponin I and myoglobin). Specifically, the authors undertook standard *in vitro* luciferase transcriptional assays in C2C12 myoblasts transfected with a myoglobin promoter–reporter construct. Transient transfection of these cells with PGC-1α and calcineurin or MEF-2 resulted in increased luciferase activity, which was attenuated when the MEF-2 binding motif was mutated. Thus, the authors concluded from their studies that PGC-1α also plays a central role in regulating myoglobin transcription *via* a calcineurin/MEF-2 dependent mechanism.

Collectively, the results from various transcriptional assays and the engineering of transgenic promoter–reporter mice have clearly demonstrated there exists a complex synergistic interaction between various transcription factors in regulating muscle-specific transcription of the myoglobin gene under normoxic conditions (Fig. 5). In addition, these studies indicate that the regulation of myoglobin transcription is similar in both skeletal and cardiac muscle. It must be noted here that the existing data regarding the transcriptional regulation of myoglobin expression comes from either *in vitro* transient transfections of promoter–reporter constructs or from *in vivo* transgenic mice expressing promoter–reporter constructs. There is a high degree of congruency between the promoter–reporter activity, myoglobin transcript levels and myoglobin protein levels; thus suggesting that myoglobin expression is regulated by transcriptional mechanisms. However, due to inherit limitations in transient transfection assays (i.e. transfection efficiency, transience, lack of incorporation into the genome, etc.) and transgenic mice (i.e. transgene copy number, transgene incorporation site), these techniques are imperfect read-outs of endogenous gene activity. Therefore, based on the current literature one cannot conclusively conclude that endogenous myoglobin expression is regulated solely at the transcriptional level and that it is possible post-translational mechanisms may also contribute to the regulation of myoglobin expression within a myocyte.

Calcium, muscle fiber type and myoglobin expression

Evidence has already been presented in the previously paragraphs regarding the importance of the calcineurin pathway in regulating myoglobin expression. Because calcineurin activity is regulated by calcium, this element plays an indirect role in regulating myoglobin expression within a myocyte. Calcium serves an essential role in multiple steps of muscle development from embryogenesis to adulthood. Differences in motor nerve activity differentially affect calcium fluxes and intracellular concentrations, which ultimately determines the fiber type composition within a specific skeletal muscle. Tonic motor nerve activity leads to a sustained elevation of intracellular calcium, which stimulates the calcineurin/NFAT pathway and the expression of slow oxidative muscle fibers. Associated with these fibers is the expression of fatigue-resistant, slow-contracting myosins, oxidative enzymes and myoglobin (Chin et al., 1998; De Arcangelis et al., 2005; Lin et al., 2002). Under normoxic conditions, changes in muscle fiber type are usually associated with changes in the protein expression of myoglobin, such that stimuli that bring about a contraction within slow-twitch oxidative muscle fibers are associated with increases in myoglobin protein levels, while stimuli that trigger contraction within fast-twitch glycolytic fibers are associated with a decrease in myoglobin levels.

The association of myoglobin with the slow-twitch fiber programme has been observed in multiple vertebrate family members. A surprising but significant result from our recent work reveals that, under hypoxic conditions as the sole stimuli, there is a significant decrease in the protein expression of myoglobin in the tibialis anterior muscle of mice with no changes in muscle fiber type distribution (Kanatous et al., 2009). The simple interpretation of this data is that under hypoxic conditions there exists unidentified regulatory control elements that differentially modulate myoglobin and fiber type expression. Two alternative explanations may also account for the observation made in this study. One possibility is that the decrease in myoglobin expression
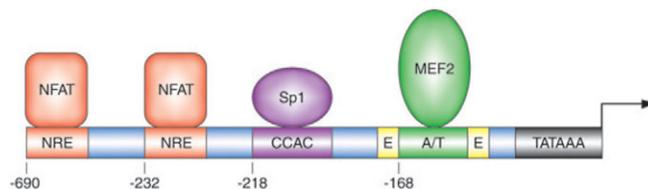

Fig. 5. Network of transcription factors regulating myoglobin transcription. Although the 2 kb 5′ upstream region of the myoglobin gene has been demonstrated to recapitulate endogenous myoglobin expression within mammalian striated muscle, the essential transcriptional regulatory motifs are located within the proximal portion of this putative promoter region. The schematic identifies the network of transcription factors that recognize and bind to these evolutionarily conserved motifs and thus are responsible for the muscle-specific transcriptional regulation of the myoglobin gene (MEF2, myocyte enhancer factor-2; NFAT, nuclear factor of activated T-cell; NRE, NFAT response element) (modified from Garry et al., 2003).

in the hypoxic tibialis anterior muscle may be due to a decrease in myoglobin mRNA stability. Another possibility is that the transcriptional regulation of myoglobin during myogenesis is regulated by the well-described calcineurin/NFAT and calcineurin/MEF2 pathways, while the maintenance of myoglobin levels (*via* ubiquitination) is regulated by factors that are independent of factors modulating muscle fiber type specification.

Role of hypoxia and calcium fluxes in regulating myoglobin expression

As mentioned earlier in this review, myoglobin's role in regulating intracellular O_2 homeostasis has been clearly established. The abundance of myoglobin in the skeletal muscles of hypoxia-adapted animals and humans suggest that hypoxia plays a role in regulating myoglobin transcription. This observation is further supported by the demonstration that a variety of cellular and molecular adaptations regulated by the transcription factor, hypoxia inducible factor 1 (HIF-1) are upregulated in the myoglobin null mouse, presumably to compensate for the lack of myoglobin and thus maintain viability of these mice (Garry et al., 1998; Godecke et al., 1999; Grange et al., 2001; Meeson et al., 2001). While the transcriptional machinery necessary to mediate muscle-specific transcription of the myoglobin gene has been extensively investigated under normoxic conditions, the mechanism underlying the regulation of myoglobin transcription and expression under low O_2 tension remained unknown until recent studies by Roesner et al. and Kanatous et al. (Kanatous et al., 2009; Roesner et al., 2006).

A few years earlier we had demonstrated myoglobin protein expression is increased in the working heart and weight-bearing muscles (i.e. soleus muscle) of the hypoxic mouse (Mammen et al., 2003). In addition, we observed a significant decrease in myoglobin expression within the quiescent tibialis anterior muscles. This data, in conjunction with the our observations that the skeletal muscles of diving mammals in captivity are significantly less red compared with similar muscle groups from free-ranging counterparts (S.B.K., unpublished data), as well as conflicting studies investigating myoglobin expression in response to hypoxia lead to the hypothesis that hypoxic stress in conjunction with contractile work are required for the increase in myoglobin expression in hypoxia-adapted vertebrates. As discussed in an editorial review of our recent manuscript by Dr Beatrice Wittenberg, a similar hypothesis was put forward as early as 1939 by Dr G. A. Millikan (Millikan, 1939; Wittenberg, 2009).

Analysis of the 10 kb upstream region of the myoglobin gene reveals no putative HIF-1 binding motifs. This observation is supported by *in vitro* transcriptional assays demonstrating the myoglobin promoter region is not directly responsive to either hypoxia or HIF-1 (Kanatous et al., 2009). Utilizing cellular, molecular, physiological and transgenic technologies the study demonstrates that indeed hypoxia alone does not induce an increase in myoglobin expression but instead an increase in calcium release from the sarcoplasmic reticulum activates calcineurin signaling pathways (*via* NFAT and MEF-2), which in turn increase myoglobin transcript and protein levels within hypoxic striated muscle (Kanatous et al., 2009). In fact, hypoxia alone resulted in the selective release of calcium from the endoplasmic reticulum, which inhibited myoglobin transcription by removing NFAT from the nucleus. However, it was the combination of hypoxic stress and contractile work that resulted in the preferential release of calcium from the sarcoplasmic reticulum resulting in an increase in myoglobin transcription and protein expression. Our study supported the work done by Stiber and colleagues, who

demonstrated that the calcineurin/NFAT signaling pathway is regulated by distinct intracellular calcium pools during myotube differentiation (Stiber et al., 2005). Collectively, our recent study indicates that both hypoxic stress and contractile work are required to increase myoglobin transcript and proteins levels under low O_2 tension (Fig. 6).

The role of alternative animal models and tissues for deciphering the regulation of myoglobin expression

Recently, numerous studies have described the development of diving capacity with enhanced oxygen stores within the skeletal muscle of diving mammals (Clark et al., 2007; Noren et al., 2001; Noren et al., 2005; Kanatous et al., 2008). These studies have found that at birth the skeletal muscle of pups have approximately 30% less myoglobin as compared with the adults. Although these levels are significantly lower than adults they are still higher than those seen in athletic terrestrial mammals. This unique animal model offers an alternative method to investigate the regulation of myoglobin expression.

A recent study by Kanatous and colleagues observed that the protein expression of myoglobin in Weddell seals follows an unexpected course during the development of these diving mammals (Kanatous et al., 2008). Weddell seals have three distinct life stages and during each stage the amount of myoglobin expressed within the skeletal muscle varies as a function of their diving activity. Therefore, the seal may serve as a natural *in vivo* model to decipher the regulatory factors governing myoglobin expression. As newborn pups they are primarily non-diving mammals for their first weeks of life. As juveniles (1–2 years of age) they are still relatively small in size compared with the adults and as such they are short duration, very active divers (mean dive duration is around 2 min). The myoglobin protein level within the skeletal muscle of the juvenile seals is significantly more than either the pups or the adults. Finally, as adults, they are relatively large mammals (mean mass of 450 kg) that transform into deep, long duration (mean dive duration of 20 min), relatively slow swimming divers. Therefore, the myoglobin level in adult skeletal muscle is significantly higher than in pups and approximately tenfold higher in comparison with athletic terrestrial mammals.

The increased myoglobin expression in juvenile Weddell seals is associated with predicted changes in protein expression of numerous
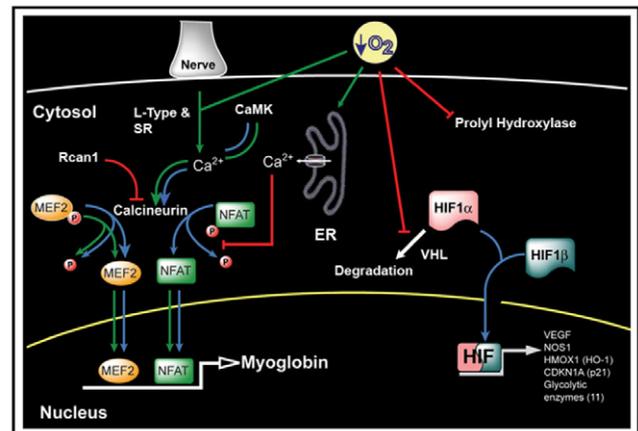

Fig. 6. Transcriptional regulation of myoglobin under hypoxic conditions. This schematic highlights the importance of the various intracellular calcium pools that regulate the calcineurin signaling pathways and in turn myoglobin transcription.

calcium regulatory and responsive proteins such as calcineurin and inositol phosphate 3 receptors (Kanatous et al., 2008). The unique role this animal model could play in deciphering the regulation of myoglobin expression is during the transition from the juvenile stage to the adult stage, where there is a significant increase in exposure to hypoxic environments with a significant drop in activity levels, yet these animals still maintain extremely high myoglobin levels as compared with terrestrial mammals. Therefore, we hypothesize that the marked abundance of myoglobin protein within the striated skeletal muscles of these diving mammals is the result of a complex interplay between physical activity, hypoxia, reperfusion injury and the generation of reactive oxygen species associated with diving. Thus, the regulation of myoglobin expression at least in the Weddell seal may be modulated by both transcriptional as well as post-translational regulatory factors.

Recent studies involving other diving mammals such as the grey seal (*Halichoerus grypus*) and emperor penguin (*Aptenodytes forsteri*) have found significant increases in the protein expression of myoglobin in the developing young before they undertake their first dives. The results from these studies demonstrate that in some species swimming and diving are not required to initiate changes in myoglobin expression (Noren et al., 2005; Ponganis et al., 2010).

Finally, other recent studies have demonstrated ectopic expression of myoglobin in non-striated muscle tissue (Cossins et al., 2009; Flonta et al., 2009; Fraser et al., 2006; Kanatous et al., 2009; Rayner et al., 2009; Roesner et al., 2006). These studies have observed low-level myoglobin expression in diverse tissues like the brain, breast tissue and smooth muscle cells. Ectopic myoglobin expression in these non-striated muscle tissues suggests that there may also exist other tissue-specific regulatory factors modulating myoglobin expression independent of the calcineurin signaling pathways.

These alternative animal models (Weddell or grey seals, emperor penguin or fish) represent natural, *in vivo* settings to analyze the differential regulation of myoglobin during ontogeny. In addition, as presented at the 2nd International Conference of Respiratory Science (Bonn/Bad Honnef, Germany; 9–13 August 2009), the authors have successfully isolated and cultured primary muscle cells from the developing and adult Weddell seals (S.B.K., unpublished data). These novel immortalized seal muscle cell lines offer a unique *in vitro* method for investigating the regulation of myoglobin expression (at both the transcriptional as well as the post-translational level) under varying environmental stress conditions. Thus, these alternative and novel *in vitro* and *in vivo* models offer unique methods to study the regulation of myoglobin expression and explore alternative pathways that may govern myoglobin expression in species other than the human and mouse. Collectively, these model systems open new frontiers into the study of the transcriptional and potentially post-translational regulation of myoglobin expression.

Conclusion

Over the past two decades significant progress has been made towards enhancing our understanding of the regulatory mechanisms governing myoglobin expression during embryogenesis, postnatal development, adulthood and hypoxic stress. The regulation of myoglobin transcription is a highly coordinated process that involves the intracellular release of select pools of calcium resulting in the activation of calcineurin signaling pathways and the interaction between a network of transcription factors (i.e. MEF-2, NFAT and Sp1) and transcriptional co-activators (i.e. PGC-1α). Although significant advances regarding our understanding of the regulation of myoglobin expression have been made, several unanswered questions remain. Future studies addressing whether there exists differential transcriptional regulatory pathways or post-translational modifications governing myoglobin expression in the heart *versus* skeletal muscle and amongst different vertebrate species will need to be undertaken. In addition, studies are needed to further define the molecular mechanism by which hypoxic stress and contractile activity regulate select pools of calcium resulting in the induction of myoglobin expression. Finally, further scientific investigation into the molecular mechanisms modulating myoglobin expression under various myopathic processes may identify myoglobin as a novel therapeutic target for the treatment of various cardiac and skeletal myopathies.

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