Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/modo

The RNA-binding protein Rbm24 is transiently expressed in myoblasts and is required for myogenic differentiation during vertebrate development

Raphaëlle Grifone [a,b], *Xin Xie* [a,b], *Adeline Bourgeois* [a,b], *Audrey Saquet* [a,b], *Delphine Duprez* [a,b], *De-Li Shi* [a,b,*]

[a] *Sorbonne Universités, UPMC Univ Paris 06, UMR 7622, Laboratory of Developmental Biology, Paris F-75005, France*
[b] *CNRS, UMR 7622, Laboratory of Developmental Biology, Paris F-75005, France*

ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form
5 August 2014
Accepted 22 August 2014
Available online 16 September 2014

Keywords:
Rbm24
MyoD
Embryonic myogenesis
Myogenic differentiation
Mouse
Chick

ABSTRACT

RNA-binding proteins (RBP) contribute to gene regulation through post-transcriptional events. Despite the important roles demonstrated for several RBP in regulating skeletal myogenesis in vitro, very few RBP coding genes have been characterized during skeletal myogenesis in vertebrate embryo. In the present study we report that *Rbm24*, which encodes the RNA-binding motif protein 24, is required for skeletal muscle differentiation in vivo. We show that *Rbm24* transcripts are expressed at all sites of skeletal muscle formation during embryogenesis of different vertebrates, including axial, limb and head muscles. Interestingly, we find that Rbm24 protein starts to accumulate in MyoD-positive myoblasts and is transiently expressed at the onset of muscle cell differentiation. It accumulates in myotomal and limb myogenic cells, but not in Pax3-positive progenitor cells. *Rbm24* expression is under the direct regulation by MyoD, as demonstrated by in vivo chromatin immunoprecipitation assay. Using morpholino knockdown approach, we further show that Rbm24 is required for somitic myogenic progenitor cells to differentiate into muscle cells during chick somitic myogenesis. Altogether, these results highlight Rbm24 as a novel key regulator of the myogenic differentiation program during vertebrate development.

1. Introduction

In the developing vertebrate embryo, skeletal muscles of the body are derived from transitory segmented structures called somites, budding off from unsegmented paraxial mesoderm alongside of the neural tube. Pax3-positive muscle progenitor cells are located in the dermomyotome, which constitutes the dorsal epithelial layer of each somite. These cells fall down from the four edges of the dermomyotome to form the underneath myotome. This myotome, considered as the first skeletal muscle to form in the embryo, expands dorsally and ventrally to build all the musculature of the trunk. At the brachial and lumbar levels, Pax3-positive progenitor cells delaminate from the ventrolateral dermomyotome to migrate into the developing limb buds and differentiate into appendicular muscle

* Corresponding author. UPMC Univ Paris 06, UMR 7622, Laboratory of Developmental Biology, Sorbonne Universités, Paris F-75005, France. Tel.: +33 1 44272772; fax: +33 1 44273445.
E-mail address: de-li.shi@upmc.fr (D-L. Shi).
http://dx.doi.org/10.1016/j.mod.2014.08.003
0925-4773/

masses (Buckingham and Vincent, 2009). Somites are not the only site of myogenesis during vertebrate development; head muscles are also formed by cells originating from the prechordal and the pharyngeal mesoderm. Consistent with their different embryological origins, the genetic hierarchies operating upstream of myogenic specification are divergent in head and trunk mesoderm (Grifone and Kelly, 2007). However, at all sites of myogenesis, entry of specified progenitor cells into the myogenic program depends on the action of the myogenic regulatory factors (MRF) of the MyoD family of basic helix–loop–helix (bHLH) transcription factors: Myf5, MyoD and Mrf4 (Buckingham, 2006). Definitive muscle identity is acquired when threshold levels of these transcription factors are attained and downstream genes are activated (Weintraub, 1993). While upstream regulators that coordinate lineage specification through the activation of *Myf5* and *MyoD* have been extensively characterized, the downstream mechanisms by which MyoD and Myf5 could specifically stabilize the muscle fate and promote muscle differentiation are not well understood. *Myogenin*, the fourth MRF member, whose expression is detected later during the myogenic program, is considered as a differentiation gene that promotes myoblasts to differentiate into functional mature myofibers by directly activating the expression of genes coding for contractile proteins (Davie et al., 2007). *Myogenin*-null mouse embryos indeed form *Myf5*- and *MyoD*-expressing myoblasts, but they are deficient in differentiated muscles (Venuti et al., 1995). *Mrf4*, which is expressed in myoblasts, also regulates their differentiation and subsequent myotube maturation (Braun and Arnold, 1995; Patapoutian et al., 1995). In addition, signals mediated by the evolutionarily conserved Notch pathway have been implicated downstream of MyoD in the regulation of myogenic differentiation in vertebrate embryos (Wittenberger et al., 1999). *Delta1* is expressed in myoblasts as well as in differentiated myocytes and may provide signals that regulate and sustain skeletal muscle differentiation (Delfini et al., 2000; Schuster-Gossler et al., 2007; Vasyutina et al., 2007). As a downstream target of MyoD, *Vgl2* has also been shown to be associated with skeletal muscle differentiation in chick myogenesis (Bonnet et al., 2010). Moreover, in addition to positively regulate *Myf5* and *MyoD* expression in the myotome and the limbs, six homeoproteins have been shown to act downstream of MyoD to activate the expression of *Myogenin* and muscle specific genes during embryogenic myogenic differentiation (Grifone et al., 2005; Relaix et al., 2013; Richard et al., 2011).

Global coordination of gene expression not only depends on transcriptional regulation, but also to a large extent on post-transcriptional events. RNA-binding proteins (RBP) are involved in modulating the metabolism of mRNAs at all stages of their lifetime thus appearing as key regulators of post-transcriptional gene expression. By controlling RNA metabolism, RBP have been implicated in different tissue-specific processes during embryonic development and the significance of post-transcriptional mechanisms for the regulation of cell differentiation becomes increasingly evident during embryogenesis (Graindorge et al., 2008; Huot et al., 2005; Spagnoli and Brivanlou, 2006). However, the possible contribution of post-transcriptional regulation by RBP to muscle development is only beginning to come to light. For example, HuR RNA-binding protein has been shown to enhance myogenic differentiation in vitro by binding to and stabilizing *MyoD* and *Myogenin* mRNA (Figueroa et al., 2003). The Lin-28 RNA-binding protein has also been identified as an essential regulator of differentiation of cultured myoblasts, by increasing IGF2 mRNA translation efficiency (Polesskaya et al., 2007). Moreover, IMP2 RNA-binding protein influences C2C12 myoblasts motility by post-transcriptional regulation of cell adhesion proteins during myogenic differentiation (Boudoukha et al., 2010). More recently, RNA biogenesis defect caused by aberrant expression of RBP coding genes has emerged as a new pathogenic mechanism underlying a number of inherited diseases including myotonic dystrophies. Facioscapulohumeral muscular dystrophy (FSHD) has been linked to an altered expression of FXR1P, an RNA-binding protein highly expressed in skeletal muscle and whose inactivation in mice leads to a reduced musculature phenotype at birth (Mientjes et al., 2004). The FRG1 RNA-binding protein, whose upregulation has been demonstrated in FSHD muscle, is another candidate for this human muscle disease (Davidovic et al., 2008). Transgenic mice overexpressing FGR1 display a post-natal muscle growth defect and exhibit impaired muscle regeneration (Xynos et al., 2013). Whereas the involvement of RBP during myogenic differentiation has been mostly investigated in vitro, no expression and functional analyses have been carefully conducted in vivo during somitic myogenesis in vertebrates. Recently, the RNA-binding protein Hoi Polloi has been reported to play a role in the regulation of myotube elongation during *Drosophila* embryogenesis. This study highlights the essential role of post-transcriptional gene regulation during embryonic myogenesis (Johnson et al., 2013).

Rbm24 gene encodes an RNA-binding protein harboring in its N-terminal part a single RNA-Recognition Motif (RRM) that is highly conserved in *C. elegans, Xenopus*, mice and human (Fetka et al., 2000). *Seb4*, the orthologous gene of *Rbm24* in *Xenopus*, was shown to be expressed in the early gastrula, concomitantly with *MyoD*, in presomitic paraxial mesoderm and later developing somites (Fetka et al., 2000). We have reported that *Seb4*, as a target gene of MyoD, is required for *Xenopus* primary myogenesis, which takes place in presomitic mesoderm. This first wave of myogenesis is a *Xenopus* specificity that is not shared by avian and mammalian system (Li et al., 2010). Our results conferring a myogenic potential to Seb4 protein during early *Xenopus* development was supported by an in vitro study showing that mouse Rbm24 protein binds to the 3′-UTR region of *Myogenin* mRNA. This interaction stabilizes *Myogenin* mRNA and promotes myogenic differentiation in C2C12 mouse myoblast cell line (Jin et al., 2010). In the present study, we have carried out a detailed analysis of *Rbm24* expression during development in representative animal models including *Xenopus*, chick and mice. By in situ hybridization we show that *Rbm24* is expressed in all sites of skeletal muscle formation, including head, trunk and limbs. Using specific antibodies we have compared the pattern of Rbm24 protein accumulation with that of known muscle markers during mouse embryonic myogenesis and established that Rbm24 is transiently expressed in a narrow time window in differentiated myogenic cells, which have entered in the myogenic program as assayed by the expression of *MyoD*. By chromatin immunoprecipitation assays we have demonstrated that MyoD is recruited in vivo to the conserved *Rbm24* 5′-untranslated region in mouse embryo. Moreover, we show that *Rbm24* ex-