AAP China Scholarship Council - CSC 2023
PROJET DE RECHERCHE DOCTORALE (PRD)

Titre du PRD : Mechanistic analysis of post-transcriptional regulation in muscle cell differentiation

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Ecole doctorale de rattachement : ED515 - Complexité du vivant

Nombre de doctorants actuellement encadrés : 0

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Unité de recherche : Code (ex. UMR xxx) et Intitulé : CNRS-UMR7622, Laboratory of Developmental Biology

Ecole doctorale de rattachement : ED515 - Complexité du vivant

Nombre de doctorants actuellement encadrés : 0

CO-TUTELLE INTERNATIONALE envisagée : ☐ OUI ☒ NON

DESCRIPTIF du PRD :

*Ce texte sera affiché en ligne à destination des candidates et candidats chinois : il ne doit pas excéder **2 pages** doit être rédigé en **ANGLAIS***

Mechanistic analysis of post-transcriptional regulaion in muscle cell differentiation

Skeletal muscle development is a temporally and spatially regulated process. Compared with the transcriptional regulation of gene expression mediated by myogenic factors, the role of RNA-binding proteins (RBPs) in the post-transcriptional regulation of muscle differentiation is far less understood. RBPs play key roles in muscle development, regeneration and disease [1]. They control RNA metabolism at multiple levels, including pre-mRNA splicing, mRNA transport, localization, stability, polyadenylation, and translation [1]. Mutations or dysfunctions of many RBPs disrupt the spatiotemporal pattern of protein synthesis and cause muscle-related diseases such as muscular dystrophy [1]. Therefore, RBPs are key players orchestrating gene expression networks to promote muscular differentiation and function. Given the growing number of diseases associated with RBPs, understanding their functional implication in muscle development may have the potential to use them as therapeutics.

Our recent works show that RNA-binding motif protein 24 (Rbm24) displays highly conserved tissue-specific expression patterns across vertebrate species [2-4], as well as dynamic subcellular localization and function during muscle differentiation and regeneration [2,5]. We also made the first demonstration that Rbm24 is required for cellular differentiation by regulating poly(A) tail length and translational efficiency of tissue-specific mRNAs [6]. Based on these unprecedented findings, the present project is aimed at understanding the post-transcriptional mechanism by which Rbm24 regulates muscle development. We will use zebrafish as a model, which is particularly attractive for studying early developmental processes due to its transparency for live imaging, suitability for genome editing, and availability of large amounts of embryos for biochemical and functional assays. This work is original and innovative because it will investigate an important novel aspect of post-transcriptional regulation of muscle differentiation. The results should shed light on the etiology of muscle diseases linked to dysfunction of RBPs-mediated gene expression.

Objective 1. Identifying muscle-specific Rbm24-regulated post-transcriptional events

We have generated zebrafish rbm24 mutants by CRISPR/Cas9 technology. These mutants show defective somite organization. RNA-seq analyses have allowed us to identify several muscle-related genes with reduced expression in the mutants, for example hspb5, hoxa5a, actn2b, mef2ca, xirp2b, smpx and myh7ba. We will use RT-qPCR and in situ hybridization to further validate the RNA-seq data and analyze temporal changes of their expression in rbm24 mutants.

Our first aim is to understand the post-transcriptional mechanism by which Rbm24 regulates muscle gene expression. Based on our original finding that loss of Rbm24 affects cytoplasmic polyadenylation in a subset of muscle mRNAs, we will use RNA-seq and TAIL-seq analysis combined with poly(A) tail length assay to identify muscle-related mRNAs with reduced poly(A) tail lengths following loss of Rbm24. These mRNAs should be targets of Rbm24 in muscle cells. Since the poly(A) tail length of an mRNA can influence its stability and/or translation efficiency, we will select mRNAs of interest to further examine their stability and translation efficiency. We will also perform functional analyses of the selected genes after overexpression or CRISPR/Cas9-mediated knockout, to examine the effects on muscle differentiation. This will open the door to new insight into Rbm24-regulated muscle-specific post-transcriptional events.

Objective 2. Deciphering the mechanisms of Rbm24 in cytoplasmic polyadenylation

Rbm24 also interacts with other proteins to regulate target gene expression. Our recent works show that Rbm24 forms a complex with two key proteins involved in regulating poly(A) tail length: the cytoplasmic polyadenylation element-binding protein (Cpeb) and the cytoplasmic poly(A)-binding protein (Pabpc). This suggests that Rbm24 may be a new component of the cytoplasmic

polyadenylation complex [6]. Thus, the second aim of this project is to determine how Rbm24 functionally interacts with Cpeb and Pabpc during muscle differentiation. We will perform knockdown or knockout of Cpeb and Pabpc either alone or along with rbm24, and examine how poly(A) tail length and translational efficiency of muscle-specific mRNAs identified above are affected. The consequence on myogenic differentiation will be analyzed using specific markers. We will also examine how loss of Rbm24 affects the interaction between Cpeb and Pabpc, which is important for poly(A) tail elongation. In parallel, we plan to use proteomic approaches to identify novel Rbm24 interaction partners by co-immunoprecipitation (using Rbm24-GFP transgenic embryos already available in the laboratory) and mass spectrometric analysis. The expected results from this aim will contribute to better understand the mechanism regulating cytoplasmic polyadenylation in muscle.

Feasibility of the research project

We have original findings on the post-transcriptional regulatory role of Rbm24 in muscle gene expression and differentiation. The project is well positioned with regards to international competition in the field, as no other groups are focusing on the possible role of cytoplasmic polyadenylation in muscle differentiation. Importantly, our project is funded by AFM (grant 23545), ensuring its success. The proposed research is built on a wealth of preliminary data:

1) We have generated zebrafish mutants for rbm24 and a zebrafish Rbm24-GFP line, which recapitulates the endogenous expression pattern of Rbm24 during various stages of development. These lines are important for the achievement of the project.

2) RNA-seq analysis has allowed us to identify several muscle genes with decreased expression and likely reduced poly(A) tail lengths. Thus, we are fully confident that the proposed TAIL-seq analysis will allow us to identify Rbm24 targets and determine how loss of Rbm24 affects cytoplasmic polyadenylation of muscle-specific mRNAs, and as a result, their stability and/or translation efficiency. The second objective is based on our recent identification of Cpeb and Pabpc as interaction partners of Rbm24. We will further study how these proteins functionally interact with Rbm24 and how their activity is regulated by Rbm24 during muscle development, using knockdown or knockout and biochemical approaches.

3) Our group is in the Developmental Biology Laboratory of the Institut de Biologie Paris-Seine (IBPS). We have strong expertise in studying Rbm24 function during muscle differentiation and regeneration using zebrafish and other vertebrate embryos as models. In recent years, we have published a number of original and review articles in very good journals and with high visibility.

4) For the research design, we have prior experience in the proposed methods, demonstrating past success using such methods. We also have well-established collaborations on genome editing, which are important for generating zebrafish mutant and transgenic lines if necessary. All equipments and most reagents related to this project are already available. Other large-scale analyses can be performed in the facility of the IBPS institute. These guarantee that different tasks will be completed and deliverables will be produced in a timely manner.

References

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2. Grifone R, et al (2014). The RNA-binding protein Rbm24 is transiently expressed in myoblasts and is required for myogenic differentiation during vertebrate development. Mech Dev 134:1-15.
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4. Grifone R, et al (2018). Expression patterns of Rbm24 in lens, nasal epithelium, and inner ear during mouse embryonic development. Dev Dyn 247:1160-1169.
5. Grifone R, et al (2021). Rbm24 displays dynamic functions required for skeletal muscle regeneration. Sci Rep 11:9423.
6. Shao M, et al (2020). Rbm24 controls poly(A) tail length and translation efficiency of crystallin mRNAs in the lens via cytoplasmic polyadenylation. Proc Natl Acad Sci USA 117:7245-7254.

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