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## RESEARCH ARTICLE

# miR-210 expression is associated with methionine-induced differentiation of trout satellite cells

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## ABSTRACT

In fish, data on microRNAs (miRNAs) involved in myogenesis are scarce. In order to identify miRNAs involved in satellite cell differentiation, we used a methionine depletion/replenishment protocol to synchronize myogenic cell differentiation. Our results validated that methionine removal (72 h) from the medium strongly decreased *myoD1* and *myogenin* expression, indicating differentiation arrest. In contrast, methionine replenishment rescued expression of *myoD1* and *myogenin*, showing a resumption of differentiation. We performed a miRNA array analysis of myogenic cells under three conditions: presence of methionine for 72 h (control), absence of methionine for 72 h (Meth<sup>-</sup>) and absence of methionine for 48 h followed by 24 h of methionine replenishment (Meth<sup>-/+</sup>). A clustering analysis identified three clusters: cluster I corresponds to miRNA upregulated only in Meth<sup>-/+</sup> conditions; cluster II corresponds to miRNA downregulated only in Meth<sup>-/+</sup> conditions; cluster III corresponds to miRNAs with high expression in control, low expression in Meth<sup>-</sup> conditions and intermediate expression after methionine replenishment (Meth<sup>-/+</sup>). Cluster III was very interesting because it fitted with the data obtained for *myoD1* and *myogenin* (supporting an involvement in differentiation) and contained seven miRNAs with muscle-related function (e.g. miR-133a) and one (miR-210) with unknown function. Based on our previously published miRNA repertoire (Juanchich et al., 2016), we confirmed miR-133a was expressed only in white muscle and showed that miR-210 had strong expression in white muscle. We also showed that miR-210 expression was upregulated during differentiation of satellite cells, suggesting that miR-210 was potentially involved in the differentiation of satellite cells.

**KEY WORDS:** Methionine depletion, miRNA, Myo-miR, Myoblast, Myogenesis

## INTRODUCTION

Many teleosts have the extraordinary ability to grow muscle throughout life, termed indeterminate muscle growth, as a result of the continuous formation of new fibers (hyperplasia) and increase in size of existing fibers by hypertrophy. The muscle stem cells (called satellite cells) are essential for these two processes of hyperplasia and hypertrophy. The satellite cells differentiate during skeletal muscle development to form multinucleated myofibers (Chargé and

Rudnicki, 2004). During this myogenic program, the satellite cells express markers of differentiation such as MyoD and myogenin (Rescan et al., 1994) early in the process, with subsequent expression of myosin heavy chain and muscle creatine kinase (Chargé and Rudnicki, 2004) after terminal differentiation occurs. This process has been studied extensively in mammals, but specific factors related to the induction and control of differentiation in indeterminate growing species like rainbow trout (*Oncorhynchus mykiss*) are still largely unknown.

Recently, the role of microRNAs (miRNAs) in regulating the expression of transcription factors and signaling molecules involved in skeletal muscle differentiation has been reported (Townley-Tilson et al., 2010; van Rooij et al., 2008). Of particular interest is the suite of miRNAs that are exclusively found or highly enriched in muscle. These miRNAs have been coined muscle-specific miRNAs or myo-miRs and include miR-133a/b, miR-206, miR-499, miR-208, miR-486 and miR-1 (McCarthy and Esser, 2007; Small et al., 2010; Townley-Tilson et al., 2010). These myo-miRs are controlled by myogenic regulatory factors (MRFs), such as myogenic differentiation 1 (MyoD1), myogenin, or serum response factor (SRF) (Chen et al., 2006; Rao et al., 2006). myo-miRs have established roles in controlling both proliferation and differentiation by controlling key genes involved in the myogenic program (Chen et al., 2006; Kim et al., 2006; Rao et al., 2006). Recent comparative genomics analyses demonstrated that myo-miRs have a well-conserved synteny that suggests a conserved functionality (Nachtigall et al., 2014). In fish, few data are available on the role of miRNA in muscle growth or myogenesis. In tilapia (*Oreochromis niloticus*), it has been shown that miR-206 regulates muscle growth by targeting the 3'-UTR of IGF-1 transcript (Yan et al., 2013). In *Paralichthys olivaceus*, it has been shown that miR-133a targets SRF mRNA (Duran et al., 2015; Su et al., 2015) as in mammals. Recently, we published a miRNA repertoire of a wide variety of tissue that identifies some muscle-specific miRNAs (Juanchich et al., 2016).

Satellite cells have been extracted from several fish species (Bower and Johnston, 2009; Fauconneau and Paboeuf, 2000; Koumans et al., 1990) and have often been used to decipher molecular mechanisms of myogenic differentiation (Garikipati and Rodgers, 2012; Seiliez et al., 2012). However, myogenic cell differentiation has been reported to start while cell proliferation remains high and our previous cell culture conditions failed to fully block differentiation initiation (Gabillard et al., 2010). Thus, these common culture conditions prevent accurate study of initiation of differentiation. In the mammalian myogenic cell line C2C12, it has been shown that removal of methionine from the medium blocks proliferation and differentiation (Kitzmann et al., 1998), but it was unknown whether the same approach could be successfully applied to fish satellite cells.

The objective of this paper was first to evaluate the efficiency of methionine restriction to synchronize satellite cells and second to

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identify new miRNAs potentially involved in satellite cell differentiation by high-throughput analysis.

## MATERIALS AND METHODS

### Animals

Fish used in this study were reared and handled in strict accordance with French and European policies and guidelines of the Institutional Animal Care and Use Committee (no. R-2011-JCG-01), which approved this study. All experiments were carried out on juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792). Fish were maintained in 12°C recirculating water under artificial light–dark conditions to mimic natural photoperiod. Chemical parameters were monitored routinely and oxygen levels always remained above 98% saturation.

### Cell culture

Satellite cells were extracted from the white muscle of trout (5–10 g), as previously reported (Froehlich et al., 2014; Gabillard et al., 2010). Briefly, after several enzymatic digestion and cell filtration steps, the cells were seeded at a density of 160,000 cells cm<sup>-2</sup> and left for 40 min. For the first 3 days, the cells were cultured in DMEM (D7777, Dulbecco's modified Eagle's medium, Sigma, St Louis, MO, USA) supplemented with 10% fetal calf serum (FCS). Afterwards, the medium was replaced by methionine-depleted DMEM (D9785, DMEM/nutrient mixture F-12 Ham, Sigma) supplemented with 2% FCS, with or without 100 μmol l<sup>-1</sup> methionine (M5308, Sigma).

For kinetic analysis of differentiation, cells were cultured in F10 medium (N6635, nutrient mixture Ham's F10, Sigma) supplemented with 10% FCS for 3 days to stimulate cell proliferation, and then in DMEM (D7777, Sigma) supplemented with 2% FCS for 3 days to stimulate differentiation.

### Immunofluorescence analysis

Cells on glass coverslips were washed twice with phosphate-buffered saline (PBS) and fixed for 10 min with 4% paraformaldehyde in PBS. For permeabilization, cells were incubated for 3 min in 0.1% Triton X-100/PBS and saturated for 1 h with 3% bovine serum albumin (BSA), 0.1% Tween-20 in PBS (PBST). Cells were incubated for 3 h with anti-myogenin primary antibody diluted in blocking buffer (Gabillard et al., 2010). The secondary antibodies (A-21442, Molecular Probes, Eugene, OR, USA) were diluted in PBST and applied for 1 h. Cells were mounted with Mowiol 4-88 (475904, Calbiochem, Merck Millipore, Billerica, MA, USA) containing Hoescht (0.5 μg ml<sup>-1</sup>). Cells were photographed by using a Canon digital camera coupled to a Canon 90i microscope.

### RNA extraction, cDNA synthesis and quantitative (q)PCR

Cells were collected using Tri reagent® (T9424, Sigma), and total RNA was extracted according to the manufacturer's instructions. A 1 μg sample of total RNA was used for miRNA cDNA synthesis using a MystiCq MicroRNA cDNA RT kit (MIRRT, Sigma). For miRNA expression assays, forward primer sequences for miRNAs (miR-133a: TTGGTCCCCTTCAACCAGCTG; miR-210: AGCC-ACTGACTAACGTACATTG; miR-375: TTTGTTTCGTTTCGGC-TCGCGTTA) were taken directly from Juanchich et al. (2016), and the universal reverse primer used for all miRNA expression analysis was provided with the MystiCq cDNA reverse transcription kit. For real-time RT-PCR assays of miRNA targets, a Step One Plus thermocycler (Applied Biosystems, Foster City, CA, USA) was used. The RT products were diluted 1:50 for miRNA and the assays

were performed using a reaction mix of 12 μl per sample: 5 μl cDNA, 5 μl SYBR® Green I Master mix, 1.5 μl RNase/DNase-free water, 0.5 μl primer mix (300 nmol l<sup>-1</sup>). The PCR protocol was 20 s at 94°C for initiation, 40 cycles of 3 s at 94°C and 30 s at 60–66°C according to the primer set. Melt curves were monitored to confirm specificity of the amplification reaction. Each PCR assay included duplicate samples and negative controls. The relative abundance of target cDNA within the sample set was calculated from a serially diluted (1:1 to 1:256) cDNA pool using Step One Plus software (Applied Biosystems). The qPCR signal for miRNA was normalized by comparison with a reference miRNA (miR-375) found to be stable in all conditions.

For mRNA cDNA synthesis, 0.5 μg of total RNA was used with the High Capacity cDNA Reverse Transcription kit (4368813, Applied Biosystems) following the manufacturer's instructions. The relative abundance of target cDNA within the sample set was calculated from a serial dilution (1:1 to 1:256, standard curve) of pool cDNA using StepOne™ Software v2.0.2 (Applied Biosystems). Subsequently, qPCR data were normalized by dividing the raw data by the 18S gene expression value.

### miRNA microarray hybridization and data processing

The miRNA microarray [gene expression omnibus (GEO) platform GPL21776] was prepared according to Juanchich et al. (2013). Briefly 200 ng of total RNA from cells was collected and dephosphorylated for 30 min at 37°C then denatured with dimethyl sulfoxide for 7 min at 100°C. The RNA was labeled using terminal ligation of cyanine 3-pCp using T4 ligase for 2 h at 16°C. Purification was performed using Micro Bio-Spin columns to remove free cyanine 3-pCp. RNA was hybridized to the slides with Spike-In at 55°C, 20 rpm for 20 h. Slides were washed and immediately scanned using an Agilent DNA Microarray Scanner (Agilent Technologies, Santa Clara, CA, USA). Signal intensity was quantified using Feature Extraction software (Agilent v10.7.3.1). Data were processed using GeneSpring software (Agilent v.11.5.0) using GmedianSignal values. Data were normalized using the quantile method. Corresponding data were deposited in the GEO database under reference GSE90960.

### Statistical analysis

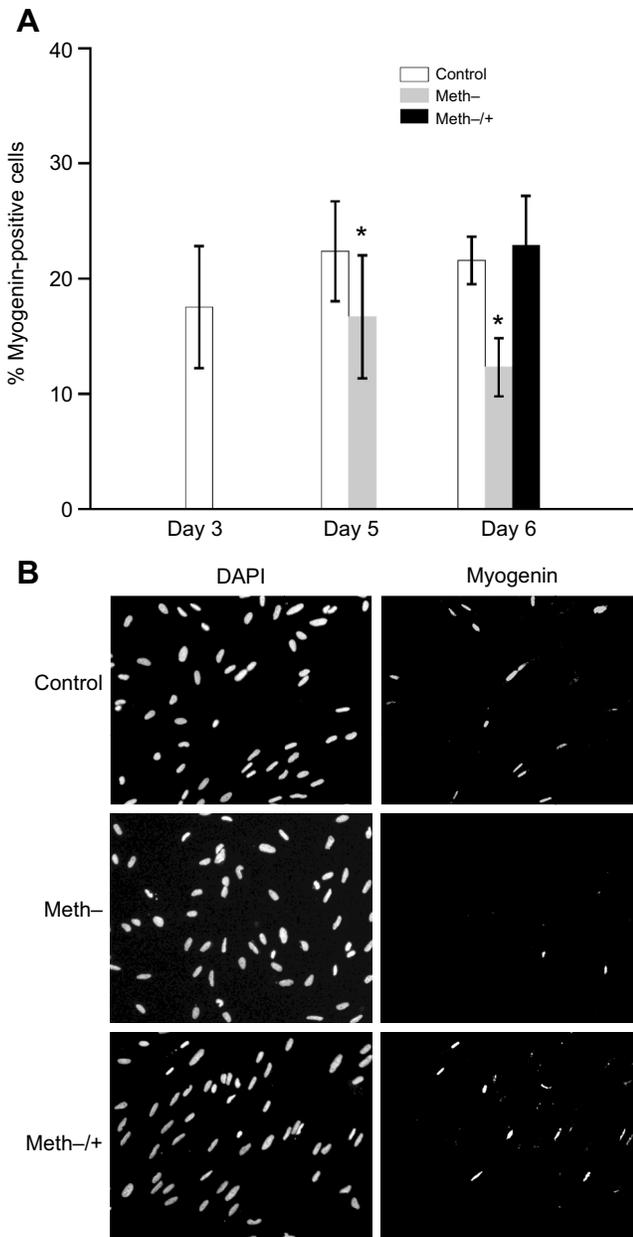
An ANOVA analysis with a Benjamin–Hochberg correction was performed to detect differential expression stages in the microarray experiments. All samples were tested against each other with a corrected  $P=0.05$  for the miRNA array. An ANOVA analysis with a Tukey's *post hoc* multiple comparisons test was performed on miRNA data obtained by qPCR with  $P=0.05$ . For the clustering analysis, the data were median-centered and an average linkage clustering was carried out using CLUSTER software and visualized using TREEVIEW.

## RESULTS

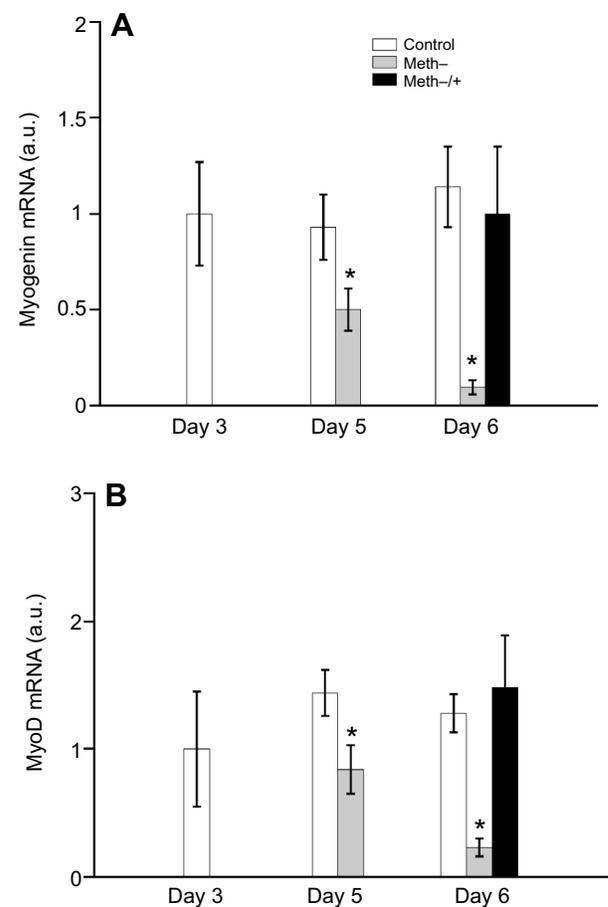
### Methionine depletion blocks the differentiation of trout satellite cells *in vitro*

To investigate the effect of methionine restriction on myogenic differentiation, trout satellite cells were cultured from day 3 to day 6 in methionine-depleted DMEM (DMEM+2% FCS) supplemented (control) or not (Meth–) with methionine. To test the possibility of a resumption of differentiation by methionine replenishment, we also added methionine for a 24 h period after 48 h of methionine restriction (Meth–/+). Quantification of myogenin-positive cells (differentiation index) by immunocytofluorescence showed that methionine restriction decreased differentiation index as soon as

day 5 (Fig. 1A). At day 6, this index was decreased 2-fold in satellite cells cultured without methionine compared with control ( $12.3 \pm 2.5\%$  versus  $21.6 \pm 2.0\%$ ,  $P < 0.05$ ). After 48 h of depletion, the addition of methionine for 24 h restored the differentiation index to a level comparable to the control ( $22.9 \pm 4.2\%$  versus  $21.6 \pm 2.0\%$ ; Fig. 1). In order to determine whether methionine restriction also altered the expression of MRFs, we measured the mRNA levels of *myod1* and *myogenin* under the same conditions. As shown in Fig. 2, methionine depletion for 72 h strongly decreased the mRNA levels of *myod1* and *myogenin* (6- and 10-fold, respectively, at day 6). Interestingly, 24 h after methionine replenishment, the expression of *myod1* and *myogenin* was similar to that of the control.



**Fig. 1. Immunocytochemical staining of cells for myogenin.** Cells were assigned to one of three treatments: presence of methionine for 72 h (control), absence of methionine for 72 h (Meth<sup>-</sup>) and absence of methionine for 48 h followed by methionine replenishment for 24 h (Meth<sup>-/+</sup>). (A) Percentage of myogenin-positive cells. (B) Immunofluorescence analysis of myogenin expression. \*Significant difference (one-way ANOVA,  $P < 0.05$ ;  $N = 4$ ).



**Fig. 2. mRNA expression of myogenin and myoD following methionine restriction *in vitro*.** Data (means  $\pm$  s.d.) are for cells grown under control, Meth<sup>-</sup> and Meth<sup>-/+</sup> conditions (see Fig. 1). mRNA levels were estimated using qPCR and normalized to levels of 18S mRNA. (A) Myogenin expression. (B) MyoD expression. \*Significant difference (one-way ANOVA,  $P < 0.05$ ;  $N = 6$ ).

Altogether, these data clearly validate the use of methionine restriction/replenishment to synchronize myogenic differentiation in our *in vitro* model.

### Methionine replenishment induces strong variation in miRNA expression

To identify miRNAs regulated by methionine and associated with satellite cell differentiation, we performed miRNA microarray analysis with samples obtained from the methionine restriction experiment described above (Fig. 2) but focused on day 6, where stronger differential expression was observed. Thus, we compared the expression of miRNAs under the three conditions: presence of methionine (control), absence of methionine for 72 h (Meth<sup>-</sup>) and absence of methionine for 48 h followed by 24 h of methionine replenishment (Meth<sup>-/+</sup>). For subsequent analyses, only miRNAs annotated and validated (Juanchich et al., 2016) were conserved. After bioinformatic and statistical analyses, comparison of the three conditions indicated that 53 miRNAs were differentially expressed in at least one condition (Table 1). A clustering analysis identified three clusters (Fig. 3): the first cluster (36 unique miRNAs) corresponds to miRNAs upregulated only in Meth<sup>-/+</sup> conditions; the second cluster (9 unique miRNAs) corresponds to miRNAs downregulated only in Meth<sup>-/+</sup> conditions; the third cluster (8 unique miRNAs) corresponds to miRNAs with high expression in control conditions, low expression in the absence of methionine

**Table 1. MicroRNAs (miRNAs) differentially expressed and present in one of the three clusters identified by clustering analysis**

Cluster I		Cluster II		Cluster III
miR-103	miR-199a-5p	miR-30d-5p	miR-125a-3p	miR-1-3p
miR-106a	miR-19a-3p	miR-455-3p	miR-1260b	miR-125a-5p
miR-107	miR-19b-3p	miR-455-5p	miR-202-3p	miR-133a-3p
miR-10a-5p	miR-20a	miR-99a-5p	miR-205-5p	miR-181b-5p
miR-10c-5p	miR-20b-5p		miR-3149	miR-210-3p
miR-126-3p	miR-214-5p		miR-466f-3p	miR-26a-5p
miR-128-3p	miR-22-3p		miR-466i-3p	miR-26b
miR-130b	miR-221		miR-466m-5p	miR-27a-3p
miR-130c	miR-222		miR-513a-5p	
miR-140-3p	miR-23b-3p			
miR-143-3p	miR-24b-3p			
miR-145-5p	miR-27b-3p			
miR-15a	miR-29a-3p			
miR-16-5p	miR-29b-3p			
miR-181a	miR-29c-3p			
miR-196a-5p	miR-29d			

(Meth–) and intermediate expression after methionine replenishment (Meth–/+). Cluster I, but not cluster II, includes members of the miR-29 family as well as miR-27b, miR-221 and miR-222, which are known to be involved in the myogenesis process. Among miRNAs included in cluster II, miR-466 and miR-125a have been shown to be involved in myoblast differentiation. Cluster III contains seven miRNAs with muscle-related function (e.g. miR-1, miR-133a) and only miR-210 has not previously been reported to function in muscle differentiation.

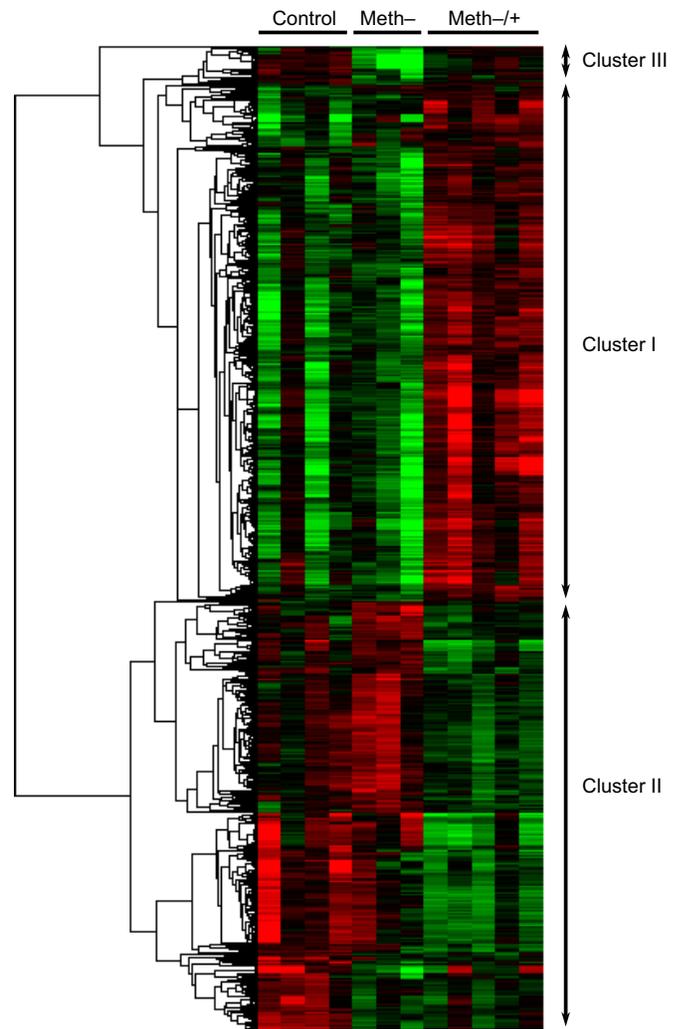
Based on our already published miRNA repertoire (Juanchich et al., 2016), we confirmed that miR-133a is expressed only in white muscle and heart (Fig. S1A) and showed that miR-210 is strongly expressed in white muscle (Fig. S1B). In an attempt to validate our microarray results, we performed qPCR analysis of miR-133a and miR-210 under the same experimental conditions. As shown in Fig. 4, the expression of miR-133a was downregulated in the absence of methionine, and 24 h of methionine replenishment increased its expression but not significantly, as seen in the microarray (Fig. 4). The expression of miR-210 decreased (2-fold) during methionine restriction and was completely restored by 24 h methionine replenishment, in accordance with the microarray results.

#### miR-133a and miR-210 expression is upregulated during differentiation of satellite cells

In order to better characterize the expression of these miRNAs, we measured their expression during the differentiation of trout satellite cells *in vitro*. The qPCR results presented in Fig. 4 indicate that the expression of miR-133a started to increase 24 h after the induction of differentiation. After 3 days in differentiation medium, the expression of miR-133a was 6-fold higher (Fig. 4C) than that in proliferative myoblasts. In a similar pattern, the expression of miR-210 increased during the differentiation of satellite cells but to a lesser extent (1.5-fold; Fig. 4F).

#### DISCUSSION

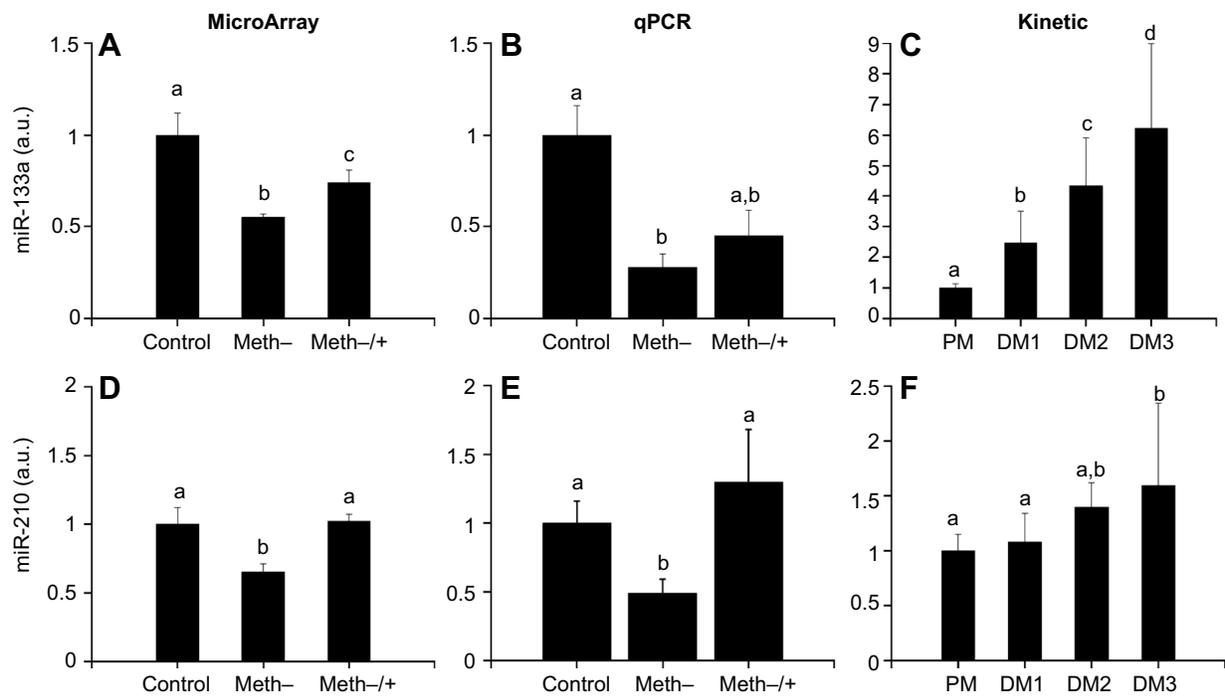
Most teleosts exhibit continuous muscle growth throughout their life, but the underlying mechanisms remain largely unknown. While the role of miRNAs in myogenesis is well documented in mammals, in teleosts the data are scarce and few miRNAs have been shown to be specific to muscle. Our work aimed at identifying miRNAs mainly expressed in muscle and regulated during myogenic differentiation. Satellite cell cultures are often used to



**Fig. 3. Hierarchical clustering of differentially expressed microRNA (miRNA) following methionine restriction.** Each row represents the expression pattern of miRNA and each column corresponds to a single sample from cells grown under control, Meth– and Meth–/+ conditions (see Fig. 1). The expression levels are represented by different colors, with red indicating the highest levels of expression and green the lowest.

decipher molecular mechanisms of myogenic differentiation. However, fish satellite cells spontaneously differentiate *in vitro* (Fauconneau and Paboeuf, 2001; Gabillard et al., 2010) without synchronization, which prevents accurate analysis of differentiation onset. In mammals, it has been shown that removal and replenishment of methionine allows synchronous resumption of differentiation (Kitzmann et al., 1998). The objective of this paper was first to evaluate the efficacy of methionine restriction to synchronize cells and second to identify new miRNAs potentially involved in satellite cell differentiation.

Our first experiment showed that satellite cells deprived of methionine for 72 h did not undergo differentiation, as illustrated by significantly lower levels of both *myogenin* and *myod1* mRNA abundance. Myogenin and MyoD1 are known hallmarks of muscle myogenesis (Davis et al., 1987; Edmondson and Olson, 1989; Wright et al., 1989) and play critical roles in the transition from proliferation to differentiation. In addition, 24 h of methionine replenishment fully restored differentiation as shown by the upregulation of *myod1* and *myogenin* expression. Therefore, our



**Fig. 4. Expression of miR-133a and miR-210 following methionine restriction and during satellite cell differentiation.** For expression studies (A,B,D,E), cells were grown under control, Meth<sup>-</sup> and Meth<sup>-/+</sup> conditions (see Fig. 1). For kinetics of differentiation (C,F), cells were grown in proliferation medium (PM) and then in differentiation medium for 3 days (DM). Data (means±s.d.) were extracted from the microarray (A,D) or obtained by qPCR (B,C,E,F). Different letters indicate a significant difference between treatments (one-way ANOVA and Tukey's multiple comparisons test,  $P < 0.05$ ;  $N = 6$ ).

results show for the first time in fish that methionine regulates the differentiation of satellite cells. This result is in agreement with data obtained with C2C12 myoblasts showing that methionine depletion blocks proliferation and differentiation in association with a decrease in myogenin protein (Kitzmann et al., 1998). Together, our results show that methionine removal can be used to synchronize the onset of satellite cell differentiation.

To identify miRNAs potentially involved in satellite cell differentiation, we performed a high-throughput analysis applied to a methionine-replenishment experiment. A clustering analysis showed the presence of three distinct clusters: cluster I miRNAs were upregulated after 24 h of methionine replenishment, cluster II miRNAs were downregulated after 24 h of methionine replenishment, and cluster III miRNAs showed high expression in control conditions, low expression in the absence of methionine (Meth<sup>-</sup>) and intermediate expression after 24 h of methionine replenishment. Cluster I consist of 36 miRNAs including several miRNAs (miR-27, miR-29, miR-221, miR-222, miR-214) known to have a role in the differentiation of myoblasts (Cardinali et al., 2009; Feng et al., 2011; Lozano-Velasco et al., 2015; Wang et al., 2008). This enrichment in myo-miRs in cluster I is in agreement with the resumption of myogenic differentiation observed after methionine replenishment. In contrast to cluster I, cluster II did not contain key myo-miRs but miRNAs such as miR-466 and miR-125a. Interestingly, miR-466 has been shown in mice to target the doublecortin protein (Shyamasundar et al., 2013) known to be involved in myoblast fusion (Ogawa et al., 2015). Therefore, a decrease of miR-466 expression following methionine replenishment could participate in the resumption of differentiation. miR-125a has been shown to inhibit proliferation of C2C12 myoblasts (Song et al., 2015), suggesting that methionine replenishment downregulates miR-125a, stimulating the resumption of the cell cycle. Cluster III contained only eight

miRNAs that were downregulated in absence of methionine and stimulated by methionine replenishment. This expression pattern fits very well with those of *myod1* and *myogenin*, indicating that the third cluster is mainly associated with the resumption of differentiation. Indeed, cluster III mainly consisted of key myo-miRs such as miR-133a and miR-1. In fact, only one (miR-210) out of the eight miRNAs of cluster III has not previously been reported to play a role in myogenic differentiation, whereas it is strongly expressed in muscle.

To further validate the results obtained by microarray, we repeated the methionine-replenishment experiment and performed qPCR analysis. The halting of differentiation, as shown by the strong decrease of *myogenin* and *myod1* expression, was also associated with a decrease of miR-133a expression. Following methionine addition, the expression of this miRNA increased, and expression also increased during the differentiation of satellite cells, suggesting a key role in differentiation, as in mammals. Indeed, miR-133a is known to be regulated by Myogenin and MyoD1 in mammals (Chen et al., 2006; Rao et al., 2006) and strongly increases during satellite cell differentiation (Chen et al., 2006, 2010). miR-133a is also known to target the Serum Response Factor (SRF), an inhibitor of proliferation (Chen et al., 2006). Although analysis of the 3'-UTR targets of trout miR-133a failed to identify SRF as target, we identified another inhibitor of proliferation, WNK2 (Moniz et al., 2007). Together, these results strongly suggest a conservation of function of miR-133a in trout satellite cell differentiation.

For the first time in vertebrates, we report that miR-210 is strongly expressed in muscle and actively regulated by methionine. This miRNA has previously been shown to be induced during the hypoxic response in cells (Chan et al., 2012; Huang et al., 2010), which has no obvious link to satellite cell differentiation. However, very recently, miR-210 has been reported to be involved in the

regulation of cell cycle progression in neural progenitors as well as in promotion of their differentiation (Abdullah et al., 2016), which is reminiscent of our results indicating a decrease in miR-210 under methionine restriction and an increase during satellite cell differentiation. We looked for putative targets of miR-210 using Mennigen's database (Mennigen and Zhang, 2016). We identified several putative targets, none of which are known to act directly on skeletal muscle differentiation. Among them, the myosin phosphatase rho-interacting protein is an activator of SRF (Mulder et al., 2005) known to repress myoblast differentiation but promote proliferation (Soulez et al., 1996). Interestingly, SRF is a target of miR-133 (Chen et al., 2006), which suggests that miR-210 regulates myogenesis in the same way as miR-133. Together, these results strongly suggest that miR-210 has a key role in satellite cell differentiation.

## Conclusion

This study clearly demonstrates that methionine restriction blocks cell differentiation by decreasing *myoD1* and *myogenin* expression, as well as specific miRNAs. Methionine deficiency synchronized cells in a state of halted differentiation that allowed the identification of specific miRNAs controlling myogenic differentiation. We showed that miR-133a expression is conserved in this process and identified miR-210, a novel miRNA potentially involved in satellite cell differentiation. Further work will be necessary to validate the targets of miR-210 and to identify at which step of the myogenic program miR-210 acts.

## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: M.L., I.S., P.B., J.C.G.; Methodology: N.S., A.L., I.S., P.B., J.C.G.; Software: A.L.; Validation: M.L., N.S., P.B., J.C.G.; Formal analysis: M.L., N.S., I.S., P.B.; Investigation: M.L., J.C.G.; Resources: A.L.; Data curation: A.L.; Writing - original draft: M.L.; Writing - review & editing: M.L., I.S., P.B., J.C.G.; Visualization: M.L.; Supervision: I.S., P.B., J.C.G.; Funding acquisition: P.B., J.C.G.

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## Data availability

Data have been deposited in the Gene Expression Omnibus (GEO) database under reference GSE90960.

## Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.154484.supplemental>

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