DDX5/DDX17 RNA helicases : Two master genes controlling skeletal myogenesis

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DDX5 and DDX17 are two highly related RNA helicases. They have been involved at several levels of gene regulation such as transcription, alternative splicing, miRNA maturation and nuclear export. In particular, they are known co-activators of MyoD, the main transcription factor involved in muscle differentiation. However the others aspects of gene regulation controlled by the two proteins have not yet been studied in this model. To answer this biological question, we chose to use Affymetrix exon microarrays as they allow to analyze gene expression both at global and splicing level. Moreover the limited cost of this type of technology and mature analysis strategies make microarrays a good choice to establish which known genes and pathways require DDX5/DDX17 during this process.

Using the myoblastic mouse cell line C2C12, we showed that DDX5/DDX17 depletion by siRNAs inhibited myogenesis. This was highlighted by an absence of differentiation morphology and a repression of common muscle markers. Furthermore chromatin immunoprecipitation (ChIP) experiments confirmed the recruitment of DDX5 onto muscle genes promoters. In silico analysis revealed that nearly 45% of genes regulated during C2C12 differentiation required DDX5/DDX17.

More interestingly, a large proportion of splicing events occurring during C2C12 differentiation was also predicted to be dependent on the presence of DDX5/DDX17. Analysis of muscle-specific splicing factors revealed that their expression was drastically decreased in the absence of DDX5/DDX17. This suggests that the activity of DDX5/DDX17 on splicing is at least partly indirect via their modulation of specific splicing factors. To confirm this hypothesis, nearly 60% of DDX5/DDX17 dependant-events present muscle splicing factors motifs in their sequences.

DDX5/DDX17 have been implicated in muscle pathologies, in particular myotonic dystrophy (DM). Using exon microarrays of normal and DM patients, we established a set of DDX5/DDX17-dependent splicing events that are expressed specifically in both adult mouse and human muscle and are deregulated in DM patients. We believe that these events are of huge interest to understand the muscle-related clinical phenotype of this disease. Four of those exons will be functionally analyzed in a next step.

Paradoxically considering the importance of DDX5/DDX17 in myogenesis, we observed that their endogenous expression decreased during differentiation. Our results showed that induction of muscle-specific miR-1/206 during differentiation is impaired in the absence of DDX5/DDX17, and ChIP experiments showed the recruitment of DDX5 on miR-1/206 promoters. Conversely, overexpression of miR-1 or miR-206 decreased the protein-level of DDX5/DDX17. These results suggest the existence of a feedback control loop involving DDX5/DDX17 and miR-1/206,which could be necessary for both the initiation and the completion of myogenesis.

In agreement with this, microarrays analysis showed that nearly 20% of transcriptional and splicing events occurring during differentiation were also induced by the depletion of DDX5/DDX17 in C2C12 cells in growth conditions. Several events specific of a late differentiation state, both at global and splicing level, have been biologically validated (Myf5, Mylk2, Pkm2...). We still have to understand how the decrease of DDX5/DDX17 expression may be important for terminal differentiation.

Our results indicate that (i) DDX5/DDX17 are necessary for the initial muscle program (probably as co-activators of MyoD) (ii) The expression of DDX5/DDX17 decreases during muscular differentiation due to a feedback regulatory loop involving muscle miRNAs (iii) the drop of DDX5/DDX17 expression may contribute to terminal differentiation.