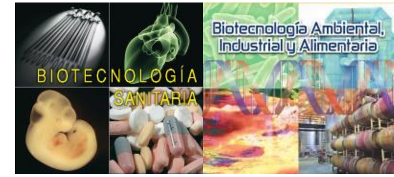
Poster



Unravelling the function of Myf5 in the developing limb musculature

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ABSTRACT

Motivation: Myogenesis is a complex process controlled by different networks, depending on the origin of different muscles, and that's why there are different types of muscle distrophies. However, the myogenic cascade is always regulated by the same Myogenic Regulatory Factors (MRFs): Myf5, Mrf4, MyoD and MyoG. These transcription factors bind DNA and activate the expression of specific genes in particular progenitor cells that will give rise to the different muscles in the adult body. The MRFs' cascade is initiated by Myf5, the first MRF to be expressed in the embryo. We and others have extensively studied the complex transcriptional regulation of the *Myf5/Mrf4* locus using transgenic mice. Nowadays, we know that there are more than 25 regulatory elements controlling the expression of *Mrf4* and *Myf5* in a specific time frame and in particular embryonic progenitors.

The limb enhancer is located 57 kb upstream of the *Myf5* transcriptional start site. This enhancer controls Myf5 expression in limbs during development. While the mechanisms involved in the spatiotemporal regulation of *Myf5* have been extensively studied at single-enhancer and global regulatory levels, the function of Myf5 in different subpopulations of muscle progenitor cells is still not clear. This project focuses on the characterization of a new allele in which the limb enhancer has been removed from the genome.

Methods: To unravel the function of Myf5 in the developing limb musculature we have generated a new knock-out (KO) allele with CRISPR/Cas9 in which the limb enhancer has been targeted. Then, we have prepared RNA probes for *In Situ* Hybridisation (ISH) of genes that are expressed in limbs and are potential targets of Myf5 in other muscle progenitors (López-Mayorga *et al.*, unpublished data), to test if the expression patters of these genes are modified in the KO allele. We are going to study three different embryonic stages: 10.5, 11.5 and 12.5 days *post-coitum* (d*pc*) by ISH using KO and wild type (WT) embryos. This time window was chosen to maximise the probability of detecting any pattern changes before the phenotype is rescued by the activity of MyoD, as previously shown. We are also preparing total RNA of fore-limbs from KO and WT embryos at 11.5 d*pc* to perform microarrays, which will give us some information about the genes regulated by Myf5 direct or indirectly. Finally, we will validate the results from microarrays by ISH and qPCR.

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