Onecut gene regulatory network in chordate development and evolution

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by

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ABSTRACT

Onecut genes have been identified in all major groups of metazoans and are expressed in the nervous system and in some endodermal derived tissues. Their function in liver and pancreas differentiation in mammals has been quite well studied, while almost nothing is known about their function in neurogenesis and eye formation in chordates. My PhD project focused in understanding the role and the genetic cascade of *OC* genes in photoreceptor cells and in eye formation during chordate evolution, by using the tunicate *Ciona intestinalis* and vertebrate zebrafish *Danio rerio* as model systems. To acquire novel insights into the degree of *OC* genes functional conservation across chordates, I performed *OC* targeted perturbation by transgenic approach in *Ciona* and morpholino-mediated knockdown in zebrafish. The *Ciona* genome contains a single *OC* gene, while the analysis of the zebrafish genome revealed the presence of five *OC* orthologue *OC1*, *OC2, OClike* and two gene copies of *OC*3, named *OC3a and OC3b*.

To study OC function in tunicate eye development, I performed electroporation of newly generated dominant active and dominant repressive forms of the gene in *Ciona* fertilized eggs. The expression of these two transgenes led to specific and opposite effects on the differentiation of photoreceptor cell types and of the pigmented photosensitive ocellus. My study indicated that *OC* is involved in the molecular pathway controlling photoreceptor and pigment cells differentiation and confirmed its role as an upstream regulator of *Rx*, a key gene for ocellus development. With the aim of studying its genetic cascade, I performed cell sorting from transgenic embryos and set the ground for an ongoing RNAseq project aiming to the analysis of differentially regulated OC target genes.

The analysis of three *OC* morphant phenotypes in zebrafish revealed an *OC* conserved role in eye specification. Indeed, similarly to mouse *OC*1/*OC*2 double knockout, the eye size of zebrafish *OC* morphants was reduced and the expression levels of several specific eye markers were altered. Furthermore, I obtained preliminary results on the evolutionary

conservation of the *OC/Rx* pathway in zebrafish, already demonstrated in *Ciona*, but not in vertebrates.

My data insert a new piece in the genetic cascade controlling the specification of the ocellus and eye structures and highlight a conserved and important role played by *OC* genes in this process during chordate evolution.