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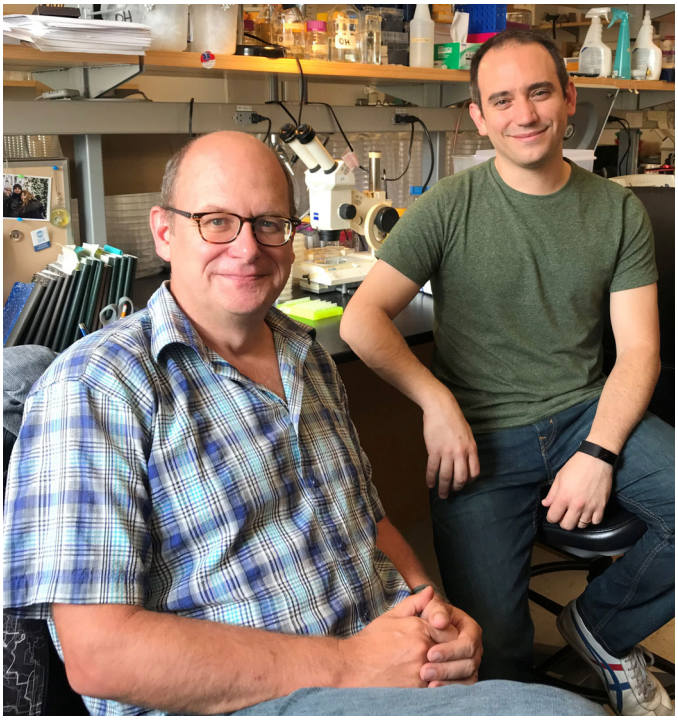
INTERVIEW

# The people behind the papers – Eduardo Leyva-Díaz and Oliver Hobert

Transcriptional autoregulation occurs when transcription factors bind their own *cis*-regulatory sequences, ensuring their own continuous expression along with expression of other targets. During development, continued expression of identity-specifying transcription factors can be achieved by autoregulation, but until now formal evidence for a developmental requirement of autoregulation has been lacking. A new paper in Development provides this proof with the help of CRISPR/Cas9 gene editing in the *C. elegans* nervous system. We caught up with the paper's two authors: postdoc Eduardo Leyva-Díaz and his supervisor Oliver Hobert, Professor of Biological Sciences and HHMI Investigator at Columbia University, New York, to find out more about the work.



Oliver Hobert (L) and Eduardo Leyva-Díaz (R).

**Oliver, can you give us your scientific biography and the questions your lab is trying to answer?**

**OH** I started out investigating signal transduction for my PhD with Axel Ullrich and Gerhard Krauss in Germany, and then moved to the USA for my postdoc with Gary Ruvkun. In Gary's lab, I started working with *C. elegans* on transcription factor regulation and specification of neuronal fates. In my own lab, we have continued to pursue our interest in understanding the molecular mechanisms that control the generation of diverse cell types in the nervous system. More recently, we are also becoming more and more interested in understanding how neuronal identity features are modulated by certain factors, such as environmental conditions or sexual identity.

**And Eduardo, how did you come to work in the Hobert lab, and what drives your research today?**

**EL-D** My fascination with science began in biology laboratory classes in high school, with a very dedicated and passionate teacher. Since then, I've been always attracted to genetics and molecular biology, and my first research experience as an undergraduate student was in Prof. Jose Luis Micol's lab working on *Arabidopsis thaliana* genetics. Towards the time of my graduation, I became interested in the nervous system, specifically in learning and memory, although I have never really worked on that field. The one thing I was not interested in at all at that time was developmental neurobiology, but funnily enough, after my rotation in different labs at the Instituto de Neurociencias de Alicante, I was totally captivated by it, and devoted my next 6 years to studying mouse brain development in Guillermina Lopez-Bendito's lab. After my thesis defense, I stayed for a few months in the lab and worked on a new research line aimed at reprogramming endogenous astrocytes into different projection neurons. With this experience in identity reprogramming and transcriptional regulation, I developed a deep interest in neuronal identity specification, particularly regarding the maintenance of neuronal features. The Hobert lab was then a clear perfect match, with *C. elegans* representing an excellent model system to study neuronal identity specification and maintenance.

**When did you first become interested in transcriptional autoregulation? And given it has been known about for decades, why do you think it has taken so long to formally test its functional requirement?**

**OH & EL-D** A key characteristic of several terminal selectors, identity-specifying transcription factors, is their role in the maintenance of neuronal identity, which is thought to be achieved by transcriptional autoregulation. However we, as well as others, had only inferred transcriptional autoregulation from the presence of binding sites of a transcription factor in its own genomic locus, and from genetic loss-of-function studies in which the activity of a transcription factor is removed and a loss of transcription of this locus is consequently observed. Formal proof for the functional relevance of autoregulation has been sparse, however. The advent of CRISPR/Cas9 technologies has been key to providing formal proof for this requirement, because it enabled us to disrupt autoregulation, but not other functions of a specific transcription factor. We could therefore precisely ask what it is that autoregulation actually does – and we came up with a surprise that we had not anticipated.

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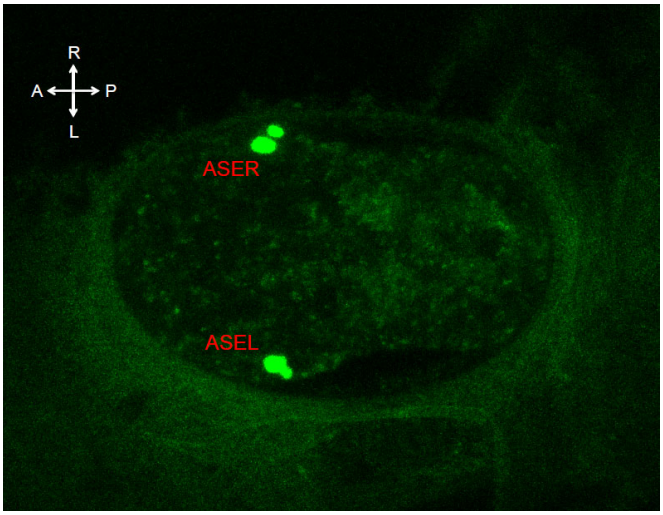


***C. elegans*** **embryo in which the *che-1* locus has been tagged with *gfp* through CRISPR/Cas9 genome engineering. *che-1::gfp* expression can be observed in the bilaterally symmetric ASE neuron pair (ASEL + ASER) and their sister cells, which are in the process of undergoing apoptotic cell death.**

**Can you give us the key results of the paper in a paragraph?**

**OH & EL-D** In this paper, we use CRISPR/Cas9 to remove a cis-regulatory motif from a cell identity-specifying transcription factor, showing that the disruption of transcriptional autoregulation leads to a failure to maintain the differentiated state of the cell. Upon regulatory motif mutation, we observe a gradual decrease in neuronal function and cell identity marker expression. This was an expected result that provided formal proof for the importance of identity-triggering transcription factors in maintaining the identity state of a cell. However, we also found that transcriptional autoregulation is not only required to maintain a specific cellular state, but is also required during development to amplify the expression levels of the autoregulating transcription factor to a critical threshold level in order to allow it to initiate expression of its target genes, which will define the differentiated state of the cell.

**Do you think the early function in initiation of *che-1* expression is likely to be a general feature of autoregulation?**

**OH & EL-D** In general, we think that if a gene can autoregulate it makes sense that this autoregulation is also used early in development. However, we have found in the literature examples of other autoregulating transcription factors for which maintenance relies on autoregulation, while the initial amplification is achieved by different means. Interestingly, this dual role of autoregulation, early amplification/late maintenance, seems to be modular and context dependent, since in some cases the autoregulation of other factors is only important early in development. Nonetheless, it does not seem far-fetched to propose that the functional duality of transcriptional autoregulation constitutes a widely used gene regulatory principle during animal development.

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**When doing the research, did you have any particular result or eureka moment that has stuck with you?**

**EL-D** For me, the eureka moment was when we realized about the function of transcriptional autoregulation in early development. We were very satisfied with the close correlation between *che-1* expression and neuronal functional performance through the different developmental stages. But when we looked earlier, we were at first surprised by finding already low levels of *che-1* expression in the embryo. Then we realized that it would only make sense if autoregulation also contributed to transcription factor initial amplification and, consequently, acquisition of the differentiated state.

**And what about the flipside: any moments of frustration or despair?**

**EL-D** Without any doubt, the moments of frustration and despair were at the very beginning of the project. Generating precise motif mutations in the *che-1* promoter was key for this story, and obtaining some of the *cis*-regulatory mutations took longer than expected. The application of CRISPR/Cas9 engineering to different projects was just becoming established in the lab at that point, and we were at the initial phase of standardization and protocol set up. Of course, we got our mutants, and the road was mostly paved after that.

**So what next for you after this paper?**

**EL-D** I am intensively working on a second project, where we are trying to understand how the expression of pan-neuronal genes is controlled. Neuronal identity is determined by the expression of neuron-type specific genes and pan-neuronal genes, which are shared by all neurons in the nervous system. We now know several examples about neuron-type specific gene regulation, but not that much about pan-neuronal genes. Previous work form the Hobert lab has shed some light into the how, and now I am trying to find the who, identifying key factors controlling pan-neuronal gene expression. And then, job hunting.

**Where will this work take the Hobert lab?**

**OH** This work will hopefully not present the endpoint of studying transcriptional autoregulation. While there's plenty of evidence to suggest that positive autoregulation is a widespread phenomenon, we also know that some identity-specifying terminal selectors do not autoregulate, even though their expression is maintained throughout the life of a neuron. How does this work? In at least one other case, we also have reason to believe that there is negative autoregulation, in which a terminal selector dims down its own expression. We would love to understand how and why this is.

**Finally, let's move outside the lab – what do you like to do in your spare time in New York?**

**EL-D** New York is an amazing place and I love to explore the city and its surroundings with my wife and friends. I especially enjoy discovering all the culinary options, and I try to take advantage of the different cultural activities that the city has to offer. I also like to stay active, running and playing different sports. Finally, I love to travel when possible, to discover new places or back to Spain to enjoy the weather, food, family and friends.

**OH** I don't have much to add to this. New York is an amazing, dynamic and constantly changing place that leaves new things to discover even if one has lived in the city for a while.

**Reference**

**Leyva-Díaz, E. and Hobert, O.** (2019). Transcription factor autoregulation is required for acquisition and maintenance of neuronal identity. *Development* **146**, dev177378. doi:10.1242/dev.177378