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INTERVIEW

# The people behind the papers – Milica Bulajić, Divyanshi Srivastava, Esteban Mazzoni and Shaun Mahony

Hox genes instruct positional identity along the anterior-posterior axis of the animal body. A new paper in Development addresses the question of how similar Hox genes can define diverse cell fates, using mouse motor neurons as a model. To hear more about the work, we caught up with the paper's two first authors, PhD students Milica Bulajić and Divyanshi Srivastava, and their respective supervisors Esteban Mazzoni (Associate Professor of Biology at New York University, USA) and Shaun Mahony (Assistant Professor of Biochemistry & Molecular Biology at Penn State University, USA).

Milica, Divyanshi, Esteban and Shaun (clockwise from top L).

**Esteban and Shaun, what questions are your labs trying to answer, and how did you come to collaborate on this project?**

**EM:** To understand cell differentiation, we focus on investigating how transcription factors control transcription and establish long-lasting epigenetic memories. With this knowledge, we then aim to control cell fate at will for clinical applications.

**SM:** We develop machine learning applications to understand gene regulatory systems. We particularly focus on understanding how transcription factors find their binding sites and drive regulatory responses in dynamic contexts such as development.

**EM & SM:** We began collaborating as postdocs more than a decade ago when ChIP was emerging (back when it was ChIP-chip!), and there were few computational tools. Even back then, we collaborated at a distance, with EM in New York and SM in Boston. EM was developing cellular models to understand cell differentiation at scales and purity compatible with the technology, and SM was developing tools to analyse the data, extract meaningful information and generate hypotheses. This cycle has been going strong ever since: the analyses carried out in SM's lab have proposed hypotheses about transcription factor selectivity that EM's lab has tested, and the systems and technologies developed in EM's lab have inspired many of the computational tools developed in SM's lab.

**Milica and Divyanshi - how did you come to work in the Mazzoni and Mahony labs, and what is the main drive behind your research?**

**MB:** I finished my undergraduate studies in Molecular Biology at the University of Belgrade, Serbia, where I am from. I joined the PhD program at the Department of Biology at New York University in 2014. After spending my first year rotating in different labs, I joined the Mazzoni lab because I really liked the research and enjoyed my rotation project, which was Hox related. I knew that I wanted to continue working on Hox genes and felt supported by Esteban in choosing questions to work on.

**DS:** When I started my PhD at Penn State, I was keen to work on computational regulatory genomics. I am very excited by the potential of novel computational methods to elucidate complex biology. Therefore, the Mahony lab was a great fit, with Shaun's expertise in computational biology and the Mazzoni lab's exciting work on the regulatory biology of cellular differentiation!

**How has your research been affected by the COVID-19 pandemic?**

**EM:** Like most institutions, we closed down with two days' notice. The situation really dawned on me when we turned off equipment for the first time since I opened the lab. However, the hiatus made us focus and plan, and execute the most informative experiments now that we are at 50% output. Thus, it has had a positive side effect.

**MB:** We were out of the lab for about 3 months so there were some experimental delays, but I'm very lucky that I didn't lose any work, or need a long time to start things up again. I also had plenty of data to analyse and manuscript edits to incorporate so that has been keeping me busy.

**SM:** As a computational lab, we were fortunate that we could continue making progress when others lost access to their facilities. But it has still been challenging to adapt to remote research; we miss the conversations and spontaneous debugging sessions that drive computational research forward. As with many others, I've personally found it difficult to devote enough time to research while also dealing with remote elementary school and adapting my own courses to a remote format.

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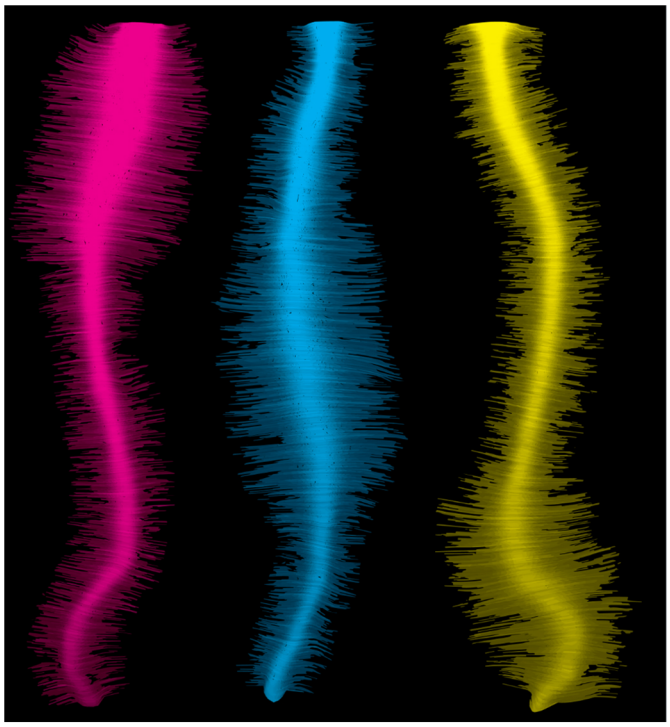

This piece of art was made by Dylan Iannitelli, a PhD student in the Mazzoni lab, from ChIP-seq data for Hox binding.

**DS:** COVID-19 has been challenging due to the remote nature of all computational work, but I was fortunate that we had continued access to computational resources, as well as a supportive lab environment, which made it easier to work through the more difficult days.

**What was known about the relationship between Hox binding and chromatin accessibility prior to your work?**

**MB, DS, SM, EM:** When we planned these experiments, not much was known about their differential ability to bind inaccessible chromatin. Soon after that, in 2016, Robert White's group described how some *Drosophila* Hox factors bind to chromatin. And then, around the time we were writing our paper last summer, a few relevant papers came out. The White group published a more extensive evaluation of all *Drosophila* Hox proteins showing that accessibility has a role in Hox selectivity, and out of all of the central and posterior Hox proteins, Abd-B stood out in having a higher ability to bind inaccessible sites. This was really interesting for two reasons: first, Hox proteins do have different abilities to bind to inaccessible chromatin; second, it primed our work – how do vertebrate posterior Hox genes (Hox9-13), all of which are fly Abd-B orthologues, behave? Coincidentally, Marie Kmita's group published a preprint showing that Hox13 paralogs are required to open specific sites during limb development. Finally, Denis Duboule's group showed similar results in genital development. Thus, the field was coming together.

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

**MB, DS, SM, EM:** We investigated the binding, transcriptional targets, sequence and chromatin preferences of seven different mammalian Hox proteins in a relevant cell type patterned by Hox genes. We discovered that the ability to engage with inaccessible sites is an important factor that drives Hox binding specificity. This ability seems to be driven by the DNA-binding domain and C-terminus. These results show that Hox specificity models should incorporate sequence preference, co-factor interactions and intrinsic abilities to bind inaccessible chromatin. We believe this can be extended to other homeobox genes (and perhaps other paralogous transcription factor groups) as a binding diversification strategy.

**Where Hox proteins show high affinity for inaccessible chromatin, do you think they are acting as so-called 'pioneer' factors?**

**MB, DS, SM, EM:** Our results and other studies show clearly that some Hox proteins play a role in 'opening' some regions of relatively inaccessible chromatin during differentiation. However, in the strict sense, the term 'pioneer factor' is reserved for those transcription factors that have been demonstrated to bind to DNA wrapped around nucleosomes, which subsequently evict nucleosomes. Our data is compatible with some posterior Hox proteins acting as pioneers, but it is now a good hypothesis to test.

**What explains the different chromatin affinities – even among paralogs – of the various posterior Hox proteins?**

**MB, DS, SM, EM:** We used multiple different approaches to characterize sequence preferences and found no evidence that sequence explains the different chromatin affinities. For example, we found no sequence preference differences between HOXC9 and HOXC10, or HOXC9 and the other HOX9 paralogs. Our results with the chimeras, made by swapping HOXC10 and HOXC13 DNA-binding domains, show that chromatin affinities seem to be controlled by the homeodomain and C-terminus. As shown with the bHLH family, the different homeodomains could engage the DNA-nucleosome complex in slightly different ways.

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

**MB:** I think for me, the most impactful thing was seeing the binding results for HOXC13, and finding that it binds to very inaccessible chromatin. Similarly, when I made the chimeric Hox proteins, seeing that this ability is controlled by the DNA-binding domain and C-terminus.

**DS:** For me, observing the difference in chromatin accessibility at HOXC9-only sites compared with other differentially bound Hox transcription factor sites was an exciting moment. And of course, the binding results for HOXC13 were striking.

**Observing the difference in chromatin accessibility at HOXC9-only sites compared with other differentially bound Hox transcription factor sites was an exciting moment.**

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

**MB:** Waiting for reviews during the publication process can be stressful. There are always ups and downs when writing a paper, but when it's finally written and then accepted for publication, it's a great feeling.

**DS:** It was challenging to design a differential binding strategy for multiple transcription factors. We took a long time to arrive at analyses that were robust and reproducible, and that could overcome biases related to technical and experimental noise.

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**What next for you two after this paper?**

**MB:** I am writing another manuscript and scheduling my PhD defence for early 2021. I'm also in the process of looking and applying for jobs.

**DS:** I am working on developing computational approaches that can interpretably model transcription factor binding sites. I also plan to defend in early 2021, and pursue research-related positions after my PhD.

**Where will this story take the Mahony and Mazzoni labs?**

**EM:** For us, it has two logical future paths. First, gaining insights into Hox-dependent positional identity allows for the precise control of *in vitro* differentiated motor neuron positional fate. Second, it opened a new dimension within homeodomain transcription factor diversification. The small sequence preference variation was always hard to reconcile with their diverse functions. Now, we hypothesize that the ability to engage inaccessible sites provides an orthogonal mechanism for homeobox genes to diversify their binding and, thus, gene regulation.

**SM:** This project has really brought home the importance of pre-existing chromatin environments in determining transcription factor binding specificity during development. In a parallel project, Divyanshi has also developed neural networks that can interpret how sequence and pre-existing chromatin features predict the binding specificity of a transcription factor. So, the use of these types of approaches to understand how chromatin shapes transcription factor binding (and vice versa) will continue to be a big focus in our lab, especially in terms of being applied to understand the dynamic systems studied in Esteban's lab.

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

**MB:** Going for long walks and hikes, and sitting in a park with a good book.

**EM:** I am an avid sailor, taking me beyond the lab, the city and the continent. Last October, I participated in a trans-Atlantic race.

**DS:** I like to go cycling, with the rolling hills of central Pennsylvania providing some lovely terrain.

**SM:** We're very fortunate in central Pennsylvania to have lots of beautiful parks and trails, and that's where my family and I like to spend our spare time.

**Reference**

**Bulajić, M., Srivastava, D., Dasen, J. S., Wichterle, H., Mahony, S. and Mazzoni, E. O.** (2020) Differential abilities to engage inaccessible chromatin diversify vertebrate Hox binding patterns. *Development* **147**, dev.194761. doi:10.1242/dev.194761