# Systematic Analysis of Posterior HOXA/HOXD Binding in Mesenchymal Cells

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Abstract: *Hox* genes encode transcription factors that determine vertebrate body plan and pattern structures and organs in the developing embryo. Despite decades of effort and research, little is known about the HOX-DNA binding properties *in vivo*. This lack of knowledge is mainly due to the absence of appropriate antibodies to distinguish between different HOX transcription factors. To tackle this problem, we adapted a cell culture system that allowed us to investigate HOX-DNA binding on a genome-wide scale. With this approach, we defined and directly compared genome-wide binding sites of nine posterior HOXA and HOXD transcription factors. We report that in cellular environment HOX binding specificity differs from the *in vitro* specificity and find that HOX-TFs largely rely on co-factor binding and not only on direct HOX-DNA binding. Finally, we identify a novel HOX co-factor, a genome architecture protein, CTCF suggesting a possible interplay between HOX-TF function and chromatin architecture.

To better understand gene regulation in the aspect of genome architecture it is imperative to precisely define the relationship between chromatin architecture and transcription. However, although there is ample evidence of concomitant changes in transcription and chromatin architecture in differentiation and development, the mechanistic and functional understanding of this relationship is still lacking. We aim to examine this relationship more closely using a well-established cell-culture neuronal differentiation system where we will assay the potential protein culprits involved in this process. Furthermore, using the combination of genome engineering, genomics and biochemistry we aim to investigate the interplay between chromatin architecture, in particular insulation, and transcription. The results obtained in this study will help us better understand the functional element of chromatin architecture and to more precisely define the relationship between recurring transcription at the sites of insulation and the insulation itself.