Contribution ID: **82** Type: **Plenary Talk**

Investigating enhancer function by generating synthetic Ihh regulatory landscapes

Thursday, September 19, 2024 4:30 PM (30 minutes)

Cis-regulatory elements such as promoters and enhancers coordinate the cell-type specific transcription of their target genes. Promoters sit directly upstream of the transcription start site, while often many partially redundant enhancers are distributed in extended regulatory domains surrounding their target gene. Despite their significance, the mechanisms by which enhancers cooperate and interact within regulatory domains, particularly to drive developmental gene expression patterns, remain poorly understood.
For example, how position and spacing between seemingly randomly distributed enhancers affects their functionality remains a poorly understood aspect of a key characteristic of mammalian genomes. Likewise, the contribution of the majority of DNA sequence, all intervening inter-enhancer sequences, remains unknown. The reason for these largely unexplored features lies in our limited ability to systematically alter large genomic regions. Traditional approaches are mostly restricted to investigating individual elements or require repeated targeting of the same locus, hindering a systematic analysis. We recently established a synthetic biology derived workflow that overcomes current limitations, combining methods from microbiology with integrase-based genome engineering for mESCs. This setup allows us to effectively synthesize any DNA sequences of dozens to hundreds of kb and integrate them site-specific into mESCs.
In this project, we employ this approach to investigate the enhancer function of the regulatory domain of *Indian hedgehog* (*Ihh*), a gene coding for a key developmental signaling molecule. We created several synthetic regulatory landscapes of varying sizes containing previously described *Ihh* enhancers with alternate spacing, order, or inter-enhancer sequence. Using multiplex whole-mount fluorescent in situ hybridization, we find that omitting inter-enhancer regions or altering enhancer order does not significantly affect the expression pattern or level of *Ihh*. However, maintaining the spacing of the wild-type configuration, while changing (reversing) only the inter-enhancer sequences has strong effects on *Ihh* expression. Our findings reveal a surprising robustness of developmental gene expression to severe pertubations in the arrangement of cis-regulatory elements, prompting new questions about the "mode-of-action"of non-coding sequence composition in mammalian genomes.

Primary author: MAGG, Andreas (Mundlos Lab, MPI for Molecular Genetics, BIH Center for Regenrative Therapies (Ibrahim lab))

Co-authors: SCZAKIEL, Henrike (Institute of Medical and Human Genetics - Charité Universitätsmedizin Berlin); BEHNCKE, Rose (BIH Center for Regenrative Therapies (Hägerling lab)); WITTLER, Lars (Transgenic Core Unit, MPI for Molecular Genetics); HÄGERLING, René (BIH Center for Regenrative Therapies); IBRAHIM, Daniel (Mundlos Lab, MPI for Molecular Genetics / BIH Center for Regenrative Therapies)

Presenter: MAGG, Andreas (Mundlos Lab, MPI for Molecular Genetics, BIH Center for Regenrative Therapies (Ibrahim lab))

Session Classification: Plenary Talk