Konrad Hochedlinger, PhD

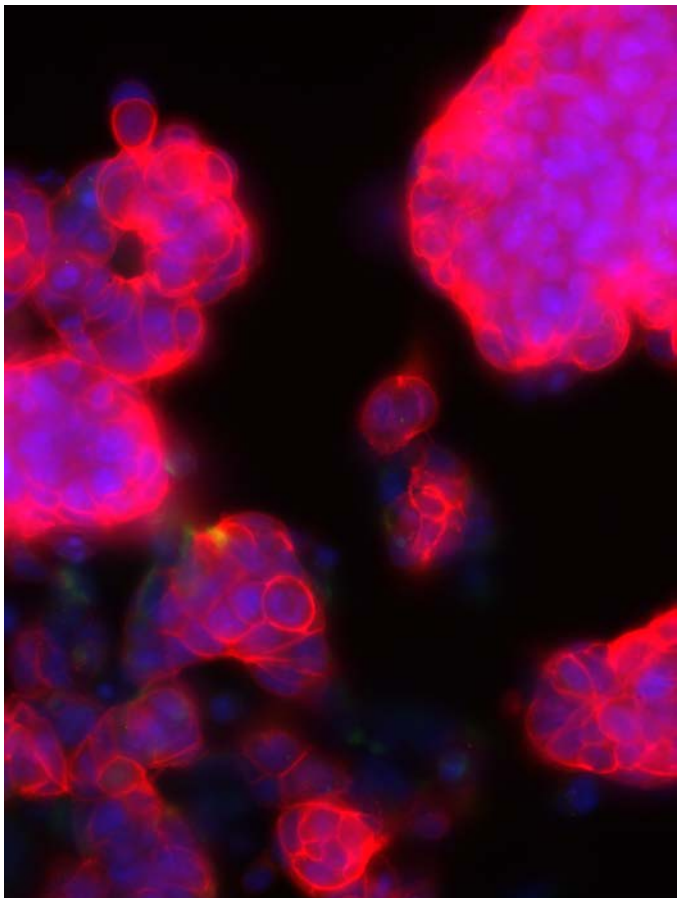
Hochedlinger Laboratory

Andres Binker Cosen**
Julie Finn*
Rebecca Gorelov**
Joy Horng**
Konrad Hochedlinger, PhD
Nikolaos Tsopoulidis, PhD
Aaron Weiner, PhD
Masaki Yagi, PhD

* Administrative Assistant
** Graduate Student

The Hochedlinger laboratory explores the fundamental question of how cells maintain their identity. We hypothesize that factors that reinforce specific cell states, such as pluripotency and differentiation, continue to play functional roles in other cellular contexts including development, tissue homeostasis and cancer. Using stem cell models and reprogramming systems as discovery tools ex vivo, our laboratory has elucidated novel mechanisms that maintain cell identity and function upstream of cell type specific transcription and chromatin factors. Specifically, work from our lab over the past five years revealed that common cellular processes such as protein sumoylation, chromatin assembly, alternative mRNA polyadenylation and P-body homeostasis play key roles in the maintenance of cell identity across distinct lineages. We now aim to probe the functional conservation of these mechanisms across physiological cell fate transitions in vivo using animal models and cell transplantation. As our strategy is not confined to one particular cell type or tissue, we are in a position to uncover shared regulatory principles crucial for the maintenance of cell identity across different developmental contexts.

While development and cellular differentiation were long thought to be irreversible processes, our ability to reprogram differentiated cells to an embryonic-like state revealed that mechanisms that safeguard cell identity and thus restrict developmental plasticity can be overcome through experimental manipulation. Indeed, seminal somatic cell nuclear transfer (SCNT) experiments proved that the nuclei of terminally differentiated cells and even certain cancer cells retain full developmental potential. While SCNT is a powerful assay to test the developmental potential of a given genome, it does not allow one to study *how* differentiated cell states are established and maintained. By contrast, transcription factor-induced reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) is a molecularly defined and tractable system to dissect fundamental questions of cell state. Our lab initially used this approach to provide crucial insight into the basic mechanisms by which transcription factors and chromatin signaling establish and maintain identity in either pluripotent or differentiated cells, and we began to probe the conservation of these principles in other cellular contexts. For example, we discovered that the transcription factor Sox2, which is essential for the establishment and maintenance of pluripotent stem cells, is re-expressed in adult gastric stem cells where it maintains tissue identity by suppressing an alternative intestinal cell program and tumorigenesis. Similarly, we demonstrated that the manipulation of safeguard mechanisms previously identified during iPSC reprogramming in other cellular contexts facilitate the derivation of self-renewing muscle stem-like cells, which have been notoriously difficult to capture using conventional strategies. Most recently, our lab uncovered two post-transcriptional processes, alternative polyadenylation (APA) and Processing body (P-body) turnover, as novel safeguard mechanisms using unbiased screens. While APA and P-bodies are thought to control different aspects of gene regulation in the nucleus (APA) and cytoplasm



Immunofluorescence images showing fibroblasts undergoing reprogramming to induced pluripotent stem cells upon forced expression of Oct4, Sox2, Klf4 and c-Myc in the presence of the histone mutant H3.3K36M. Note that cells express the epithelial marker Epcam (red) homogeneously but no longer express the fibroblast marker Vimentin (green), demonstrating that loss of H3K36 methylation is sufficient to endow the majority of somatic cells with an epithelial state that subsequently gives rise to iPSCs (See Hoetker et al., Nat Cell Bio, in press).

Image: Michael Hoetker, MD

(P-bodies), a key commonality that emerged from our work is that both processes regulate the protein homeostasis of hundreds of fate-instructive genes. Together, these examples underscore the power of our approach to gain insights into tissue identity through the study of pluripotency and cellular reprogramming.

Considering that several of the safeguard mechanisms we previously identified in reprogramming converge on chromatin regulators, we have recently developed versatile transgenic tools to directly probe the physiological role of chromatin modifications in cell fate change. This approach has allowed us to uncover previously unappreciated functions of H3K9 and H3K36 methylation in the regulation of pluripotency, reprogramming, tissue homeostasis and aging, which is the basis for ongoing work in the lab.

Thus, by pursuing our hypothesis that different physiological as well as experimentally induced cell fate transitions utilize common mechanisms, our lab has uncovered novel epigenetic, transcriptional and post-transcriptional regulators of cell identity. As we pursue a deeper understanding of how these underexplored regulators and processes guide cell fate transitions in vivo, we are poised to discover shared principles by which they safeguard cell identity during development and tissue homeostasis and how this knowledge may be exploited in a therapeutic setting to alter cell fate.

Selected Publications:

Hoetker, M. S., M. Yagi, B. Di Stefano, J. Langerman, S. Cristea, L. P. Wong, A. J. Huebner, J. Charlton, W. Deng, C. Haggerty, R. I. Sadreyev, A. Meissner, F. Michor, K. Plath, and K. **Hochedlinger K**. H3k36 Methylation Maintains Cell Identity by Regulating Opposing Lineage Programmes. *Nat Cell Biol* 2023 (in press)

Huebner, A. J., R. A. Gorelov, R. Deviatiiarov, S. Demharter, T. Kull, R. M. Walsh, M. S. Taylor, S. Steiger, J. T. Mullen, P. V. Kharchenko, and K. **Hochedlinger K**. Dissection of Gastric Homeostasis in Vivo Facilitates Permanent Capture of Isthmus-Like Stem Cells in Vitro. *Nat Cell Biol* 2023 (25), no. 3 (Mar): 390-403.

Yagi M, Ji F, Charlton J, Cristea S, Messemer K,…Goldhamer DJ, Wagers AJ, Michor F, Meissner A, Sadreyev RI, **Hochedlinger K**. Dissecting dual roles of MyoD during lineage conversion to mature myocytes and myogenic stem cells. *Genes Dev*. 2021 Sep 1;35(17-18):1209-1228.

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Di Stefano B, Luo EC, Haggerty C,… Gygi SP, Sadreyev RI, Meissner A, Yeo GW, **Hochedlinger K**. The RNA Helicase DDX6 Controls Cellular Plasticity by Modulating P-Body Homeostasis. *Cell Stem Cell*. 2019 Nov 7;25(5):622-638.e13.

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