The intestinal epithelium has emerged as a leading model to study cellular lineage differentiation in a renewing tissue. Elegant studies using mouse transgenics have led investigators to decipher a beautiful system in which crypt base columnar cells, nestled at the base of the crypts of Lieberkühn, either undergo stem cell renewal or differentiate and give rise to absorptive enterocyte progenitor cells or secretory progenitor cells. A series of transcription factor knockouts showed critical regulators of subsequent lineage decisions; these key genes govern distinct aspects of differentiation into absorptive enterocytes, mucus-producing goblet cells, hormone-producing enteroendocrine cells, or Paneth cells, which support the stem cell niche as well as secrete antimicrobial peptides. Although many genetic regulators of intestinal epithelial differentiation have been identified, the transcriptional mechanisms through which these key factors direct differentiation are largely unknown, and identifying the hierarchical relationship between lineage-regulating factors remains a challenge, particularly in vivo.

Two transcription factors that have been shown to have roles in the secretory lineages are Atonal basic Helix-Loop-Helix transcription factor 1 (ATOH1) and SAM pointed domain containing ETS transcription factor (SPDEF). ATOH1 is a basic helix–loop–helix transcription factor previously shown to be required, and in some contexts sufficient for differentiation to intestinal secretory lineages. Atoh1 functions via Notch-mediated lateral inhibition, and deletion of ATOH1 even in established secretory cells can trigger their conversion to enterocytes. Previous work, also based largely on mouse genetic models, has shown that SPDEF works to promote goblet cell identity downstream of ATOH1. Although the genetic functions of these transcription factors are well documented, their regulatory mechanisms are less clear. In this issue, Lo et al leveraged a series of mouse genetic tools and cell isolation and sorting techniques to explore the epigenomic regulatory underpinnings of how Atoh1 and Spdef mediate secretory cell fate.

To look under the hood at how ATOH1 mediates lineage fate, Lo et al. took advantage of a green fluorescent protein-tagged Atoh1 locus, which provides both an epitope tag for chromatin immunoprecipitation (ChIP) and a fluorescent marker for cell isolation. The model provides a robust signal in goblet and Paneth cells, and a detectable signal in enteroendocrine cells. They also use Atoh1floX/floX mice and the CreERT2 driven by the villin promoter to conditionally inactivate Atoh1 in intestinal epithelia. These genetic tools allowed Lo et al. to compare the transcriptional profiles of whole crypts, GFP-expressing ATOH1-expressing secretory cells isolated from the crypts, or whole crypts that received conditional inactivation of ATOH1. Subsequent analysis confirmed the expected depletion of secretory lineage genes in ATOH1-deficient cells, and the enrichment of secretory genes in GFP+ cells. More interestingly, Lo et al. took the next step to identify which of these regulated transcripts had loci bound by ATOH1–GFP using ChIP-sequencing in the ileum and colon. ATOH1 genomic binding events showed strong concordance in the ileum and colon, were enriched at transcriptional start sites, and at regions showing the active chromatin mark H3K27ac. They also identified potential co-regulatory partners for ATOH1 based on DNA motifs enriched at ATOH1-bound regions, including SPDEF. Finally, they integrated their RNA sequencing and ChIP sequencing analysis to propose ATOH1’s likely direct regulatory targets; these genes were enriched for roles that might hint at a secretory cell’s function, including activities such as intracellular transport. ATOH1’s targets also included other transcriptional regulators, consistent with ATOH1’s role atop the regulatory hierarchy of secretory cell lineages.

Identification of the likely direct targets of ATOH1 function will be important for cell biologists to decipher the molecular steps required for the secretory cell’s product assembly and secretion. It will also help refine sets of genes specific to secretory lineages. In addition, using an impressive knockout-plus-overexpression model, Lo et al. began to describe the co-regulatory relationship between ATOH1 and its downstream partner SPDEF. They observed that SPDEF overexpression can cooperate with ATOH1 to amplify its target gene expression levels, but cannot stimulate transcription of these co-targets in the absence of ATOH1. Thus, SPDEF activity, at least at the genes analyzed, is dependent on ATOH1. Why is ATOH1 upstream of SPDEF-mediated activation? Is ATOH1 required for SPDEF binding to the genome? For chromatin activation? For transcriptional elongation of shared target genes? Understanding the mechanisms behind the ATOH1-SPDEF regulatory hierarchy will be important next steps in appreciating the details of secretory cell differentiation.

It is particularly important that these studies all were performed on freshly isolated intestinal epithelial subpopulations because investigators building upon this work can trust that the measurements captured by Lo et al. were performed under near-physiological conditions. Indeed, as mouse genetics, cell isolation, and epigenomic technologies continue to advance, so will our appreciation of the beautiful arbors of the intestinal epithelial lineage tree.
Reference

1. Lo Y-H, Chung E, Li Z, et al. Transcriptional regulation by ATOH1 and its target SPDEF in the intestine. Cell Mol Gastroenterol Hepatol 2017;3:51–71.

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Conflicts of interest
The author discloses no conflicts.

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