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An Epigenomic Road Map for Endoderm Development

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While studies of organ development have traditionally relied on model organisms, recent advances in embryonic stem cell (ESC) culture allow investigation of organogenesis in human cells. Wang et al. (2015) employ this system to map the dynamic enhancer landscape during ESC differentiation to the endoderm derivatives pancreas and liver.

Through decades of studies in model organisms ranging from nematodes to mice, a general framework has emerged that explains the transitions from the pluripotent zygote to hundreds of specific cell fates in its differentiated descendants. Thus, both extrinsic, inductive signals, as well as the intrinsic regulation of gene expression by stage-specific DNA binding transcription factors, have been shown to direct lineage specification and organ development. A complex assortment of epigenetic marks accompanies these fate transitions. In this issue of *Cell Stem Cell*, Wang and colleagues employ comprehensive mapping of two key enhancer marks during the in vitro differentiation of human embryonic stem cells (hESCs) toward the pancreatic and hepatic lineages to unravel the epigenomic landscape of stepwise endoderm differentiation into these alternative cell fates (Wang et al., 2015).

Wang and colleagues employ a well-validated, stepwise in vitro differentiation system, pioneered by D'Amour and colleagues (D'Amour et al., 2006), in which ESCs are first induced to form "definitive endoderm," the germ layer from which the gut-associated organs thyroid, lung, liver, pancreas, and the epithelia of the entire gastrointestinal tract are derived. These definitive endoderm cells are then further differentiated, using various growth factors and medium supplements, into "gut tube," "foregut endoderm," and finally "pancreatic endoderm" cells. At each of these stages, the authors determined the global transcriptome using RNA-seq, the approximate transcriptional rate by global run-on sequencing, and the distribution of two key "enhancer marks." The histone H3 lysine 4 mono-methyl (H3K4me1) modification, while also found near active promoters, is widely accepted as a mark of enhancers, regardless of whether they are "poised" or "active." The H3K27Ac (histone H3 acetylated on lysine 27) is typically present at the subset of enhancers that are active, increasing transcription from a nearby or even distant target promoter. By performing this comprehensive analysis, the authors confirmed that on a global level, transcription at enhancers, producing so-called "eRNAs," occurs mostly when these carry the H3K27Ac mark.

When cataloguing active versus poised enhancers during their differentiation protocol toward the pancreatic lineage, the authors noted that a large fraction of poised enhancers present at the definitive endoderm or gut tube stages never become activated during the directed differentiation process. Considering that the gut tube gives rise to multiple organs, this finding is not surprising. Indeed, the authors demonstrate that many of these poised enhancers become activated when they differentiate gut tube cells toward either liver or lung fates. It is likely that yet other subsets of enhancers that are poised in the gut tube stage would become active if the cells were differentiated into thyroid-like or colonic epithelium-like cells. Altogether, these findings suggest that the establishment of a large set of poised enhancers contributes to the acquisition of developmental competence during stem cell differentiation.

So how are these gut tube enhancers specified in the first place? Using both transcription factor recognition motif analysis and chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) for selected transcription factors, the authors find that the FOXA transcription factors occupy a large fraction of the enhancers that are poised at the gut tube stage, but they become active after differentiation to either pancreas or liver lineages. The FOXA proteins are the founding members of a large class of "winged helix transcription" factors that play essential roles in multiple tissues and were discovered almost 3 decades ago (Hannenhalli and Kaestner, 2009). Seminal work by Zaret and colleagues showed early on that the FOXA proteins, which are structurally related to linker histones, can displace nucleosomes from their target sites in an in vitro assay (McPherson et al., 1993), and are therefore referred to as pioneer transcription factors. More recently, the FOXA proteins were shown to be important in global nucleosome repositioning during in vitro differentiation of ESCs to pre-hepatic endoderm (Li et al., 2012).

In the current paper, Wang and colleagues employ shRNA against FOXA1, one of the three FOXA proteins, to show that this gene is required for full induction of pancreatic marker gene expression in the in vitro differentiation system. This finding confirms genetic data in mice, which demonstrated that in vivo ablation of Foxa1 and Foxa2 in the pancreatic primordium blocked pancreas development and the induction of Pdx1 (Gao et al., 2008). In fact, the Foxa proteins are required for the proper development of all gut-tube-derived organs where their contribution has been tested, such as lung, liver, and pancreas (Gao et al., 2008, 2010; Lee et al., 2005; Wan et al., 2005), and the current manuscript provides the molecular explanation for these findings from mouse genetics by documenting that the FOXA proteins bind to a large fraction of enhancers that become

activated during lineage development from gut endoderm.

In their shRNA gene suppression experiment, reducing FOXA1 expression by 50%, the authors also analyzed the enrichment for the H3K4me1 mark at six selected enhancers and found no difference compared to controlled cells. They suggest that FOXA1 might not be involved directly in establishing this mark. While this is of course possible, the data on this point are not definitive, as genetic experiments have shown partial redundancy among the Foxa factors in multiple instances (Gao et al., 2008; Li et al., 2009; Wan et al., 2005). Thus, it is likely that a 50% reduction in FOXA1 levels as achieved by the authors is compensated in part by continued presence of FOXA2 and FOXA3. Therefore, it remains to be seen if these FOXA bound enhancers can be established during hESC differentiation without any FOXA protein present.

In summary, the comprehensive study by Wang and colleagues demonstrates that the establishment of developmental competence during hESC differentiation toward the endoderm-derived organs occurs in a stepwise manner, with entire sets of enhancers first poised and then activated depending on which lineage is targeted. In addition, these enhancers are first occupied by the winged helix transcription factors FOXA1 and FOXA2 during the acquisition of developmental competence, while lineage-specific transcription factors are recruited at subsequent steps. These findings suggest that effective reprogramming of somatic cells might require both the action of pioneer factors, such as the FOXA proteins, and lineage-specific transcription factors for full activation of the transcriptome appropriate for a given lineage.

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The lncRNA Pnky in the Brain

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Long noncoding RNAs (lncRNAs) influence diverse cellular processes and have been implicated in regulating stem cell properties. Now in *Cell Stem Cell*, Ramos et al. (2015) demonstrate that the neural-specific lncRNA Pnky regulates neuronal differentiation from neural stem cells and mediates RNA splicing through interactions with polypyrimidine tract-binding protein 1 (PTBP1).

lncRNAs are noncoding transcripts that are increasingly appreciated as important regulators of cellular function. They are greater than 200 nucleotides in length and, similar to protein-coding transcripts, undergo capping, polyadenylation, and splicing. lncRNAs are often expressed in a highly cell type-dependent or tissue-specific manner, but they exhibit lower conservation at the sequence level compared to protein-coding RNAs (Batista and Chang, 2013). While previously considered as transcriptional junk, recent evidence suggests that lncRNAs participate in many cellular regulatory processes including X chromosome inactivation, epigenetic chromatin modification, RNA processing, and transcriptional and post-transcriptional control of gene expression (Batista and Chang, 2013). Importantly, recent studies have demonstrated that lncRNAs are crucial players in the pluripotency network. The lncRNAs *Gomafu* (AK028326) and AK141205 are involved in the maintenance of pluripotency in mouse embryonic stem cells (mESCs) (Sheik Mohamed et al., 2010), while others, for instance *Mistral*, promote mESC differentiation (Bertani et al., 2011). Now in *Cell Stem Cell*, Ramos et al. (2015) demonstrate that the lncRNA Pnky regulates differentiation of embryonic and postnatal neural stem cells (NSCs).