Chromatin “pre-pattern” and epigenetic modulation in the cell fate choice of liver over pancreas in the endoderm

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Extra View to: Xu CR, Cole PA, Meyers DJ, Kormish J, Dent S, Zaret KS. Chromatin “prepattern” and histone modifiers in a fate choice for liver and pancreas induction in the endoderm. The information about chromatin states from embryonic studies can be used to predict lineage-specific developmental potential and chromatin modifiers to enhance particular cell fate transitions from stem cells.

Chromatin States in Pluripotent Cells

Epigenetic regulation, particularly through chromatin modification and DNA methylation, plays a critical role in controlling gene regulation and cell differentiation in the development of multicellular organisms. Various studies revealed that chromatin alterations accompany cell lineage specification during mammalian development. In the mammalian embryo, early pluripotent cells initially develop into the endoderm, ectoderm, and mesoderm germ layers. Germ layer cells are multipotent and each layer further differentiates into certain tissues and organs, but not others. Fully understanding the changes of chromatin states in cell lineage specification, which is fundamental to the studies of organogenesis and regeneration medicine, will allow the efficient induction of stem cells into desired cell lineages.

Much has been learned about the chromatin states in pluripotent cells, specifically embryonic stem (ES) cells and how such states endow the competence to self-renew or initiate particular cell programs. Master transcription factors control self-renewal and pluripotency by extensive chromatin binding and auto-regulatory loops. trimethylation of histone H3 on lysine residues K4 and K27 often marks silent genes with the potential to be activated in early development, pluripotent chromatin is more loosely structured and accessible than differentiated cell chromatin and P300, H3K27me3 and H3K4me1 are associated with poised enhancers at genes involved in early development.

While the combined pattern of transcription factors and histone modifications helps explain the competence for all tissue programs, it is not clear how such chromatin states will be relevant to multipotent cells, in which a restricted set of cell fates can be activated and other cell fates cannot. Furthermore, it is not known whether a common “open” chromatin state exists for silent genes in multipotent cells or if the silent genes for different fates are packaged into markedly different states. Superimposed upon these issues is the question of how chromatin states allow or deny the effect of inductive signals that promote cell fates. Lineage-restricted, multipotent progenitors exist at numerous stages of development, they are...
Chromatin States in Embryonic Endoderm Development

To address the above issues, we identified and tested the function of chromatin states in the embryonic endoderm. The ventral foregut endoderm of the mammalian embryo gives rise to liver and ventral pancreas progenitors and is an experimental model for investigating chromatin and inductive signaling.12,13 The multipotency of the ventral foregut endoderm has been shown by the ability of mouse embryo tissue to activate either the earliest liver or pancreas genes in explants,14 by the ability of improperly positioned mouse endoderm to acquire a hepatic instead of a pancreatic fate in vivo15 and by the ability of individually labeled zebrafish endoderm cells to give rise to hepatic and pancreatic descendants.16 Endoderm cells contain occupied DNA binding sites for FoxA and GATA factors at an enhancer of the silent DNA binding sites for FoxA and GATA causing compaction of the local chromatin. FoxA2 can recruit the corepressor Grg3, activate the hepatic program22 and could remain competent but silent by their ability to open chromatin has led to the proposal that such factors function in regulating the states at the liver vs. pancreas regulatory elements, except area IV of the Pdx1 gene,27–31 which are necessary for both the liver bud development.23–25 The early developmental binding of the factors and enhancers in the endoderm should provide insight into the regulation of the liver or pancreas fate choice. Due to the small cell numbers, 400–800 per embryo, the chromatin states in liver vs. pancreas regulatory elements is retained in hepatoblasts. Currently, the low cell numbers preclude a sequential ChIP assay on the native embryonic cells. However, the persistence of both H3K9acK14ac and H3K27me3 marks on the silent Pdx1 gene in sorted Liv2+ hepatoblasts is consistent with their co-existence on individual genes. Such co-existence has been seen in embryonic liver and pancreatic regulatory elements, where the genes are poised but not active, in undifferentiated endoderm.41 H3K9K14 marks tested, only H3K9acK14ac showed enhancers in the endoderm, we discovered a mark- edly different “pre-pattern” of chromatin states at the liver vs. pancreas regulatory elements, where the genes are poised but not active, in undifferentiated endoderm.45 In contrast to the liver regulatory elements in undifferentiated endoderm, which were devoid of the positive (H3K9acK14ac) and negative (H3K27me3) marks, the pancreas regulatory elements, except area IV of the Pdx1 gene, contained both marks. Despite the diversity of chromatin marks tested, only H3K9acK14ac showed a consistent, significant increase at the liver genes when the undifferentiated endoderm cells differentiated into hepatoblasts. The co-existence of H3K9K14 hyperacetylation and H3K27me3 at the Pdx1 elements is retained in hepatoblasts. 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SMAD proteins are downstream effectors of BMP signaling and can interact with P300. To directly test the role of SMAD4 in regulating P300 and histone acetylation, we conditionally disrupted Smad4 in the foregut endoderm. The study showed that the liver-inducing BMP signal is mediated by SMAD4 and P300 and results in histone acetylation at liver target elements and liver gene activation (Fig. 1). Our studies reveal chromatin “pre-programming” for different lineages in multipotent progenitor cells, they provide approaches that can be applied to other systems, and they provide new landmarks and molecular targets to track and modulate liver and pancreas specification from stem cells.

**Distinct Types of Positive-Negative Components at Poised Genes**

We found the co-existence of the positive and negative marks of H3K4me3/H3K27me3, respectively, at the silent Pdx1 elements in the endoderm, where the Pdx1 gene is competent or poised, to be activated. As described above, bivalent regulatory elements in H3K4me3/H3K27me3 often mark poised early developmental genes. Also, the positive and negative effectors FoxA/GATA and Groucho/Grg3, respectively, appear to co-exist at the silent liver regulatory elements in the endoderm, where the genes are poised to be activated.

Other examples of positive and negative components at poised genes include transcription factors bound to enhancers or promoters of silent genes in ES and iP cells that are activated late in differentiation. In ES cells, the enhancer of the hypomethylated, silent Alb1 gene is occupied by FoxD3, which represses transcription. During endoderm induction, FoxD3 is replaced by FoxA1, all prior to Alb1 activation. Similarly, in nascent endoderm, the induced FoxA1 binds within the methylation-free Afp distal promoter, which is necessary for Afp activation during stem cell differentiation. The positive factors are bound at unmethylated Cpg residues amidst many methylated CpGs, which are typically considered repressive; thus together constituting a distinct kind of positive/negative mark. In ES cells, an intergenic enhancer of 5-VpreB1 genes, which are expressed in pro- and pre-B cells, is occupied with Sox2 and FoxD3. Sox2 acts as a positive factor, contributing to the establishment of the H3K4me2 mark, whereas FoxD3 is a negative factor, repressing intergenic transcription from the enhancer. Another example is the pre-loading of RNA polymerase II, but its pausing, at silent genes with the potential to be activated in development. Since the poised of genes by simultaneous positive and negative chromatin components may be general, but involving diverse mechanisms, we suggest by defining the “bivalency” in stem and progenitor cell chromatin to refer to the simultaneous presence of positive and negative components at poised genes, and not solely to the original H3K4me3/H3K27me3. By having the poised state result from a dynamic equilibrium between positive and negative effectors, it may favorize the synchronous activation of genes in the rapidly developing embryo.

**Future Directions**

While in principle it should be possible to characterize chromatin states in germ layer cells derived from embryonic stem cells in vitro, early in this study we found that the chromatin states in ES-derived endoderm can be different from those we have characterized in vivo (C.-R. Xu, P. Gadue, C. Nuzzo, G. Keller and K.Z. unpublished data). Knowing the chromatin states in native embryonic tissue can be used to define benchmarks of proper progenitor cell programming in stem cell studies and molecular targets for enzymatic modifiers that function in the cell fate transitions. It will be informative to perform genome-wide analysis for H3K9/K14 acetylation and H3K27me3 in the undifferentiated endoderm, as the marks were found to be different between in vitro and in vivo populations. There may be key genes that demonstrate altered chromatin marks in ESC-derived endoderm compared with in vitro-derived endoderm that could limit in the in vitro differentiation process. The initial analysis of the genome-wide ChIP data will focus on genes known to be involved in pancreatic and liver.

The mechanistic studies to date have focused on the chromatin regulation of hepatic specification. We don’t yet know how the repressive chromatin mark of H3K27me3 is lost from Pdx1 regulatory elements, which appears to be required for initiation of the pancreatic program. We hypothesize that cell signaling regulates the recruitment of a histone demethylase to Pdx1 elements upon pancreas specification. Such information could provide insight into ways to enhance pancreas specification from stem cells.
Our work has provided a paradigm for using antagonism of Hex homeobox factors at specific developmental stages to alter cell specification. This was enabled by a careful analysis of chromatin patterns of native progenitor cells in vivo. Such approaches can be taken with any stem or progenitor cell type to predict developmental potential and enhance differentiation of stem cells.

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