Published in final edited form as:

Semin Cell Dev Biol. 2019 July ; 91: 111–118. doi:10.1016/j.semcdb.2018.08.014.

Epigenetic regulation of renal development

Samir S. El-Dahr*, Zubaida Saifudeen*

Tulane University School of Medicine, 1430 Tulane Avenue, Department of Pediatrics, Section of Pediatric Nephrology, New Orleans, LA, 70112, USA

Abstract

Developmental changes in cell fate are tightly regulated by cell-type specific transcription factors. Chromatin reorganization during organismal development ensures dynamic access of developmental regulators to their cognate DNA sequences. Thus, understanding the epigenomic states of promoters and enhancers is of key importance. Recent years have witnessed significant advances in our knowledge of the transcriptional mechanisms of kidney development. Emerging evidence suggests that histone deacetylation by class I HDACs and H3 methylation on lysines 4, 27 and 79 play important roles in regulation of early and late gene expression in the developing kidney. Equally exciting is the realization that nephrogenesis genes in mesenchymal nephron progenitors harbor bivalent chromatin domains which resolve upon differentiation implicating chromatin bivalency in developmental control of gene expression. Here, we review current knowledge of the epigenomic states of nephric cells and current techniques used to study the dynamic chromatin states. These technological advances will provide an unprecedented view of the enhancer landscape during cell fate commitment and help in defining the complex transcriptional networks governing kidney development and disease.

1. Introduction

Immediately after fertilization the parental genomes in the mammalian zygote undergo drastic chromatin reprogramming first to establish totipotency and subsequently to establish pluripotency in the embryonic cells. DNA demethylation/methylation, histone mobility, histone variants, histone modifications and higher order chromatin organization all play essential roles [1–6]. Observation of open chromatin architecture in embryonic stem cells (ESCs) and in 2-cell stage zygotes associates cellular plasticity with open chromatin. Differentiated cells at the blastocyst stage show more compact chromatin than the pluripotent cells from the epiblast [7]. The requirement of spatiotemporal control of developmental genes expression demands precisely orchestrated accessibility of transcription factors to chromatin at both enhancers and promoters. In this review we discuss the epigenetic regulation of lineage specification with respect to kidney development and nephrogenesis, highlighting the vital role of developmental enhancers in the stage-specific control of gene expression and technological advances that facilitate these studies at the single cell and gene level.

*Corresponding authors. seldahr@tulane.edu (S.S. El-Dahr), zubisaif@tulane.edu (Z. Saifudeen).

2. Kidney development

The kidney develops from the intermediate mesoderm (IM), the germ layer that lies between the paraxial and lateral plate mesoderm. The IM gives rise to the entire urogenital system, including the kidneys and the gonads. Genetic models and explant studies in multiple model systems (zebrafish, xenopus, chick and mice) have delineated the transcriptional and signaling networks that are critical for nephric lineage specification and commitment, nephron induction, patterning and differentiation [8–11] (and references therein). With the derivation of the nephric duct and the metanephric mesenchyme from the IM at E8.5 (mouse), kidney development ensues. Essential to this process is the GDNF-Ret signaling pathway that results in the outgrowth of the ureteric bud (UB) [12,13]. The precise localization of the site of budding from the caudal end of the nephric duct requires the concerted action of growth factors (GDNF, Fgf, Bmp) and receptors (Ret, Fgfr, Alk) [14–16], signaling pathway modulators (Spry1/2) [17] and transcription factors (Etv4/5, Smad) [16,18]. The UB then invades the adjacent metanephric mesenchyme (MM, the source of GDNF), and reciprocal induction that ensues between these two derivatives of the IM will sustain the growth and branching of the collecting duct system (UB-derived), and self-renewal and differentiation of the MM-derived nephron progenitors into nephrons [8,11] (Fig. 1).

Cell plasticity is progressively restricted with developmental age, as cells undergo commitment in response to the changing microenvironment. The chromatin landscapes and epigenetic networks that specify transcriptional changes during nephric lineage commitment and differentiation remain to be defined.

3. Chromatin states of promoters and enhancers define gene function

Promoters and enhancers are cis-regulatory elements classically defined by their proximity to or distance from the transcription start site, respectively, with clusters of transcription factor binding sites. The promoter specifies accurate transcription initiation whereas enhancers characteristically augment basal transcription in orientation- and promoter-independent manner [19]. Typically, active enhancers and promoters are devoid of nucleosomes over several hundred base pairs, and these regions are flanked by nucleosomes. Data gathered from genome-wide studies reveal distinct patterns of histone modifications, transcription factor and co-activator occupancies at promoters and enhancers [20,21]. Histone modifications can impact gene expression by physically altering the affinity of DNA to histone proteins, thus making DNA less or more accessible for transcription. Additionally, the modifications may act as binding sites for effector proteins and remodeling complexes that regulate local chromatin function [22]. Details on cell-type specific chromatin landscapes and genome organization have emerged as a result of epigenomic maps of multiple cell types. Detailed chromatin state maps of active, repressed or poised promoters and enhancers as well as the expressed gene body regions are available for numerous cell types (Fig. 2A) [23].

3.1. The (epigenomic) hallmarks of promoters Fig. 2B

Active gene promoters are characterized by the presence of tri-methylated lysines 4 and 36 at histone H3 (H3K4me3 and H3K36me3), and in the case of K36me3 also within the body of the gene that is associated with transcriptional elongation [19,24,25]. Although both enhancers and promoters are both decorated with H3K4me1, a distinguishing feature of active promoters is the low H3K4me1/me3 ratio [26]. In contrast, di- and tri-methylation of lysines 9 and 27 (H3K9me2/3 and H3K27me2/3) are representative of gene repression [27,28]. Presence of both H3K4me3 and H3K27me3 denote bivalent promoters, often found in the epigenomic landscapes of promoters of developmentally regulated genes, poised either for use under appropriate signals or for inactivation [29,30].

A distinguishing mark of promoters, especially of housekeeping genes, is the presence of CpG islands (CGIs) [31]. Whereas greater than two-thirds of the CpG (5'-Cytosine-P-Guanine-3' sites are methylated at the 5'-Cytosine (DNA methylation), the CGIs are usually hypomethylated and serve as binding sites for MLL (H3K4me3) and PRC2 (H3K27me3) complexes and bestow bivalency [32]. Methylated CGIs recruit the H3K9 methyltransferase, the modification that denotes repressed promoters and heterochromatin [32,33].

3.2. The (epigenomic) hallmarks of enhancers Fig. 2B

Primed enhancers are marked by high levels of H3K4me1/2. The mixed-lineage leukemia 4 (MLL4/KMT2D) factor is a H3K4 mono-/di-methyltransferase that primes enhancers with H3K4 mono/di-methylation (H3K4me1/2) serving as a hallmark for primed enhancers. MLL3/4 (redundancy between MLL 3 and 4) activity is essential for cell fate transition and for inducing cell identity-specific gene expression [34]. For example, transition of embryonic stem cells (ESCs) from self-renewal to differentiation requires MLL function, as does reprogramming fibroblasts to iPSCs and cell differentiation during adipogenesis and myogenesis [34]. Acetylation of histone H3K27 (H3K27ac) by the acetyltransferase p300/CBP activates the primed enhancer. As histone acetylation creates a transcriptionally permissive chromatin state p300/CBP occupancy on chromatin and presence of its target modification H3K27ac is often used as a surrogate for identifying active enhancers [35]. Enhancers, like promoters, also recruit transcription factors and RNA Polymerase II (RNA PolII). Moreover, like active promoters, active enhancers with RNA PolII also produce RNA transcripts called enhancer RNAs (eRNAs) [36]. Enhancers that are clustered in large domains comprised of multiple units or modules of enhancers are termed super-enhancers. Each module is often occupied by terminal transcription factors from Wnt (Tcf3), TGFb (Smads), LIF (Stats) and Notch (Rbpj) signaling pathways [37]. The independent modules serve as signaling platforms that communicate with each other to regulate stimulus-driven gene expression. Super-enhancers typically drive cell-type-specific gene expression during development or in tumorigenesis [19,37].

Instead of acetylation of H3K27 at a primed enhancer, tri-methylation of H3K27 (H3K27me3) by the Polycomb complex (PRC2) is observed at enhancers of poised (H3K27me3/K4me1) and repressed (H3K27me3) genes. The two mutually exclusive modifications at H3K27, viz. acetylation vs methylation, identify active versus poised/inactive enhancers, respectively. Poised enhancers are typically associated with bivalent

promoters that contain both H3K27me3 and H3K4me3 modifications. The K4me3 modification in the absence of K27me3 at histone H3 is found at active promoters [19]. A recent study by Soldi et al combined ChIP-Seq with mass spectrometry for comprehensive identification of enhancer-specific histone marks in activated macrophages [38]. Integrating proteomics with histone ChIP-Seq and transcriptional data revealed distinct combinatorial codes of transcribed enhancers (H3K4me1/K36me2) and intronic enhancers (H3K4me1/K36me3 and H3K4me1/K79me2), with the former combination tagging particularly super-enhancers. Figs. 3 and 4 should be cited elsewhere in the text. See below

3.3. Dynamic control of the enhancer landscape drives cell fate decisions

Cells at a particular functional state possess a defined set of cis-regulatory elements that are accessible to transcription factors, which defines the chromatin regulatory network of the cell state. Genomewide maps of nucleosome-free regions (NFR), i.e., open (accessible) chromatin domains, representing promoters and enhancers [6] established that NFR are progressively established during preimplantation development from the 2-cell to the Morula stage. Moreover, distal NFR appear to be more dynamic compared to proximal sites suggesting that enhancers are more likely to undergo chromatin remodeling during early mouse development. Notably, functional annotation revealed that the NFR landscape is closely linked to intracellular functions of preimplantation embryos. Specifically, the density and abundance of NFR correlated with the state of transcription with actively transcribed genes displaying wide-open enhancers while silent “primed” genes that are poised for activation had smaller enhancer chromatin peaks. Analysis of transcription factor motif enrichment in NFR footprints and gene knockdown revealed that two cell type-specific transcription factors contribute to NFR establishment during mouse preimplantation [6]. Similar studies conducted during T-cell development [39] also identified the gene regulatory networks shaping transcription during cell-type specific differentiation. There is currently no knowledge of the enhancer landscape dynamics of nephron progenitor cells or their progeny.

4. Methods to study epigenomic and epigenetic states (Fig. 3)

4.1. Chromatin landscapes: direct vs indirect chromatin accessibility assays

4.1.1. Direct assays: ATAC vs DNase1 vs Faire—Enhancers and promoters are similar in architecture demonstrating reduced nucleosome density and increased DNase1 hypersensitivity. Cis-regulatory elements in active or poised enhancers and promoters are bound by regulatory factors instead of nucleosomes. The modified chromatin states with decreased/absent nucleosomes are readily accessible to nucleases and have classically been identified upon deep sequencing as DNase I hypersensitive sites (DHS, DNase-Seq) [40]. DHSs are flanked by nucleosomes, with histone modification patterns reflective of the genes' expression status, i.e. active or bivalent. Thus DNase I hypersensitivity offers a sensitive readout of open (active) versus closed (repressed) chromatin landscape on a global scale that accompanies lineage commitment, or differentiation or diseased states. The limitation to this technique is the requirement for large cell number, typically in the tens of millions [41]. A relatively new technique Assay for Transposase Accessible Chromatin (ATAC) -Seq, maps genome wide chromatin accessibility or open chromatin landscape similar to profiles generated by DNase-Seq [42]. Using a hyperactive transposase, this

method inserts sequencing adapters, or tags, preferentially into accessible or open chromatin regions. As the accessible regions will be amplified, open chromatin maps can be obtained on as few as 500 cells using ATAC-Seq, orders of magnitude lower than the number required for DNase-Seq [41]. Sequencing and mapping the reads will reveal regions of increased accessibility that are represented as peaks (Fig. 2), identifying active promoters and enhancers by peak location with respect to the transcription start site. It is important to note that small peaks of accessible chromatin can also mark poised or primed enhancers. Another protocol that identifies open chromatin regions is Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) analysis coupled with deep sequencing (FAIRE-Seq) [41,43]. The advantage of FAIRE over DNase-Seq, MNase-Seq (below) and ATAC-Seq is its independence from enzymatic cleavage efficiency. However, while both DNase-Seq and ATAC-Seq generate TF footprint data, FAIRE-Seq does not. Additionally, the low background and sharp peaks obtained with ATAC-Seq generate a higher signal-to-noise ratio than obtained with FAIRE-Seq has resulted in the emergence of ATAC-Seq replacing DNase-Seq as the gold standard for probing chromatin accessibility.

4.1.2. Indirect assays: MNase-Seq—Whereas the assays described above directly isolate open chromatin regions, micrococcal nuclease (MNase)-Seq evaluates chromatin accessibility indirectly. MNase digests accessible chromatin in formaldehyde cross-linked chromatin, leaving behind mononucleosomes that are subsequently deproteinized, DNA sequenced and mapped to the genome. The location of open chromatin regions are inferred by absence of nucleosomal peaks [41].

4.2. Chromatin occupancy and histone modifications: ChIP-Seq

The chromatin immunoprecipitation assay is used to study what is bound to chromatin, such as transcription factors and co-activators/repressors, histones and histone modifications. The four histone proteins in the core nucleosome can be post-translationally altered by at least 80 known covalent modifications that modulate gene activity [41,44]. Transcription factor binding is generally the initiating step that recruits the histone modification enzymes, characterized as co-activators or co-repressors of gene expression as they do not bind DNA. The general principle is to use a crosslinking agent such as formaldehyde to fix the interaction between DNA-protein complexes, fragment the cross-linked chromatin to 200–600bp fragments and IP the DNA-protein complex with a specific antibody directed against the protein of interest. After crosslink reversal the co-IP'd DNA fragments are purified, sequenced and mapped to the genome to locate the site of interaction relative to a gene's transcription start site. Efficient fragmentation and high quality antibodies are key steps for accurate and reproducible ChIP assays, along with the inclusion of a non-specific IgG from the same host species as a negative control [44]. Under appropriate cross-linking conditions the presence/occupancy of modifying enzymes (writers and/or erasers) and interacting proteins that facilitate modifications at enhancers and promoters can be reliably assessed. Improved sonication, IP and DNA purification methods have evolved that minimize sample loss, resulting in the technique being adapted to smaller sample size.

4.3 Single cell/single locus histone modifications: ISH-PLA

ChIP analysis provides genome-wide and locus-specific temporal factor occupancy and modification profiles. However, spatial resolution is not preserved since the assay is not performed in situ. To overcome this limitation of ChIP, the Owens' lab devised a method to visualize histone modifications at single genomic loci at single cell resolution in situ, in formaldehyde-fixed paraffin embedded tissue sections [45]. This single cell ChIP assay combines in situ hybridization and proximity ligation assays (ISH-PLA). First, the gene of interest is hybridized with a biotin-tagged probe. Next, antibodies against a histone modification and against biotin are applied. The antibodies are tagged with overlapping complementary single strand of DNA. If the antibodies are in close enough proximity the overlapping ends of DNA can be ligated, and PCR amplified with a fluorescent probe. In the absence of ligation (if the two antibodies are not in proximity) PCR amplification will not occur and fluorescence is not observed [45].

In summary, numerous techniques are available to query the epigenome and identify epigenetic changes at specific loci. As each technique has advantages and limitations, most researchers utilize multiple assays to generate a more complete map of epigenomic and epigenetic states. Development of single-cell techniques to study histone modifications, cytosine modification, DNA accessibility and chromatin landscapes and three dimensional chromatin organization between enhancers and promoters offer critical tools to study epigenetic heterogeneity that is key to understanding cell fate decisions and disease [46].

5. Epigenomic states of the nephric lineage

Although the transcriptional specification of the nephric lineage from the intermediate mesoderm has been studied extensively (described above), the role of epigenome in these early fate decisions remains unknown. Initiation of a developmental program by a master regulator requires the factor to recognize and bind sequence specifically to regulate target gene expression. The conundrum of how a transcription factor binds closed chromatin was answered with the discovery of pioneer factors. The 'pioneer' transcription factor has the ability to bind DNA that is inaccessible in nucleosomes, or closed and DNase I resistant [47]. Pioneer factor recruits chromatin modifiers such as MLL3/4 to elicit chromatin opening to allow additional factors to bind and activate gene expression. In doing so, pTFs initiate lineage commitment and enable changes in cell-fate. The indispensable element of pioneer activity was demonstrated to be the inherent ability of the factor's DNA binding domain (DBD) to recognize and access silent chromatin. For example, the DBD of the paradigm pTF FoxA resembles linker histone which facilitates its binding to partial DNA motifs displayed on nucleosome surface [48]. Alternatively, binding to naive (unmodified) chromatin may involve coordinate binding between factors as was demonstrated between Oct4 and Sox2 during reprogramming of fibroblasts to iPSCs [48].

Although a bona fide pioneer factor has not been identified for the nephrogenic lineage, based on available data Osr1 and Pax2 are attractive candidates. Osr1 is the earliest expressed factor that marks the IM at E7.5, and absence of Osr1 results in renal agenesis [49]. Interestingly, Osr1 was recently shown to interact with the histone variant H2A.Z which is specifically found in nucleosomes associated with transcriptional regulatory regions

like enhancers and promoters [50]. While core nucleosomes in inaccessible chromatin are comprised of dimers of histones H2A, H2B, H3 and H4, accessible chromatin contains H2A.Z in place of H2A. Osr1 can also mediate incorporation of H2A.Z in nucleosomes at target enhancers [50]. The Pax transcription factors demonstrate variability in their DNA binding patterns and recruit chromatin remodelers to establish activating marks and open chromatin, recently demonstrated by Pax7 [51]. Identification of functional Pax2 binding sites, or Pax2 occupancy determined by ChIP-Seq as a part of a complex, at or around open enhancers of nephric lineage genes prior to their induction would offer insight to Pax2's role in establishing the IM, and in lineage specification (see below).

Lineage commitment of pluripotent cells is contingent on dynamic changes that occur in the transcriptome mediated by cis-elements and trans-factor regulators of key genes. As mentioned above, resolution of modifications at bivalent (H3K4me3/K27me3) genes will occur to either allow gene expression or not (Fig. 4). Several essential transcription factors with lineage-specific expression patterns are described in the IM, such as Pax2, Six2, Sall1, WT1, Osr1 and Hox11 [10]. Characteristically the TFs bind to enhancers to modulate lineage-specific expression. Both activating and repressive epigenetic regulators are critical to restrict cell fate (Polycomb and Trithorax). The precise mechanisms of how these TFs recruit chromatin modifying enzymes to establish and maintain lineage commitment are emerging. Role of Pax2, which is essential to specify the renal epithelial lineage from the IM, is best characterized. DNA-bound Pax2 recruits the transcriptional co-activator Pax Transactivation Domain Interacting Protein (PTIP) [52]. Interaction of PTIP with the histone methyltransferase complex MLL3/4 (mixed lineage leukemia 3/4, Trithorax-like protein complex) facilitates acquisition of H3K4 mono-, di- and tri-methylation, hallmarks of active/primed enhancers and promoters. On the other hand, Pax2 interactions with Grg4/Tle4 recruit Polycomb complexes to establish repressive modifications [53]. WT1 is another key TF required at multiple stages of kidney development. Its requirement in regulating expression of the de novo DNA methyltransferase 3A (DNMT3a) was shown in loss- and gain-of-function studies demonstrating altered levels of specific promoter methylation and consequently gene expression of critical modulators of cell fate [54]. How Osr1, Hox11 and Lhx1 regulate the epigenome of the IM remains to be elucidated.

6. Signal-dependent transcription factors (SD-TF) enable distribution of epigenetic marks in nephrogenic vs differentiation zones

The heterogeneous population of the cap mesenchyme (CM) consists of Six2⁺ nephron progenitor cells (NPCs) that are behaviorally and functionally different. The self-renewing Cited1⁺/Six2^{high}/β-catenin^{low}/BMP^{MAPK-responsive} subpopulation requires Wnt/β-catenin signaling for renewal [55–57], whereas the transit Cited1⁻/Six2^{high}/Wnt4⁻/BMP^{Smad-responsive} subpopulation is highly responsive to β-catenin-mediated differentiation [55,57]. Reduction in Six2 levels and activation of Wnt4 expression (Cited1⁻/Six2^{low}/β-catenin^{high}/Wnt4⁺) permits differentiation of the progenitors to pretubular aggregates that will undergo mesenchyme-to-epithelial transition to nascent nephrons. The immature structures undergo lumen formation, elongation, polarization, segmentation, and function acquisition. The ability of the NPCs to rapidly respond to the dynamic niche by rapidly

restricting expression of progenitor genes while turning on differentiation genes points to an exquisitely poised system that is able to rapidly change the transcription profile and thus the fate of the cell. Indeed, as posited by El-Dahr [23], and also by Surendran and Kopan [58], epigenetic mechanisms have been implicated in cell fate decisions of cap mesenchyme cells.

A spatial examination of H3K methyl marks and expression of their respective histone methyltransferases (HMTs) was undertaken in E15.5 mouse kidneys by McLaughlin et al [59]. This study expounds the global epigenetic states of this lineage-committed progenitor population. The data show that the chromatin marks associated with transcriptional repression, viz., H3K9me2 and H3K27me3, are more enriched in Six2⁺ progenitors than nascent nephrons. These modifications correlate with the differential expression of the respective histone modifiers, G9a and Ezh2, in these two compartments. In comparison to the progenitor population in the CM, the differentiating cells in nascent nephrons showed high H3K4me3 and those in renal epithelia acquire high levels of H3K79me2. Interestingly, while the global levels of most histone H3 marks remain stable during nephrogenesis, H3K79me2/3 and its writer Dot1l showed remarkable upregulation during terminal epithelial differentiation [59]. These data suggest that nephrogenesis is accompanied by dynamic stage-specific changes in the histone profile of the target cells. It is anticipated that a critical number of the histone modification changes are specific to nephrogenic genes, and will need to be confirmed genetically by stage-specific ablation of HMTs in mice.

As discussed above, another important regulator of chromatin accessibility is histone acetylation mediated by histone acetyltransferases (HATs) and deacetylation by histone deacetylases (HDACs) that are recruited to enhancers/promoters by TFs. Expression of HDAC 1–4, 7 and 9 is developmentally regulated, high prenatally and declining postnatally [60]. These HDACs are highly enriched in the mesenchymal and epithelial lineages, in the renewing and differentiating nephron progenitor cells, the ureteric tree and the stroma. HDAC3 is also present in podocytes, and HDAC 7–9 are expressed in the renal vasculature. Pharmacological or genetic disruption of acetylation regulators severely impairs key signaling pathways in kidney development, including Wnt/ β -catenin, PI3/Akt and TGF β /Smad [60]. Moreover, Osr1, Eya1, Pax2/8, WT1Lhx1, Wnt4 and FoxD1 among others are targets of HDAC regulation. Thus, conditional deletion of HDAC1 and 2 from the Six2⁺ mesenchyme shows premature depletion of the cap mesenchyme from decreased proliferation, arrested differentiation at the RV stage resulting in severe nephron deficit [61]. Loss of Lhx1 expression and that of components from the Notch pathway in the HDAC1/2 deficient NPCs suggests HDACs may be involved in activating gene expression and signaling pathways, beyond its repressive role in transcription regulation.

7. Chromatin bivalency restricts differentiation cell fates

Studies in several experimental systems have demonstrated that bivalent chromatin marks “poised” developmental regulators throughout development. In the fly, the body segments are specified by the homeotic gene cluster whose regionalized expression is epigenetically regulated by two major complexes: Polycomb (PcG, repressive) and Trithorax (trxG, activating) [62]. The Polycomb complex methylates lysine 27 on histone H3, while the Trithorax complex methylates lysine 4 on histone H3. Thus, lineage commitment and the

acquisition of positional identity are driven by epigenetic modifications that regulate chromatin structure, DNA accessibility, and ultimately gene expression at developmental loci [63]. In the pluripotent state, the promoters of lineage-specific genes carry both H3K4me3 (activating) and H3K27me3 (repressive) modifications. The “bivalent” chromatin state is believed to keep the genes transcriptionally silent yet poised for expression in response to the appropriate differentiation cues. Bivalent promoters, although more abundant in stem cells, are also observed in lineage-restricted multipotent cells and more differentiated cell types. Interference with Polycomb-Repressive Complex 2 (PRC2)-mediated H3K27 methylation via deletion of one or more of its components in ESCs has minimal effects on self-renewal capacity but greatly impairs the differentiation potential and lineage-restriction [64]. In mice, PRC2 inactivation in the germ layers disrupts endoderm fate choice into pancreatic and hepatic lineages [65], whereas PRC2 inactivation in the limb bud and retina halts differentiation into mature cell types [66–69]. Thus, interference with chromatin bivalency has wide-ranging developmental effects that are not limited to ESCs but also encompass organ progenitors.

Initial attempts into defining the promoters’ histone codes in metanephric cells were done using whole-genome Chip-seq and Chip-qPCR comparing mK3 and mK4 cell lines [70]. mK3 cells represent the self-renewing $\text{Six2}^{\text{high}}/\text{Wnt}^{\text{low}}$ NPC and mK4 cells represent early differentiating $\text{Six2}^{\text{low}}/\text{Wnt}^{\text{high}}$ NPC. In response to activation of canonical Wnt signaling, MK3 cells show a loss of repressive histone marks, H3K9me2 and H3K27me3, and the retention of the activation mark H3K4me3, on the promoters of nephrogenic genes (e.g., Pax2, Pax8, Lhx1, Jag1, and Lef1). These differentiation genes harbor a bivalent chromatin code: broad H3K27me3-enriched domains together with several peaks of H3K4me3 positioned around the transcriptional start site. Accordingly, these Wnt-responsive genes gain β -catenin/H3K4me3 and lose Ezh2/H3K27me3 at the TCF/LEF binding sites. Indeed, the requirement for PRC2 complex activity to maintain the NPCs in progenitor state and allow proper nephron differentiation was recently demonstrated genetically in a conditional mouse model lacking PRC2 activity in the NPC [71]. It remains to be determined if similar chromatin modifications occur in native NPC during Wnt-induced differentiation in vivo.

8. Future perspectives

In spite of significant strides in our understanding of the epigenetic regulation of kidney development, much remains to be learned. For example, the epigenomic landscape of the intermediate mesoderm during the patterning of the Wolffian duct and metanephric mesenchyme is unknown. And, along the same line, the chromatin landscapes of iPSC-derived ureteric bud and nephron progenitors have not defined. Equally interesting will be a more detailed view of chromatin states in native and cultured (expanded) nephron progenitors - does propagation/passaging alter the dynamic state of developmental enhancers? Finally, will epigenome editing become a reliable tool to manipulate enhancer turnover and affect developmental fates?

Acknowledgments

We thank the Tulane Hypertension and Renal Centers of Excellence Molecular Core Service. This work was partially funded by grants from National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health (RO1DK114050 to SED, R56DK104779 to ZS).

References

- [1]. Biechele S, Lin C-J, Rinaudo PF, Ramalho-Santos M, Unwind and transcribe: chromatin reprogramming in the early mammalian embryo, *Curr. Opin. Genet. Dev* 34 (2015) 17–23, 10.1016/j.gde.2015.06.003PMC4674342. [PubMed: 26183187]
- [2]. Zhou LQ, Dean J, Reprogramming the genome to totipotency in mouse embryos, *Trends Cell Biol.* 25 (2) (2015) 82–91, 10.1016/j.tcb.2014.09.006PMC4312727. [PubMed: 25448353]
- [3]. Du Z, Zheng H, Huang B, Ma R, Wu J, Zhang X, He J, Xiang Y, Wang Q, Li Y, Ma J, Zhang X, Zhang K, Wang Y, Zhang MQ, Gao J, Dixon JR, Wang X, Zeng J, Xie W, Allelic reprogramming of 3D chromatin architecture during early mammalian development, *Nature* 547 (7662) (2017) 232–235, 10.1038/nature23263. [PubMed: 28703188]
- [4]. Jukam D, Shariati SAM, Skotheim JM, Zygotic genome activation in vertebrates, *Dev. Cell* 42 (4) (2017) 316–332, 10.1016/j.devcel.2017.07.026PMC5714289. [PubMed: 28829942]
- [5]. Burton A, Torres-Padilla M-E, Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis, *Nat. Rev. Mol. Cell Biol* 15 (723) (2014), <https://doi.org/10.1038/nrm3885><https://www.nature.com/articles/nrm3885#supplementary-information><https://www.nature.com/articles/nrm3885#supplementary-information>.
- [6]. Lu F, Liu Y, Inoue A, Suzuki T, Zhao K, Zhang Y, Establishing chromatin regulatory landscape during mouse preimplantation development, *Cell* 165 (6) (2016) 1375–1388, 10.1016/j.cell.2016.05.050. [PubMed: 27259149]
- [7]. Dang-Nguyen TQ, Torres-Padilla M-E, How cells build totipotency and pluripotency: nuclear, chromatin and transcriptional architecture, *Curr. Opin. Cell Biol* 34 (2015) 9–15, 10.1016/j.ceb.2015.04.006. [PubMed: 25935759]
- [8]. Costantini F, Kopan R, Patterning a complex organ: branching morphogenesis and nephron segmentation in kidney development, *Dev. Cell* 18 (5) (2010) 698–712, 10.1016/j.devcel.2010.04.008PMC2883254. [PubMed: 20493806]
- [9]. Dressier GR, Patterning and early cell lineage decisions in the developing kidney: the role of Pax genes, *Pediatr. Nephrol* 26 (9) (2011) 1387–1394, 10.1007/S00467-010-1749-x. [PubMed: 21221999]
- [10]. Little MH, McMahon AP, Mammalian kidney development: principles, progress, and projections, *Cold Spring Harb. Perspect. Biol* 4 (5) (2012), 10.1101/cshperspect.a008300.
- [11]. McMahon AP, Development of the mammalian kidney, *Curr. Top. Dev. Biol* 117 (2016) 31–64, 10.1016/bs.ctdb.2015.10.010PMC5007134. [PubMed: 26969971]
- [12]. Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiovaara K, Suvanto P, Smith D, Ponder B, Costantini F, Saarma M, et al., GDNF signalling through the Ret receptor tyrosine kinase, *Nature* 381 (6585) (1996) 789–793, 10.1038/381789a0. [PubMed: 8657282]
- [13]. Costantini F, Shakya R, GDNF/Ret signaling and the development of the kidney, *Bioessays* 28 (2) (2006) 117–127, 10.1002/bies.20357. [PubMed: 16435290]
- [14]. Shakya R, Watanabe T, Costantini F, The role of GDNF/Ret signaling in ureteric bud cell fate and branching morphogenesis, *Dev. Cell* 8 (1) (2005) 65–74, 10.1016/j.devcel.2004.11.008. [PubMed: 15621530]
- [15]. Michos O, Cebrian C, Hyink D, Grieshammer U, Williams L, D'Agati V, Licht JD, Martin GR, Costantini F, Kidney development in the absence of Gdnf and Spry1 requires Fgf10, *PLoS Genet.* 6 (1) (2010) e1000809, 10.1371/journal.pgen.1000809PMC2797609. [PubMed: 20084103]
- [16]. Michos O, Goncalves A, Lopez-Rios J, Tiecke E, Naillat F, Beier K, Galli A, Vainio S, Zeller R, Reduction of BMP4 activity by gremlin 1 enables ureteric bud outgrowth and GDNF/WNT11 feedback signalling during kidney branching morphogenesis, *Development* 134 (13) (2007) 2397–2405, 10.1242/dev.02861. [PubMed: 17522159]

- [17]. Basson MA, Akbulut S, Watson-Johnson J, Simon R, Carroll TJ, Shakya R, Gross I, Martin GR, Lufkin T, McMahon AP, Wilson PD, Costantini FD, Mason IJ, Licht JD, Sproutyl is a critical regulator of GDNF/RET-mediated kidney induction, *Dev. Cell* 8 (2) (2005) 229–239, 10.1016/j.devcel.2004.12.004. [PubMed: 15691764]
- [18]. Kuure S, Chi X, Lu B, Costantini F, The transcription factors Etv4 and Etv5 mediate formation of the ureteric bud tip domain during kidney development, *Development* 137 (12) (2010) 1975–1979, 10.1242/dev.051656PMC2875840. [PubMed: 20463033]
- [19]. Kim T-K, Shiekhatter R, Architectural and functional commonalities between enhancers and promoters, *Cell* 162 (5) (2015) 948–959, 10.1016/j.cell.2015.08.008. [PubMed: 26317464]
- [20]. Zacher B, Michel M, Schwalb B, Cramer P, Tresch A, Gagneur J, Accurate Promoter and Enhancer Identification in 127 ENCODE and Roadmap Epigenomics Cell Types and Tissues by GenoSTAN, *PLOS One* 12 (1) (2017) e0169249, 10.1371/journal.pone.0169249. [PubMed: 28056037]
- [21]. The EPC, An integrated encyclopedia of DNA elements in the human genome, *Nature* 489 (57) (2012), <https://doi.org/10.1038/nature11247><https://www.nature.com/articles/nature11247#supplementary-information><https://www.nature.com/articles/nature11247#supplementary-information>.
- [22]. Soshnev AA, Josefowicz SZ, Allis CD, Greater than the sum of parts: complexity of the dynamic epigenome, *Mol. Cell* 62 (5) (2016) 681–694, 10.1016/j.molcel.2016.05.004PMC4898265. [PubMed: 27259201]
- [23]. Adli M, Parlak M, Li Y, El-Dahr SS, Epigenetic states of nephron progenitors and epithelial differentiation, *J. Cell. Biochem* 116 (6) (2015) 893–902, 10.1002/jcb.25048PMC4511265. [PubMed: 25560433]
- [24]. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NCT, Schreiber SL, Mellor J, Kouzarides T, Active genes are tri-methylated at K4 of histone H3, *Nature* 419 (6905) (2002) 407–411, 10.1038/nature01080. [PubMed: 12353038]
- [25]. Bannister AJ, Schneider R, Myers FA, Thorne AW, Crane-Robinson C, Kouzarides T, Spatial distribution of di- and tri-methyl lysine 36 of histone H3 at active genes, *J. Biol. Chem* 280 (18) (2005) 17732–17736, 10.1074/jbc.M500796200. [PubMed: 15760899]
- [26]. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Barrera LO, Van Calcar S, Qu C, Ching KA, Wang W, Weng Z, Green RD, Crawford GE, Ren B, Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome, *Nat. Genet* 39 (3) (2007) 311–318, 10.1038/ng1966. [PubMed: 17277777]
- [27]. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI, Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly, *Science* 292 (5514) (2001) 110–113, 10.1126/science.1060118. [PubMed: 11283354]
- [28]. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y, Role of histone H3 lysine 27 methylation in Polycomb-group silencing, *Science* 298 (5595) (2002) 1039–1043, 10.1126/science.1076997. [PubMed: 12351676]
- [29]. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES, A bivalent chromatin structure marks key developmental genes in embryonic stem cells, *Cell* 125 (2) (2006) 315–326, 10.1016/j.cell.2006.02.041. [PubMed: 16630819]
- [30]. Azuara V, Perry P, Sauer S, Spivakov M, Jorgensen HF, John RM, Gouti M, Casanova M, Warnes G, Merkenschlager M, Fisher AG, Chromatin signatures of pluripotent cell lines, *Nat. Cell Biol* 8 (5) (2006) 532–538, 10.1038/ncb1403. [PubMed: 16570078]
- [31]. Deaton AM, Bird A, CpG islands and the regulation of transcription, *Genes Dev.* 25 (10) (2011) 1010–1022, 10.1101/gad.2037511. [PubMed: 21576262]
- [32]. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE, Genome-wide maps of chromatin state in pluripotent and lineage-committed cells, *Nature* 448 (7153) (2007) 553–560, 10.1038/nature06008PMC2921165. [PubMed: 17603471]

- [33]. Rose NR, Klose RJ, Understanding the relationship between DNA methylation and histone lysine methylation, *Biochim. Biophys. Acta* 1839 (12) (2014) 1362–1372, 10.1016/j.bbarm.2014.02.007. [PubMed: 24560929]
- [34]. Wang C, Lee J-E, Lai B, Macfarlan TS, Xu S, Zhuang L, Liu C, Peng W, Ge K, Enhancer priming by H3K4 methyltransferase MLL4 controls cell fate transition, *Proc. Natl. Acad. Sci* 113 (42) (2016) 11871–11876, 10.1073/pnas.1606857113. [PubMed: 27698142]
- [35]. Plank Jennifer L., Dean A, Enhancer function: mechanistic and genome-wide insights come together, *Mol. Cell* 55 (1) (2014) 5–14, 10.1016/j.molcel.2014.06.015. [PubMed: 24996062]
- [36]. Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, Markenscoff-Papadimitriou E, Kuhl D, Bito H, Worley PF, Kreiman G, Greenberg ME, Widespread transcription at neuronal activity-regulated enhancers, *Nature* 465 (7295) (2010), 10.1038/nature09033182-U65.
- [37]. Hnisz D, Abraham Brian J., Lee Tong I., Lau A, Saint-André V, Sigova Alla A., Hoke Heather A., Young Richard A., Super-enhancers in the control of cell identity and disease, *Cell* 155 (4) (2013) 934–947, 10.1016/j.cell.2013.09.053. [PubMed: 24119843]
- [38]. Soldi M, Mari T, Nicosia L, Musiani D, Sigismondo G, Cuomo A, Pavesi G, Bonaldi T, Chromatin proteomics reveals novel combinatorial histone modification signatures that mark distinct subpopulations of macrophage enhancers, *Nucleic Acids Res.* 45 (21) (2017) 12195–12213, 10.1093/nar/gkx821. [PubMed: 28981749]
- [39]. Huang J, Liu X, Li D, Shao Z, Cao H, Zhang Y, Trompouki E, Bowman TV, Zon LI, Yuan GC, Orkin SH, Xu J, Dynamic control of enhancer repertoires drives lineage and stage-specific transcription during hematopoiesis, *Dev. Cell* 36 (1) (2016) 9–23, 10.1016/j.devcel.2015.12.014PMC4714361. [PubMed: 26766440]
- [40]. Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, Sheffield NC, Stergachis AB, Wang H, Vernot B, Garg K, John S, Sandstrom R, Bates D, Boatman L, Canfield TK, Diegel M, Dunn D, Ebersol AK, Frum T, Giste E, Johnson AK, Johnson EM, Kutayin T, Lajoie B, Lee B-K, Lee K, London D, Lotakis D, Neph S, Neri F, Nguyen ED, Qu H, Reynolds AP, Roach V, Safi A, Sanchez ME, Sanyal A, Shafer A, Simon JM, Song L, Vong S, Weaver M, Yan Y, Zhang Z, Zhang Z, Lenhard B, Tewari M, Dorschner MO, Hansen RS, Navas PA, Stamatoiyannopoulos G, Iyer VR, Lieb JD, Sunyaev SR, Akey JM, Sabo PJ, Kaul R, Furey TS, Dekker J, Crawford GE, Stamatoiyannopoulos JA, The accessible chromatin landscape of the human genome, *Nature* 489 (75) (2012), <https://doi.org/10.1038/nature11232> . <https://doi.org/10.1038/nature11232https://www.nature.com/articles/nature11232#supplementary-information>. <https://www.nature.com/articles/nature11232#supplementary-information>.
- [41]. Tsompana M, Buck MJ, Chromatin accessibility: a window into the genome, *Epigenetics Chromatin* 7 (1) (2014) 33, 10.1186/1756-8935-7-33. [PubMed: 25473421]
- [42]. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ, Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position, *Nat. Methods* 10 (2013) 1213, <https://doi.org/10.1038/nmeth.2688> . <https://doi.org/10.1038/nmeth.2688https://www.nature.com/articles/nmeth2688#supplementary-information>. <https://www.nature.com/articles/nmeth2688#supplementary-information>. [PubMed: 24097267]
- [43]. Simon JM, Giresi PG, Davis IJ, Lieb JD, Using formaldehyde-assisted isolation of regulatory elements (FAIRE) to isolate active regulatory DNA, *Nat. Protoc.* 7 (256) (2012), 10.1038/nprot.2011.444.
- [44]. Furey TS, ChIP-seq and beyond: new and improved methodologies to detect and characterize protein-DNA interactions, *Nat. Rev. Genet* 13 (840) (2012), 10.1038/nrg3306.
- [45]. Gomez D, Shankman LL, Nguyen AT, Owens GK, Detection of histone modifications at specific gene loci in single cells in histological sections, *Nat. Methods* 10 (2) (2013) 171–177, 10.1038/nmeth.2332. [PubMed: 23314172]
- [46]. Clark SJ, Lee HJ, Smallwood SA, Kelsey G, Reik W, Single-cell epigenomics: powerful new methods for understanding gene regulation and cell identity, *Genome Biol.* 17 (1) (2016) 72, 10.1186/s13059-016-0944-x. [PubMed: 27091476]
- [47]. Iwafuchi-Doi M, Zaret KS, Cell fate control by pioneer transcription factors, *Development* 143 (11) (2016) 1833–1837, 10.1242/dev.133900. [PubMed: 27246709]

- [48]. Soufi A, Garcia Meilin F, Jaroszewicz A, Osman N, Pellegrini M, Zaret Kenneth S., Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate reprogramming, *Cell* 161 (3) (2015) 555–568, 10.1016/j.cell.2015.03.017. [PubMed: 25892221]
- [49]. James RG, Kamei CN, Wang Q, Jiang R, Schultheiss TM, Odd-skipped related 1 is required for development of the metanephric kidney and regulates formation and differentiation of kidney precursor cells, *Development* 133 (15) (2006) 2995–3004, 10.1242/dev.02442. [PubMed: 16790474]
- [50]. Zhang Y, Ku WL, Liu S, Cui K, Jin W, Tang Q, Lu W, Ni B, Zhao K, Genomewide identification of histone H2A and histone variant H2A.Z-interacting proteins by bPPI-seq, *Cell Res.* 27 (2017) 1258, <https://doi.org/10.1038/cr.2017.112><https://www.nature.com/articles/cr2017112#supplementary-information><https://www.nature.com/articles/cr2017112#supplementary-information>. [PubMed: 28862252]
- [51]. Mayran A, Pelletier A, Drouin J, Pax factors in transcription and epigenetic remodelling, *Semin. Cell Dev. Biol* 44 (2015) 135–144, 10.1016/j.semcdb.2015.07.007. [PubMed: 26234816]
- [52]. Patel SR, Kim D, Levitan I, Dressier GR, The BRCT-domain containing protein ptip links pax2 to a histone h3, lysine 4 methyltransferase complex, *Dev. Cell* 13 (4) (2007) 580–592, 10.1016/j.devcel.2007.09.004. [PubMed: 17925232]
- [53]. Abraham S, Paknikar R, Bhumbra S, Luan D, Garg R, Dressier GR, Patel SR, The Groucho-associated phosphatase ppmlb displaces pax transactivation domain interacting protein (ptip) to switch the transcription factor pax2 from a transcriptional activator to a repressor, *J. Biol. Chem.* 290 (11) (2015) 7185–7194, 10.1074/jbc.M114.607424PMC4358138. [PubMed: 25631048]
- [54]. Szemes M, Dallosso AR, Melegh Z, Curry T, Li Y, Rivers C, Uney J, Magdefrau A-S, Schwiderski K, Park JH, Brown KW, Shandilya J, Roberts SGE, Malik K, Control of epigenetic states by WT1 via regulation of de novo DNA methyltransferase 3A, *Hum. Mol. Genet* 22 (1) (2013) 74–83, 10.1093/hmg/dd5403. [PubMed: 23042785]
- [55]. Muthukrishnan SD, Yang X, Friesel R, Oxburgh L, Concurrent BMP7 and FGF9 signalling governs AP-1 function to promote self-renewal of nephron progenitor cells, *Nat. Commun* 6 (2015) 10027, 10.1038/ncomms10027PMC4686668. [PubMed: 26634297]
- [56]. Brown AC, Muthukrishnan SD, Oxburgh L, A synthetic niche for nephron progenitor cells, *Dev. Cell* 34 (2) (2015) 229–241, 10.1016/j.devcel.2015.06.021PMC4519427. [PubMed: 26190145]
- [57]. Karner CM, Das A, Ma Z, Self M, Chen C, Lum L, Oliver G, Carroll TJ, Canonical Wnt9b signaling balances progenitor cell expansion and differentiation during kidney development, *Development* 138 (7) (2011) 1247–1257, 10.1242/dev.057646PMC3050658. [PubMed: 21350016]
- [58]. Surendran K, Kopan R, Chromatin-based mechanisms of renal epithelial differentiation, *J. Am. Soc. Nephrol* 22 (7) (2011) 1208–1212, 10.1681/asn.2010101018. [PubMed: 21700830]
- [59]. McLaughlin N, Wang F, Saifudeen Z, El-Dahr SS, In situ histone landscape of nephrogenesis, *Epigenetics* 9 (2) (2014) 222–235, 10.4161/epi.26793. [PubMed: 24169366]
- [60]. Rosenberg SL, Chen S, McLaughlin N, El-Dahr SS, Regulation of kidney development by histone deacetylases, *Pediatr. Nephrol* 26 (9) (2011) 1445–1452, 10.1007/s00467-011-1796-y. [PubMed: 21336812]
- [61]. Liu H, Chen S, Yao X, Li Y, Chen CH, Liu J, Saifudeen Z, El-Dahr SS, Histone deacetylases 1 and 2 regulate the transcriptional programs of nephron progenitors and renal vesicles, *Development* 145 (10) (2018), 10.1242/dev.153619PMC6001373.
- [62]. Geisler SJ, Paro R, Trithorax and Polycomb group-dependent regulation: a tale of opposing activities, *Development* 142 (17) (2015) 2876–2887, 10.1242/dev.120030. [PubMed: 26329598]
- [63]. Aldiri I, Vetter ML, PRC2 during vertebrate organogenesis: a complex in transition, *Dev. Biol* 367 (2) (2012) 91–99, 10.1016/j.ydbio.2012.04.030PMC3374057. [PubMed: 22565092]
- [64]. Harikumar A, Meshorer E, Chromatin remodeling and bivalent histone modifications in embryonic stem cells, *EMBO Rep.* 16 (12) (2015) 1609–1619, 10.15252/embr.201541011PMC4693513. [PubMed: 26553936]
- [65]. 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, *Science* 332 (6032) (2011) 963–966, 10.1126/science.1202845PMC3128430. [PubMed: 21596989]

- [66]. Chen H, Gu X, Su IH, Bottino R, Contreras JL, Tarakhovsky A, Kim SK, Polycomb protein Ezh2 regulates pancreatic beta-cell Ink4a/Arf expression and regeneration in diabetes mellitus, *Genes Dev.* 23 (8) (2009) 975–985, 10.1101/gad.1742509PMC2675862. [PubMed: 19390090]
- [67]. Juan AH, Derfoul A, Feng X, Ryall JG, DellOrso S, Pasut A, Zare H, Simone JM, Rudnicki MA, Sartorelli V, Polycomb EZH2 controls self-renewal and safeguards the transcriptional identity of skeletal muscle stem cells, *Genes Dev.* 25 (8) (2011) 789–794, 10.1101/gad.2027911PMC3078704. [PubMed: 21498568]
- [68]. Wyngaarden LA, Delgado-Olguin P, Su IH, Bruneau BG, Hopyan S, Ezh2 regulates anteroposterior axis specification and proximodistal axis elongation in the developing limb, *Development* 138 (17) (2011) 3759–3767, 10.1242/dev.063180. [PubMed: 21795281]
- [69]. Gibson WT, Hood RL, Zhan SH, Bulman DE, Fejes AP, Moore R, Mungall AJ, Eydoux P, Babul-Hirji R, An J, Marra MA, Consortium FC, Chitayat D, Boycott KM, Weaver DD, Jones SJ, Mutations in EZH2 cause Weaver syndrome, *Am. J. Hum. Genet* 90 (1) (2012) 110–118, 10.1016/j.ajhg.2011.11.018PMC3257956. [PubMed: 22177091]
- [70]. McLaughlin N, Yao X, Li Y, Saifudeen Z, El-Dahr SS, Histone signature of metanephric mesenchyme cell lines, *Epigenetics* 8 (9) (2013) 970–978, 10.4161/epi.25753. [PubMed: 23867747]
- [71]. Zhang L, Ettou S, Khalid M, Taglienti M, Jain D, Jung YL, Seager C, Liu Y, Ng KH, Park PJ, Kreidberg JA, Eed, a member of the Polycomb group, is required for nephron differentiation and the maintenance of nephron progenitor cells, *Development* 145 (2018), 10.1242/dev.157149dev157149.

Fig. 1.

Mammalian kidney development. The kidney originates from the *Osr1* + intermediate mesoderm (IM). The anterior IM gives rise to the Nephric Duct (pink) and the posterior IM to the metanephric mesenchyme (MM) (blue). Metanephric development begins with *Gdnf* secretion from the MM. The *Ret* + cells of the nephric duct respond to the signal with an outgrowth termed the ureteric bud (UB). The precise localization of the site of budding from the caudal end of the nephric duct requires the concerted action of growth factors (GDNF, Fgf, Bmp) and receptors (*Ret*, *Fgfr*, *Alk*) [14–16], signaling pathway modulators (*Spry1/2*) [17] and transcription factors (*Etv4/5*, *Smad*) [16,18]. The MM gives rise to the *Six2*+ nephron progenitor cells (NPC) and the *Foxd1* + stromal progenitor cells (SPC). The *Ret* + ureteric progenitor cells (UPC) are from the nephric duct lineage.

Fig. 2. Epigenomic states of promoters and enhancers. (A) A schematic depicting of histone H3 modifications associated with active or repressed promoters and enhancers as well as transcriptional elongation. (B) A schematic depicting the combinatorial H3 modifications characteristic of active enhancers (K27Ac/K4me1), poised enhancers (K4me1/K27me3), repressed enhancers (K27me3), active promoters (K4me3/K27Ac/K9Ac), poised promoters (K4me3/K27me3) and repressed promoters (K27me3/K9me2/3).

Method (Refs)	Basis	Resolution	Limitation
Epigenomic Profile			
DNase-Seq (21, 22)	Enzymatic cleavage of open, unprotected DNA (hypersensitive sites) by DNase endonuclease followed by NGS	Direct chromatin accessibility: paired- or single-end seq to map open chromatin genome wide	<ul style="list-style-type: none"> Cell number $>10^6$-10^7 Digestion optimization critical to obtain limited digestion of open chromatin regions Inherent sequence preferences can generate false signal of protected regions Transient TF footprints are missed
MNase-Seq (22)	Enzyme-based indirect evaluation of chromatin accessibility and nucleosome occupancy by MNase digestion followed by NGS	Paired- or single-end seq to generate genome wide map of nucleosome positioning and distribution; indirect assay of chromatin accessibility	<ul style="list-style-type: none"> Cell number $>10^6$-10^7 Enzyme vibrations a critical step to avoid A-T bias at low enzyme concentrations Extensive digestion and inclusion of digestion standards essential
Faire-Seq (22, 24)	Sheared formaldehyde x-linked chromatin subjected to phenol-chloroform (organic vs aqueous phase) separation of nucleosome-bound vs naked DNA	Direct chromatin accessibility: Maps open chromatin genome wide	<ul style="list-style-type: none"> Cell number $>10^3$-10^6 High background and low signal to noise ratio makes data interpretation difficult Results dependent on fixation efficiency
ATAC-Seq (22, 23)	Based on enzymatic ability of hyperactive Tn5 transposase to fragment and tag open chromatin regions with sequencing adapters	Direct chromatin landscape; currently the gold standard due to high resolution maps of open chromatin regions from low cell number (500-50K)	<ul style="list-style-type: none"> Limited ability to map nucleosomes
Epigenetic Profile			
ChIP-Seq (22, 25)	Co-immunoprecipitation of DNA fragment with antibody directed against a protein or histone modification associated with the fragment	Base pair resolution. In case of ChIP-PCR, <i>a priori</i> knowledge of binding sites necessary	<ul style="list-style-type: none"> Efficiency of chromatin immunoprecipitation is antibody dependent Low cell number protocols not streamlined, generally requires 10^6 cells per assay Efficient shearing to maintain inter-experiment reproducibility Lack of spatial resolution
Bisulfite-Seq (27)	Deamination of unmethylated cytosines to uracil by bisulfite treatment, resulting in conversion from C-G to T-A base pair upon PCR amplification	Currently the gold standard method for detecting cytosine methylation as it reveals modifications at single base resolution and is not dependent on antibody affinity	<ul style="list-style-type: none"> Difficult to discriminate between 5mC and 5-hydroxymethylcytosine (5hmC) Optimization of treatment (time, temperature, bisulfite concentration) necessary to minimize depurination and DNA degradation
ISH-PLA (26)	Combines in situ hybridization and proximity ligation assays	Single cell (spatial) and loci-specific resolution	<ul style="list-style-type: none"> Hybridization of probe must be optimized Highly antibody dependent

Fig. 3. Methods to study the epigenomic states and epigenetic modifications in chromatin. Comparison of resolution and limitations of the various methods that are currently in use to study histone modifications, cytosine modification, DNA accessibility and chromatin landscapes of enhancers and promoters.

Fig. 4.

Integrative Genome Viewer (IGV) tracks of ChIP-seq for Six2, H3K27ac, and H3K27me3 in mouse Cited1 + mouse nephron progenitor cells (Development 143: 595–608, 2016). Read alignment visualization using IGV allows integration of transcription factor (Six2) binding and histone modification (H3K27ac and H3K27me3) peaks.