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Regulation of α ENaC Transcription

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Abstract

Aldosterone is a major regulator of Na^+ absorption and acts primarily by controlling the epithelial Na^+ channel (ENaC) function at multiple levels including transcription. ENaC consists of α , β , and γ subunits. In the classical model, aldosterone enhances transcription primarily by activating mineralocorticoid receptor (MR). However, how aldosterone induces chromatin alternation and thus leads to gene activation or repression remains largely unknown. Emerging evidence suggests that Dot1a-Af9 complex plays an important role in repression of α ENaC by directly binding and modulating targeted histone H3 K79 hypermethylation at the specific subregions of α ENaC promoter. Aldosterone impairs Dot1a-Af9 formation by decreasing expression of Dot1a and Af9 and by inducing Sgk1, which, in turn, phosphorylates Af9 at S435 to weaken Dot1a-Af9 interaction. MR counterbalances Dot1a-Af9 action by competing with Dot1a for binding Af9. Af17 derepresses α ENaC by competitively interacting with Dot1a and facilitating Dot1a nuclear export. Consistently, $\text{MR}^{-/-}$ mice have impaired ENaC expression at day 5 after birth, which may contribute to progressive development of pseudohypoaldosteronism type 1 in a later stage. $\text{Af17}^{-/-}$ mice have decreased ENaC expression, renal Na^+ retention, and blood pressure. In contrast, Dot1^{AC} mice have increased α ENaC expression, despite a 20% reduction of the principal cells. This chapter reviews these findings linking aldosterone action to ENaC transcription through chromatin modification. Future direction toward the understanding the role of Dot1a-Af9 complex beyond ENaC regulation, in particular, in renal fibrosis is also briefly discussed.

1. Introduction

1.1 Aldosterone is a ligand for the mineralocorticoid receptor and glucocorticoid receptor

Members of steroid hormone family include aldosterone, glucocorticoid, estrogen, progesterone, and androgen. The hormones induce genomic and nongenomic effects. In the classical mode of action, steroid hormones diffuse into cytoplasm, where they bind their intracellular receptors. The hormone–receptor complexes then move into the nucleus, bind the hormone response elements (HREs), and stimulate or inhibit transcription of their target genes. This ligand-dependent modulation of transcription by the ligand–receptor complex has been termed a “genomic” effect and is sensitive to inhibitors of transcription

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(actinomycin D) and translation (cycloheximide). In addition to the classical mode of receptor action, steroid hormones might also act through alternative, “nongenomic” (also called nontranscriptional or nonclassical) pathways, particularly in cardiovascular systems. These actions are rapid (<1 min), independent of transcription and translation, and presumably mediated by distinct plasma membrane/cytosolic receptors that have not been identified yet (Losel, Feuring, & Wehling, 2002). Hence, this mode of hormone action is referred as nongenomic (Alangari, 2010).

Aldosterone is produced in the *zona glomerulosa* of the adrenal cortex. The hormone serves as a ligand for two distinct but similar types of nuclear receptors: mineralocorticoid receptor (MR) and glucocorticoid receptor (GR). Both MR and GR, like other members of the steroid hormone receptor family, act as ligand-inducible transcription factors. Upon binding their ligand aldosterone, the receptors activate or repress the transcription of target genes by directly binding as monomers, homodimers, or heterodimers to HREs and produce long-lasting physiological effects of aldosterone stimulation. HREs are normally presented in the 5'-flanking region of target genes.

Unique receptor complexes may target distinct *cis*-acting elements (Stockand, 2002). These modes of steroid hormone action involve the *trans*-activation and *trans*-repression via interaction with cognate DNA-binding sites, such as HRE. In contrast to direct *trans*-activation and repression, the steroid receptor may regulate gene expression by protein–protein interaction between the receptor and other *trans*-acting factors. In these cases, the receptor does not need to physically bind DNA (Stockand, 2002). Obviously, before interaction of the nuclear receptors with the cognate HRE, the ligand–receptor complex must have the accessibility to the DNA, which is compacted into the chromatin.

Interestingly, many coregulators of steroid hormone receptors are enzymes that have either histone-modifying activities or ATP-dependent chromatin remodeling activities to promote the accessibility of transcription factors to HRE (Narlikar, Fan, & Kingston, 2002; Westin, Rosenfeld, & Glass, 2000). For example, aldosterone induces the binding of RNA helicase A (RHA) to MR, then recruits a complex with histone acetyltransferase (HAT) activity that contains cAMP-response element-binding protein (CREB)-binding protein (CBP), leading to the cooperative potentiation of MR transcriptional activity by RHA and the HAT complex (Kitagawa et al., 2002).

Although many aldosterone up- or downregulated genes have been identified in different systems including the renal collecting duct (CD) (Kellner et al., 2003; Robert-Nicoud et al., 2001; Spindler & Verrey, 1999) and IMCD3 cells (Gumz, Popp, Wingo, & Cain, 2003), our understanding about the underlying molecular mechanisms by which aldosterone induces chromatin alternation and thus leads to gene activation or repression is hindered. No aldosterone-regulated genes had been identified to have a known function in histone modifications or chromatin remodeling until our finding of histone H3 K79 methyltransferase Dot1a as an integrate component of aldosterone-signaling network regulating *αENaC* transcription.

1.2 Epithelial sodium channel (ENaC) is a major target of aldosterone action and a key ion channel in regulating Na⁺ balance

The renin–angiotensin–aldosterone system plays a major role in the control of blood pressure, extracellular fluid volume, and electrolyte balance, largely through the regulation of urinary Na⁺ excretion. Perturbations in the normal or adaptive mechanisms controlling this system, which are frequently seen in clinical practice, can result in organ system dysfunction and even death. In the kidney, aldosterone-dependent regulation of ENaC takes place in the aldosterone-sensitive distal nephron (ASDN). ASDN comprises the late distal convoluted tubule (DCT2), connecting tubule (CNT), and CD. The latter includes the cortical CD (CCD) and the outer and inner medullary CD (OMCD and IMCD, respectively).

In the ASDN, transepithelial Na⁺ absorption occurs by apical Na⁺ entry via ENaC and basolateral Na⁺ exit via the Na⁺, K⁺ ATPase. ENaC, composed of three subunits α , β , and γ , constitutes the rate-limiting step in this process, and changes in the synthesis of the subunits, its open probability and/or plasma membrane abundance constitute key regulatory steps, and determine the rate of Na⁺ entry. Defects in ENaC subunits cause two human genetic diseases, Liddle's syndrome and the autosomal recessive form of pseudohypoaldosteronism type 1 (PHA-1). Liddle's syndrome is characterized by early onset of hypertension associated with normal plasma aldosterone levels and is caused by gain-of-function mutations in β ENaC or γ ENaC that produce inappropriately large Na⁺ absorption by CD. In contrast, PHA-1 is a salt-wasting syndrome with hypotension and high plasma aldosterone levels, resulting from loss-of-function mutations in any of the three subunits. Classical gene inactivation of all three genes encoding the ENaC subunits leads to perinatal-lethal phenotype, characterized by lung fluid-clearance failure, and/or by an acute PHA-1 with severe hyperkalemia and metabolic acidosis (reviewed in Hummler & Vallon, 2005).

2. Dot1a–AF9 Complex Mediates Repression of α ENaC

2.1 Histone H3 K79 methyltransferase Dot1a

2.1.1 Dot1 proteins are a unique class of histone methyltransferases—

Eukaryotic cells coil DNA around the histone octamer to form the basic unit, nucleosome, of the higher-order chromatin structures. Dynamic alternations in the chromatin structure control accessibility to regulatory factors and, thus, function critically in all DNA-based biological processes including replication, repair, recombination, and transcription. Among many mechanisms by which cells modulate their chromatin structure is the posttranslational covalent modifications of histones N-terminal tails, such as acetylation, methylation, phosphorylation, and ubiquitination. According to the histone code hypothesis (Strahl & Allis, 2000), many regulatory factors gain access to these exposed and unstructured histone tails and write/read the various modifications of specific amino acids or their combinations to control particular cellular processes. The effects of histone methylation on gene expression depend on the chromosomal location, the context of the targeted lysines and arginines, and the methyltransferase involved (Jenuwein & Allis, 2001).

Histone arginine methylation typically activates genes. In this scenario, histone methyltransferases (HMTs) are recruited as coactivators to the target promoters. One of such

examples is the coactivator-associated arginine methyltransferase, protein arginine methyltransferase (CARM1/PRMT1) family, which primarily targets histones H3 or H4 (Wang et al., 2001). In contrast, gene silencing has been attributed to the suppressor of variegation, enhancer of zeste, and trithorax (SET) domain-containing lysine HMT family. The Suvar39 enzyme represents the first identified member of this family. It methylates histone H3 K9 and serves as a corepressor (Rea et al., 2000). A third class of HMTs comprises disruptor of telomeric silencing (Dot1) family members (van Leeuwen, Gafken, & Gottschling, 2002). They do not contain a SET domain. Unlike the other two types of HMTs, which target the N-terminal tails of histones, Dot1 proteins methylate lysine residue (K79) in the globular domain of histone H3. This methylation *in vivo* strongly depends on the intact nucleosomal structure and Rad6-dependent ubiquitination of histone H2B at K123 and Paf1 protein complex (Krogan et al., 2003), which is associated with the elongating RNA polymerase II.

2.1.2 Dot1 proteins and H3 K79 methylation have diverse functions—Dot1 was originally identified as a gene affecting telomeric silencing in *Saccharomyces cerevisiae* and is highly conserved from yeast to human (Feng et al., 2002; Singer et al., 1998). In *S. cerevisiae*, deletion of *Dot1* or alteration of histone H3 K79 significantly impedes telomeric and HM silencing and Sir protein association at these loci (van Leeuwen et al., 2002). Surprisingly, overexpression of Dot1 also impairs silencing and Sir protein association at these loci (Singer et al., 1998). To accommodate these paradoxical results, two models have been proposed in which Sir proteins associate preferentially with histone H3 harboring nonmethylated (van Leeuwen et al., 2002) or methylated (Ng et al., 2002) K79. It is generally held that Dot1 HMTs specifically methylate histone H3 K79 in a nontarget manner in spite of the nonrandom methylation pattern observed (van Leeuwen et al., 2002). For instance, about 90% of all H3 K79 in yeast is methylated (van Leeuwen et al., 2002) and hDot1L aa 1–416 exhibited apparently nonsequence-specific binding with DNA *in vitro* (Min, Feng, Li, Zhang, & Xu, 2003). However, it has been reported that the level of H3 K79 methylation is low at silenced loci in both yeast and mammalian cells (Ng, Ciccone, Morshead, Oettinger, & Struhl, 2003) and is concentrated at the recombination-active rather than at the inactive loci in mammalian cells. These observations raise the possibility that Dot1-mediated H3 K79 methylation occurs in a targeted manner, which is now clearly demonstrated by our work on Dot1a–Af9-mediated repression of *αENaC* (Zhang, Yu, et al., 2013). Since the identification of Dot1 encoding a HMT, various roles of H3 K79 methylation have been proposed. The modification is considered as a conserved hallmark of active chromatin regions (Ng et al., 2003) and involved in multiple biological processes including telomeric and HM silencing, cell cycle regulation, cell proliferation, meiotic checkpoint, DNA replication, apoptosis, and leukemogenesis (reviewed in Nguyen & Zhang, 2011). The functional diversity of Dot1 and H3 K79 methylation may in part comes from the ability of the enzyme to add one, two, and three methyl groups to K79, leading to mono-, di-, and trimethylated K79, respectively (van Leeuwen et al., 2002). These methylation events are referred as H3 m1, m2, and m3K79.

2.1.3 Dot1a is the first aldosterone-regulated target with a known function in epigenetics—We cloned mouse Dot1-like (*Dot1h*) gene, characterized its mRNA

expression in the mouse kidney and IMCD3 cells (derived from the mouse inner medullary CD), identified five alternative splicing variants (Dot1a–e), and defined its methyltransferase activity specific for histone H3 K79 (Zhang, Hayashizaki, & Kone, 2004). Among these isoforms, Dot1a is the most extensively studied isoform. It contains a methyltransferase domain, an Af9/Af17-interacting domain, three potential nuclear localization signals, and a putative leucine zipper (Fig. 1). Dot1a shares 84% identity in the full length of amino acid sequence and 97% identity in the methyltransferase domain with its closest human counterpart hDOT1L.

Our subsequent analyses identified Dot1a as the first aldosterone-regulated target that has a known function in histone modifications. Aldosterone dynamically regulates Dot1a mRNA levels, leading to a delayed overall reduction of H3 K79 methylation in IMCD3 cells (Zhang, Xia, Jalal, et al., 2006). Aldosterone-treated IMCD3 cells displayed Dot1a mRNA levels that were ~30%, ~65%, and ~110% of vehicle-treated controls at the 2-, 4-, and 7-h time points, respectively. However, changes in H3m2K79 in bulk histones were not obvious until the 7-h time point, at which H3m2K79 was significantly reduced, followed by progressive recovery till 72-h time point. The effect of aldosterone on H3m2K79 seems specific, since H3 acetylated K9 as well as the total histone H3 remained constant at all time points examined (Zhang, Xia, Jalal, et al., 2006). Aldosterone also downregulated hDOT1L mRNA expression in 293T cells (Reisenauer et al., 2009), suggesting that the mechanism is likely conserved between mouse and human cells. Consistently, there are multiple putative glucocorticoid response elements (GRE) throughout the 2-kb 5'-flanking region of mouse *Dot1l* (-283, -359, -649, -716, -748, -900, -1274, -1553, -1613, -1755, -1805, and -1846; relative to the putative transcription start site 238 bp upstream of the translation initiation codon of Dot1a), as revealed by the analyses using the Transcription Element Search System (<http://www.cbil.upenn.edu/tess/>). Nevertheless, whether aldosterone decreases Dot1a mRNA levels by repressing its transcription through these GRE-like sites or by impacting its stability/degradation remains unexplored. Whether aldosterone downregulates Dot1a mRNA abundance *in vivo* in kidney and distal colon, two major physiological targets of aldosterone action, still lacks direct evidence.

2.1.4 Dot1a modulates targeted H3 K79 methylation at the α ENaC promoter and represses α ENaC in a methyltransferase-dependent manner—The physiological relevance and significance of aldosterone-induced downregulation of Dot1a are established by the identification of Dot1a as a negative regulator of α ENaC transcription. There are several lines of evidence to support this notion. First, *trans*-activation of α ENaC after aldosterone administration is associated with significantly impaired association of methylated histone H3 K79 at specific areas of the α ENaC 5'-flanking region (Fig. 2). The impairment varies to different degrees, depending on the subregions (Ra and R0–R3) examined (Zhang, Xia, Jalal, et al., 2006). Second, RNAi-mediated knockdown of Dot1a mimics aldosterone treatment, producing similar effects including increased activity of a stably integrated α ENaC promoter-luciferase construct and expression of endogenous α ENaC mRNA. Third, overexpression of WT Dot1a, but not the methyltransferase-dead mutant, causes increased association of H3m2K79 at the specific subregions of the α ENaC 5'-flanking regions, repression of α ENaC mRNA expression, and reduced activity of the

αENaC promoter-luciferase construct (Zhang, Xia, Jalal, et al., 2006). In aggregate, our data identify Dot1a as a new aldosterone-downregulated histone modification enzyme, and a negative regulator of *αENaC* transcription, most likely by directly or indirectly binding the *αENaC* promoter and hypermethylating histone H3 K79 associated with the *αENaC* promoter. These initial findings from IMCD3 cells have been replicated in 293T cells and mouse cortical CD M1 cells and extended to other aldosterone-regulated genes including *βENaC*, *γENaC*, *connecting tissue growthfactor (CTGF)*, *period* homolog, *preproendothelin*, and *Sgk1* (Reisenauer et al., 2009; Reisenauer, Wang, Xia, & Zhang, 2010; Wu, Chen, Zhou, & Zhang, 2011). Generation and characterization of mice lacking function of Dot1a and its regulators further solidify these findings *in vivo* in mouse kidneys (see below).

2.1.5 Dot1a-mediated repression apparently requires its nuclear expression as well as its methyltransferase activity and targeted H3 K79 methylation—

Transiently expressed GFP-Dot1a fusion in 293T cells was primarily located in the cytoplasm, nucleus, or both. The cellular distribution of Dot1a apparently regulated histone H3 K79 (Reisenauer et al., 2009). Sequence analysis revealed three potential NLSs spanning aa 394–416 (NLS1), 1089–1112 (NLS2), and 1165–1172 (NLS3) (Fig. 1). These NLSs are also highly conserved in rat and human with identical NLS1 sequences, one mismatch in human NLS2, rat NLS2, and rat NLS3, respectively. The biological significance of these putative NLSs in regulating Dot1a cellular distribution was evaluated by mutagenesis. Analyses of GFP-Dot1a fusions harboring various mutations in the three NLSs revealed that NLS1, NLS2, and NLS3 most likely play a role in directing Dot1a nuclear expression, with NLS1 and NLS2 being more important than NLS3. Moreover, Dot1a-mediated repression of the *αENaC* promoter-luciferase construct was largely relieved by mutations that impede Dot1a nuclear expression, remove the Af9/Af17-interacting domain, or remove the methyltransferase domain. A Dot1a mutant harboring deletions of all three NLSs was almost exclusively cytoplasmic and failed to inhibit *αENaC* promoter activity (Reisenauer et al., 2010). These results suggest that nuclear expression is essential for Dot1a to repress *αENaC* promoter. Manipulation of Dot1a nuclear/cytoplasmic distribution offers an additional strategy to remove Dot1a-mediated repression. Indeed, Af17 upregulates *αENaC* promoter by stimulating Dot1a nuclear export.

2.1.6 Dot1a-mediated repression of *αENaC* raised new questions—

Dot1a-mediated repression of *αENaC* raised three major questions regarding the function and regulation of Dot1a and H3 K79 methylation. The first question comes from Dot1a independency of aldosterone. Although it is clear that aldosterone stimulates *αENaC* transcription, it appears that Dot1a-mediated effects on *αENaC* transcription are at least partially independent of aldosterone–MR interactions with the *αENaC* promoter. Manipulation of Dot1a expression or its methyltransferase activity without aldosterone treatment is sufficient to induce changes in histone H3 K79 methylation and *αENaC* transcription.

The second question is if H3K79 methylation is active or repressive. In the broader context of other targets impacted by histone H3 K79 methylation, our work suggests that the modification may have bimodal effects on transcription. Studies in yeast of Sir-dependent

silencing loci and in mammalian cells of recombination-active versus recombination-inactive genomic loci suggest that histone H3 K79 hypermethylation functions as a marker of active chromatin regions (Ng et al., 2003). On the contrary, our data indicate that increased H3 K79 methylation is correlated with repression. Several explanations exist for these inconsistent findings. (1) Even in yeast, the role of histone H3 K79 methylation in association with the Sir proteins and thus in gene silencing is still unclear. As mentioned above, deletion or overexpression of Dot1 shares a similar phenotype: impaired Sir protein binding and disrupted silencing at telomeric and HM loci. (2) The cells contain a mixture of non-, mono-, di-, or trimethylated H3 K79 (van Leeuwen et al., 2002). A mechanistic insight of differential methylation states is mysterious. While the specificity of the anti-dimethyl H3 K79 antibody (from Upstate Biotechnology) to methylated K79 in histones has been established (Ng et al., 2002; Zhang et al., 2004), the cross-reactivity of this antibody toward mono- and trimethylated H3 K79 has not been rigorously examined. In case of *αENaC*, it remains to be determined whether hypomethylation induced by aldosterone or hypermethylation resulted from Dot1a overexpression represents a change in the relative ratio of each K79-methylated form to the non-modified one. Since the same antibody was utilized in the other studies (Ng et al., 2002, 2003), it is possible that a similar increase or decrease in histone H3 K79 methylation observed by chromatin immunoprecipitation (ChIP) with the antibody in different systems may represent a different shift of the spectrum of differential methylation status and thus point to different functions. Finally, it is also possible that histone H3 K79 methylation behaves similarly as H3 S10 phosphorylation (Cheung et al., 2000). Both of them may lead to gene activation and repression. Their ultimate effects on gene expression are dependent on the patterns of other histone modifications leading to either the association or the dissociation of distinct effector proteins.

The third question lies in the mode of Dot1a action. Dot1 family members are generally considered as nontargeted methyltransferases, although H3m2K79 is not uniformly detected throughout the genome in yeast or mammalian cells (Ng et al., 2002, 2003; van Leeuwen et al., 2002). Our data raise the additional possibility that targeted interactions of Dot1a with the *αENaC* 5'-flanking region not only occur but also are regulated in response to changes in Dot1a expression levels achieved by aldosterone treatment, overexpression, or knockdown of Dot1a. These results argue against nonsequence-specific DNA-binding activity of Dot1a in this promoter context and favor the hypothesis that Dot1a is recruited to these specific regions, presumably by Dot1a-interacting protein(s) that exerts DNA-binding activity to the implicated regions of the *αENaC* promoter. Driven by this hypothesis, we have identified Af9 as a Dot1a binding partner and a specific DNA-binding protein that also regulates *αENaC* promoter in an aldosterone-sensitive manner.

2.2 Putative transcription factor Af9

2.2.1 Af9 interacts with Dot1a—ALL1-fused gene from chromosome 9 (*hAF9*) was initially cloned from a leukemia patient containing t(9,11)(p22;q23) translocation (Iida et al., 1993). The translocation results in in-frame fusion of hAF9 aa 376–568 (corresponding to mouse Af9 aa 365–557) to mixed lineage leukemia (MLL) gene. To date, there are over 40 MLL fusion partners. These fusion events are considered as the cause of leukemogenesis. MLL-hAF9 is one of the most common forms. hAF9 is highly homologous to ENL and

interacts with AF4. Like hAF9, both AF4 and ENL are also MLL fusion partners (Erfurth, Hemenway, De Erkenez, & Domer, 2004). Other hAF9 interacting proteins include specific isoforms of BCL-6 corepressor (BCoR) (Srinivasan, de Erkenez, & Hemenway, 2003), a component of histone deacetylase complexes, and MPC3, a member of the Polycomb group multiprotein complexes involved in gene silencing by modifying chromatin structure (Hemenway, de Erkenez, & Gould, 2001). MPC3 can simultaneously interact with hAF9 and RING1, and in turn RING1 may bind other Polycomb group proteins (Hemenway et al., 2001). Thus hAF9 may acquire a dominant-negative function by recruitment of, or to, corepressors or chromatin-modifying proteins. hAF9 and its mouse homolog Af9 belong to YEATS family, which is named after “Ynk7,” “ENL,” “AF9,” and “TFIIF small subunit.” This family is characterized by the possession of a YEATS domain and thought to have a transcription stimulatory activity. Apart from these observations, little is known about the hAF9 function in normal cells.

To identify Dot1a-interacting protein that may modulate targeted H3 K79 methylation, we screened a mouse kidney cDNA library ligated to the GAL4 activation domain using Dot1a as bait in a yeast two-hybrid assay. This effort led to identification of Af9 as a specific binding partner of Dot1a. The specificity of the interaction was subsequently confirmed *in vitro* and *in vivo* by multiple assays (GST pulldown, Co-IP, colocalization, and mammalian two-hybrid). Further analyses revealed that aa 479–659 in Dot1a and the very C-terminal part (aa 397–557) of Af9 are capable of and sufficient for mediating the interaction (Fig. 1). Since the domain responsible for interacting with Af9 in Dot1a also binds Af17 (see below), it is referred as Af9/Af17-interacting domain. This domain is not overlapped with the methyltransferase domain (aa 1–416) and does not exist in yeast Dot1. Since the Af9 very C-terminal part also mediates its interaction with MR and Hsp90, it is named as Dot1a/MR/Hsp90-interacting domain. This fragment is retained in the leukemia patient used to identify hAF9 from the t(9;11)(p22;q23) translocation. Hence, our study also provided additional evidence in favor of the hypothesis that mistargeting hDOT1L is a critical event for leukemogenesis (Okada et al., 2005).

2.2.2 Af9 directly binds α ENaC promoter and represses its transcription—The interaction of Af9 with Dot1a implies that Af9 may be a component of aldosterone-signaling network and regulate α ENaC transcription. Indeed, like Dot1a, Af9 expression is also downregulated by aldosterone at both mRNA and protein levels in IMCD3 cells. Af9 overexpression causes hypermethylation of histone H3 K79 at R0–R3, but not at Ra subregions of the α ENaC promoter. It also decreases expression of the endogenous α ENaC mRNA expression and acts synergistically with Dot1a to inhibit α ENaC promoter-luciferase constructs. RNAi-mediated knockdown of Af9 induces the opposite effects. ChIP assays reveal that endogenous Af9 and Dot1a as well as overexpressed FLAG-Af9 are each associated with R0–R3, but not Ra subregions of α ENaC promoter (Zhang, Xia, Reisenauer, et al., 2006; Zhang et al., 2007). Gel shift and antibody competition assays revealed that Af9 has specific DNA-binding activity and binds a functional Af9 *cis*-element (+78/+92) in the R3 subregion of α ENaC promoter, the principal site for Dot1a–Af9 interaction, in IMCD3 cells (Zhang, Yu, et al., 2013). Mutation of the Af9 *cis*-element resulted in greatly reduced association of Af9 and Dot1a with the α ENaC promoter-reporter constructs, higher basal

αENaC promoter activity, and impaired Dot1a-mediated inhibition in *trans*-repression assays (Zhang, Yu, et al., 2013). Thus, it can be concluded that Af9 recruits Dot1a, primarily through directly binding +78/+92 of *αENaC*, to mediate targeted H3 K79 methylation and to repress basal and aldosterone-sensitive *αENaC* transcription. In addition, NAD⁺-dependent deacetylase sirtuin 1 (Sirt1) functionally and physically interacts with Dot1 to enhance the distributive activity of Dot1a on H3K79 methylation and thereby represses *αENaC* transcription in IMCD3 cells (Zhang, Li, Cruz, & Kone, 2009). Dot1a–Af9-mediated repression may be more broadly applied to at least some other aldosterone-regulated genes, including *βENaC*, *γENaC*, *CTGF*, *period* homolog, *preproendothelin*, and *Sgk1* (Wu et al., 2011; Zhang, Xia, Reisenauer, et al., 2006; Zhang et al., 2007). However, compared to *αENaC*, these genes are much less characterized in terms of Dot1a–Af9-mediated targeted H3 K79 methylation associated with their promoters.

3. Dot1a–Af9-Mediated *αENaC* Repression is Relieved by Multiple Mechanisms

3.1 Sgk1 relieves Dot1a–Af9-mediated repression by phosphorylating Af9

3.1.1 Sgk1 is an early target of aldosterone and regulates ENaC expression and activity—Prevailing evidence suggests that aldosterone increases ENaC function in two phases. The early phase involves the upregulation of preexisting transport machinery and of aldosterone-induced regulatory proteins, notably Sgk1. The delayed phase of aldosterone action involves *de novo* synthesis of ENaC, either through the liganded MR directly binding HREs in the *αENaC* promoter to activate transcription (Thomas & Itani, 2004) or through indirect mechanisms involving other proteins (Asher, Wald, Rossier, & Garty, 1996; Sayegh et al., 1999).

Studies on acute transcriptional effects of aldosterone led to identification of several transcripts, including Sgk1, induced in the early phase of aldosterone stimulation (Chen et al., 1999). Sgk1 expression can be induced as soon as 30 min after hormone administration (Chen et al., 1999). Rapid induction of Sgk1 mRNA by aldosterone is coupled to increased ENaC cell surface expression (Loffing et al., 2000, 2001), which can be achieved by enhancing ENaC trafficking from the cytosol to the apical membrane or by decreasing its removal from the membrane. How Sgk1 regulates these processes is still not fully defined. A widely accepted theme is that Sgk1 regulates ENaC abundance at the cell surface, in part through phosphorylation of the ubiquitin ligase Nedd4-2 (Debonneville et al., 2001). Nedd4-2 phosphorylation reduces its affinity with and hence binding ENaC, and induces its interaction with 14-3-3. The concerted actions of Sgk1 and 14-3-3 appear to disrupt Nedd4-2-mediated ubiquitination and subsequent degradation of ENaC, leading to accumulation of ENaC channels at the cell surface (Debonneville et al., 2001; Ichimura et al., 2005; Lee, Campbell, Cook, & Dinudom, 2008; Snyder, Olson, & Thomas, 2002). Aldosterone also induces ubiquitin-specific protease Usp2-45, which deubiquitylates ENaC and increases its surface expression and activity (Fakitsas et al., 2007; Verrey, Fakitsas, Adam, & Staub, 2008). In this regard, part of the early effect of aldosterone is mediated by inhibiting ENaC removal from the membrane (Kamynina & Staub, 2002; Snyder et al., 2002).

However, following observations support additional mechanisms for Sgk1-mediated ENaC regulation. (1) While the PY motif of ENaC is required for interaction with Nedd4-2, its requirement for Sgk1-stimulated Na⁺ transport is controversial (Alvarez de la Rosa, Zhang, Naray-Fejes-Toth, Fejes-Toth, & Canessa, 1999; Debonneville et al., 2001). For example, the isolated collecting tubules and primary cultures of CCD from a Liddle mouse model expressing a PY-missing βENaC subunit still displayed aldosterone-stimulated Na⁺ transport (Dahlmann et al., 2003; Pradervand et al., 2003). Some ion-transporters such as renal outer medullary K⁺ channel ROMK1 (Yoo et al., 2003; Yun et al., 2002) and Na⁺, K⁺ ATPase (Setiawan et al., 2002) that have neither a PY motif nor a known interaction with Nedd4-2 can also be regulated by Sgk1. (2) Sgk1 also apparently plays a role at the transcriptional level. Sgk1 has been shown to regulate the transcription of *CTGF* (Vallon et al., 2006) and *α*- and *β*ENaC (Boyd & Naray-Fejes-Toth, 2005; Zhang et al., 2007). Examination of αENaC mRNA expression in the presence or absence of corticosteroids in mouse CCD cells stably expressing either full-length Sgk1 or a kinase-dead, dominant-negative (K127M)-Sgk1 mutant revealed that Sgk1 upregulates αENaC expression in a corticosteroid-independent, but kinase-dependent manner (Boyd & Naray-Fejes-Toth, 2005). Accordingly, Sgk1 is proposed to activate unidentified transcription factors or inactivate an inhibitor of αENaC transcription (Boyd & Naray-Fejes-Toth, 2005). Our finding supports the latter. That is, Sgk1 impairs Dot1a–Af9-mediated repression of αENaC through phosphorylating Af9 to impair Dot1a–Af9 interaction.

In addition to aldosterone, Sgk1 is also rapidly induced in response to other stimuli in a tissue-specific manner (Cooper et al., 2001; Cowling & Birnboim, 2000; Waldegger, Barth, Raber, & Lang, 1997; Waldegger, Gabrysch, Barth, Fillon, & Lang, 2000). Sgk1 can be shuttled between nucleus and cytoplasm in a cell cycle- and stimulus-dependent manner (Firestone, Giampaolo, & O'Keeffe, 2003). The activation of Sgk1 is dependent upon phosphoinositide 3-kinase activity and requires the phosphorylation of the two regulatory sites (T256 and S422) (Park et al., 1999). Thus, Sgk1 serves as a functional convergence point between several types of signaling pathways and cellular phosphorylation cascades. The Sgk1 gene is highly conserved from yeast to human and expressed in a variety of tissues and cell lines in mammals. In addition to Nedd4-2, Sgk1 also phosphorylates Af9, forkhead-like transcription factor FOXO3a and several other substrates (Sahin, McCaig, Jeevahan, Murray, & Hainsworth, 2013; Zhang, Xia, Reisenauer, et al., 2006). All of these proteins contain the Sgk1 consensus phosphorylation sites (RXRXXS/T).

3.1.2 Sgk1 phosphorylates Af9 at S435 *in vitro* and *in vivo*—As mentioned above, aldosterone decreases expression of Dot1a and Af9 and thus the formation of Dot1a–Af9 complex. In addition, aldosterone rapidly induces Sgk1, which inactivates the Dot1a–Af9 complex by targeting Af9 for phosphorylation. Af9 possesses a highly conserved consensus Sgk1 phosphorylation site S435. *In vitro*, purified Sgk1 can phosphorylate recombinant GST-Af9, but not GST-Af9 S435A. *In vivo* in IMCD3 cells, the overall level of FLAG-Af9 S435A phosphorylation was about 50% of the wild-type FLAG-Af9 phosphorylation, suggesting that S435 is a major phosphorylation site. Aldosterone increased Sgk1 protein level slightly at 1-h and significantly at 1.5- and 2-h time points, which was accompanied with a significant enhancement of the endogenous Af9 S435 phosphorylation at 1 h. The

phosphorylation was peaked at 1.5 h of aldosterone treatment. Hence, Sgk1 may contribute to aldosterone-stimulated Af9 S435 phosphorylation in IMCD3 cells. RNAi-mediated knockdown of Sgk1 by ~50% resulted in ~40% reduction in Af9 S435 phosphorylation. In reciprocal experiments, transient overexpression of Sgk1, but not its kinase-dead mutant, increased by ~160% Af9 S435 phosphorylation in IMCD3 cells (Zhang et al., 2007). *Sgk1*^{-/-} versus *Sgk1*^{+/+} mice had significantly reduced Af9 S435 phosphorylation in the kidney (Zhang et al., 2007). Taken together, Sgk1 phosphorylates Af9 at S435 *in vitro* and *in vivo* in multiple experimental settings.

3.1.3 Sgk1-mediated Af9 phosphorylation decreases the Dot1a–Af9 interaction *in vitro* and at the *αENaC* promoter in IMCD3 cells—

The location of S435 in the Dot1a/MR/Hsp90-interacting domain (aa 397–557) provides the structural basis for its role in regulating the Dot1a–Af9 interaction through Sgk1-mediated phosphorylation. Consistently, the ability of Sgk1-phosphorylated GST-Af9 397–557 to retain GFP-Dot1a 479–659 was decreased to 24% of the nonphosphorylated GST-Af9 fusion. Such ability to retain the GFP-Dot1a fusion and sensitivity to Sgk1 were significantly impaired, but not abolished by S435A mutation (Zhang et al., 2007). These results suggest that Sgk1-mediated Af9 S435 phosphorylation decreases but does not completely abolish the Dot1a–Af9 interaction, and that S435 plays a critical role in binding Dot1a. Analogous to our findings, protein kinase A phosphorylation of GST-neurabin I significantly reduced, but did not completely eliminate, its interaction with protein phosphatase 1 (McAvoy et al., 1999). Similarly, the nonphosphorylated form of PC4 displayed a lower, but not eliminated, affinity for Rep proteins compared to phosphorylated PC4 (Weger, Wendland, Kleinschmidt, & Heilbronn, 1999), and phosphorylated merlin had lower, but not abolished, interactions with CD44 and hepatocyte growth factor-regulated tyrosine kinase substrate in GST-pulldown assays (Rong, Surace, Haipok, Gutmann, & Ye, 2004). In addition, S435A and S435D to mimic nonphosphorylated and phosphorylated forms, respectively, yielded a similar phenotype, indicating that phosphorylation induces changes in structure, charge, or both. Observations of identical behavior of Ala and Asp mutations of putative serine phosphorylation sites also have been reported before. For example, changing the putative phosphorylation site S33 in the intermediate filament protein keratin 18 into Ala or Asp disrupted the keratin 18 interaction with 14-3-3 proteins (Ku, Liao, & Omary, 1998). Similarly, substitution of the putative phosphorylation site S444 in the K⁺ channel KCNQ4 with Ala or Asp abolished Sgk1 modulation of KCNQ4 (Seeböhm et al., 2005). In addition, the kinase activity of Sgk1, while necessary for lessening the Dot1a–Af9 interaction, does not appear to be required for its association with the R0–R3 subregions of the *αENaC* promoter in IMCD3 cells (Zhang et al., 2007).

Since ChIP revealed that endogenous Af9 and FLAG-Af9 associated with specific subregions R0–R3, but not with Ra of the *αENaC* promoter (Zhang, Xia, Reisenauer, et al., 2006; Zhang et al., 2007), it is predicted that Sgk1 would associate with the *αENaC* promoter, decrease the association of the Dot1a–Af9 complex, and thus limit histone H3 K79 methylation at the *αENaC* promoter. These predictions were validated through ChIP and Re-ChIP in IMCD3 cells transfected with FLAG-tagged Sgk1 or the kinase-dead mutant Sgk1. In particular, Sgk1, like Af9, specifically associated with the R0–R3, but not Ra,

subregions of the *αENaC* promoter in IMCD3 cells. Sgk1 impairs the ability of Af9 to interact with Dot1a at these subregions without impacting Af9 DNA-binding activity, leading to targeted histone H3 K79 hypomethylation. In all subregions except R1, Sgk1 induced these effects in a kinase-dependent manner and presumably by phosphorylating Af9 (Zhang et al., 2007).

Mice with high salt intake versus normal salt intake exhibited comparable low levels of Af9 S435 phosphorylation and mRNA expression of Sgk1 and *αENaC* in their kidneys. Salt restriction coordinately induced Sgk1 expression and Af9 S435 phosphorylation, which was associated with increased *αENaC* mRNA levels in mouse kidney (Zhang et al., 2007). These data provide first evidence that Af9 is a bona fide substrate of Sgk1 for phosphorylation at S435 *in vivo* in mouse kidney. These findings are also consistent with previous studies showing that low-salt diet-induced renal *αENaC* mRNA expression (Hou, Speirs, Seckl, & Brown, 2002; Ono, Kusano, Muto, Ando, & Asano, 1997).

More direct evidence showing that Sgk1 phosphorylates Af9 at S435 and regulates *αENaC* transcription *in vivo* in the kidney was acquired by analysis of *Sgk1*^{-/-} mice (Huang et al., 2006; Vallon, Huang, et al., 2005; Vallon, Wulff, et al., 2005; Wulff et al., 2002). *Sgk1*^{-/-} mice exhibited significantly lower levels of renal Af9 S435 phosphorylation than WT controls under a normal- or low-salt diet, indicating that Af9 can be phosphorylated in a Sgk1-dependent or Sgk1-independent manner in mouse kidney. The reduced Af9 phosphorylation was accompanied with impaired *αENaC* expression at both mRNA and protein levels (Zhang et al., 2007).

Mechanistically, Sgk1-mediated phosphorylation of Af9 impairs but does not fully abolish the ability of Af9 to interact with Dot1a. Therefore, other factors controlling the Dot1a–Af9 complex are operative. Among these factors are MR and Af17 as described below.

3.2 MR counterbalances Dot1a–Af9 by interacting with Af9

A central question is how aldosterone coordinately activates *αENaC* through MR-dependent mechanism and removes Dot1a–Af9-mediated repression. In the unliganded state, MR is localized in the cytosol as a part of a multiprotein complex containing heat-shock protein 90 (Hsp90) (Faresse, Ruffieux-Daidie, Salamin, Gomez-Sanchez, & Staub, 2010). MR has been known to interact with GR (Liu, Wang, Sauter, & Pearce, 1995; Savory et al., 2001) and multiple coactivators and corepressors (reviewed in Yang & Young, 2009). Af9 binds Dot1a (Zhang, Xia, Reisenauer, et al., 2006), Sgk1 (Zhang et al., 2007), Af4 (Erfurth et al., 2004), Aff4 (Biswas et al., 2011), Hsp90 (Lin & Hemenway, 2010), CBX8 (Hemenway et al., 2001), and BCoR (Srinivasan et al., 2003). Because they have opposite roles in *αENaC* transcription, MR and Af9 may interact to mutually antagonize their opponent effect. Yeast two-hybrid assays revealed a specific interaction between GAL4-AD-MR and GAL4-BD-Af9 fusions (Zhang, Zhou, et al., 2013). The interaction was subsequently confirmed by Co-IP and colocalization experiments. MR coimmunoprecipitated with Af9 in the whole-kidney lysates from the WT, but not from the *MR*^{-/-} mice. This demonstrates not only the interaction but also the specificity of the MR–Af9 interaction. Further yeast two-hybrid assays revealed that MR, like Dot1a, also binds the Dot1a/MR/Hsp90-interacting domain (aa 397–557), but not the N-terminal part (aa 2–406) of Af9 (Zhang, Zhou, et al., 2013). This

suggests that MR may interfere with Dot1a–Af9 interaction by competing for Af9 binding. Such hypothesis was subsequently validated by GST-pulldown assays to investigate Dot1a–Af9 interaction with MR as a competitor. Increasing the amount of lysate containing overexpressed MR gradually reduced the amount of the GFP-Dot1a fusion retained by a fixed amount of the GST-Af9 fusion, indicating that MR inhibited Dot1a–Af9 interaction. Consistently, the Dot1a–Af9-mediated repression of α -, β -, and γ ENaC was progressively abolished and eventually reversed by increasing amounts of overexpressed MR in the presence of aldosterone. Hence, MR/aldosterone is capable of antagonizing Dot1a–Af9-mediated repression of ENaC mRNA expression, at least in IMCD3 cells under the conditions tested (Zhang, Zhou, et al., 2013). In these experiments, the aldosterone concentration in the medium (100 nM) was higher than the physiological concentration of the hormone. However, the actual effective intracellular concentration may be much lower because steroid hormones may not diffuse across cell membranes freely (Gumz et al., 2003; Pietras, Nemere, & Szego, 2001). This may explain why there is a wide range of aldosterone concentrations including 10 nM (Dooley, Angibaud, Yusef, Thomas, & Harvey, 2013), 30 nM (Faresse, Debonneville, & Staub, 2013), 1000 nM (Gumz et al., 2003; Reisenauer et al., 2009, 2010; Soodvilai, Jia, Fongsupa, Chatsudhipong, & Yang, 2012; Wu et al., 2011; Zhang, Xia, Jalal, et al., 2006; Zhang, Xia, Reisenauer, et al., 2006; Zhang et al., 2007; Zhang, Yu, et al., 2013), and even 1500 nM (Helms et al., 2005) used in different cell culture systems. Aldosterone elicited the most dramatic effect in IMCD3 cells at 1000 nM, which can be completely blocked by MR and GR inhibitors, used alone or in combination (Gumz et al., 2003).

On the other hand, reciprocal experiments demonstrated that overexpression of Dot1a–Af9 suppressed MR-mediated transcriptional activation of ENaC genes in IMCD3 cells. Transfection with the MR construct alone significantly increased α -, β -, and γ ENaC mRNA levels, compared to the vector-transfected control. Increasing amounts of the Dot1a and Af9 constructs added to the transfection mixture progressively impaired the MR-mediated activation (Zhang, Zhou, et al., 2013). Taken together, Dot1a–Af9 and MR mutually impair their opponent's effect on ENaC transcription under the conditions tested.

3.3 Af17 impairs Dot1a–Af9-mediated repression by competitively binding Dot1a and facilitating Dot1a nuclear export

3.3.1 Af17 competes with Af9 for binding Dot1a—Aldosterone-independent impairment of Dot1a–Af9 interaction and relief of Dot1a–Af9-mediated repression were first revealed by identification of ALL1-fused gene from chromosome 17 (hAF17) as another Dot1a-interacting molecule (Reisenauer et al., 2009; Wu et al., 2011). *hAF17* gene was originally cloned as a less frequent fusion partner of the *MLL* gene in t(11;17)(q23;q21) translocations from some acute myeloid leukemias (Prasad et al., 1994; Suzukawa et al., 2005). hAF17 is proposed to be a transcriptional regulator and a downstream target of the β -catenin/T-cell factor pathway and regulates G2–M progression. hAF17 binds PC2 glutamine/q-rich-associated protein (PCQAP) and CCAAT/enhancer binding protein (C/EBP) in yeast two-hybrid assay (Lin et al., 2001). However, the biological relevance and significance of these protein–protein interactions have not been well characterized.

In search of additional Dot1a partners by yeast two-hybrid screen, we identified mouse Af17 aa 635–786 is capable of and sufficient for interacting with Dot1a. Interestingly, like Af9, mouse Af17 binds the same Af9/Af17-interacting domain of Dot1a, but not the methyltransferase domain (aa 1–416), the putative leucine zipper domain (aa 576–597), or the C-terminal portion (aa 1112–1540) of Dot1a. The Dot1a–Af17 interaction was mutually confirmed by multiple other approaches (GST pulldown, Co-IP, mammalian two-hybrid, and colocalization) (Reisenauer et al., 2009).

The fact that the same domain of Dot1a can interact with Af17 and Af9 suggests that these two proteins may compete for binding Dot1a. In the GST-pulldown assays to investigate Dot1a–Af17 interaction with FLAG-Af9 as a competitor, increasing the amount of FLAG-Af9 gradually reduced the ability of GST–Af17 fusion to retain GFP-Dot1a. In competitive mammalian two-hybrid assays, the luciferase reporter activity driven by Dot1a–Af17 interaction was inversely correlated with the amount of FLAG-Af9 added. Reciprocally, the Dot1a–Af9 interaction was progressively prohibited by elevating amounts of FLAG-Af17 635–786 as a competitor in GST-pulldown assays and in competitive mammalian two-hybrid assays (Reisenauer et al., 2009). Hence, Af17 and Af9 apparently inhibited their opponent to interact with Dot1a *in vitro* and *in vivo*.

3.3.2 Af17 facilitates Dot1a nuclear export and upregulates *ENaC* genes—

RNAi-mediated depletion of the endogenous hAF17 induced a shift of GFP-Dot1a expression from cytoplasm to nucleus in 293T cells. In contrast, overexpression of RFP-hAF17 enhances cytoplasmic expression of GFP-Dot1a at the expense of its nuclear expression in 293T cells. In the absence of their partners, GFP-Dot1a and RFP-hAF17 were primarily, but not exclusively, located in the nucleus and cytoplasm, respectively. However, when these fusion proteins were coexpressed, Dot1a nuclear expression was significantly decreased, which was accompanied with a dramatic increase of Dot1a colocalization with hAF17 in the cytoplasm (Fig. 3). The redistribution of Dot1a from the nucleus to the cytoplasm was accompanied by a substantial reduction of H3m2K79 and H3m3K79, but not dimethylated H3 K9 (H3m2K9) in bulk histones. The effect of hAF17 overexpression on H3 K79 hypomethylation can be blocked by the nuclear export inhibitor leptomycin B. ChIP and Re-ChIP revealed that hAF17 overexpression significantly impaired the Dot1a–Af9 interaction at all four subregions (R0–R3) of *αENaC* promoter and upregulates *αENaC* transcription in 293T cells and in mouse cortical CD M1 cells (Reisenauer et al., 2009; Wu et al., 2011). Therefore, like Sgk1 and MR, hAF17 appears to inhibit the Dot1a–Af9 interaction at the promoter without measurably affecting the association of Af9 with the *αENaC* promoter. However, unlike Sgk1 that impairs Dot1a–Af9 interaction by phosphorylation of Af9, hAF17 and its mouse homolog Af17 do so presumably by competing with Af9 for binding Dot1a. hAF17 overexpression also led to upregulation of several other aldosterone-regulated genes examined, including β - and γ -*ENaC*, *Sgk1*, and *CTGF* (Reisenauer et al., 2009).

3.4 Hsp90 relieves Dot1a–Af9-mediated repression by directly modulating the spatial distribution of Af9

The heat-shock protein Hsp90 plays an important role in nuclear import of steroid receptors. It is also an effector of transcriptional activation through diverse mechanisms. Hsp90 was identified by mass spectrometry as one of the proteins bound a FLAG-AF9-ct column (amino acids 475–568 of hAF9, identical to mouse Af9 aa 464–557) in THP-1 whole-cell extract (Lin & Hemenway, 2010). The interaction was subsequently confirmed by Co-IP and colocalization assays and appeared to be a part of an Hsp90–Hsp70–p60/Hop chaperone complex. Both inhibition of Hsp90 function by the inhibitor novobiocin and knockdown of Hsp90 with siRNA resulted in redistribution of hAF9 from a primarily nuclear to cytoplasmic location, which was associated with a decrease in hAF9 occupancy at the *αENaC* promoter and increased *αENaC* expression (Lin & Hemenway, 2010). Interestingly, the Af9-ct fragment mediating the interaction with Hsp90 resides within the domain capable of binding Dot1a and MR, we name the later as Dot1a/MR/Hsp90-interacting domain. It remains to determine if Hsp90 can compete with Dot1a and/or MR for binding Af9. However, it is very unlikely that Sgk1-mediated Af9 S435 phosphorylation is important for regulation of Af9–Hsp90 interaction since the Af9-ct fragment lacks S435.

4. Transcriptional Changes in ENaC Genes are Translated into Changes in ENaC Activity

The physiological significance of Dot1a–Af9-mediated repression is indicated by translation of the changes in the mRNA levels of ENaC genes into the corresponding changes in ENaC activity. ENaC activity can be measured with different approaches using ENaC inhibitors such as benzamil. Single-cell fluorescence imaging with SBFI-AM is often used to measure benzamil-sensitive intracellular sodium concentration ($[Na^+]_i$) as an index of ENaC activity. With this approach, we found that aldosterone-induced ENaC mRNA upregulation led to elevated ENaC activity, as seen by an increase in the benzamil-sensitive $[Na^+]_i$ in IMCD3 cells (Wu et al., 2011). Both transcription inhibitor actinomycin D and translation inhibitor cycloheximide largely blocked this effect (Wu et al., 2011). These results indicate that new mRNA and protein synthesis is required for aldosterone-induced ENaC-mediated Na^+ uptake in IMCD3 cells. Furthermore, overexpression of Dot1a and Af9 attenuated the benzamil-sensitive $[Na^+]_i$, while knockdown of Dot1a and Af9 increased Na^+ uptake in IMCD3 cells (Wu et al., 2011).

The second approach is the measurement of the benzamil-sensitive equivalent short-circuit current (I_{sc}) of the confluent monolayer of cells on filter units. Af9 overexpression significantly lessened the benzamil-sensitive I_{sc} (I_{sc}) in IMCD3 and M1 cells. While Dot1a-transfected cells were able to transiently overexpress Dot1a, they failed to maintain Dot1a overexpression during the formation of confluent monolayers on permeable support. This excluded the possibility to evaluate the effect of Dot1a overexpression on the benzamil-sensitive I_{sc} (Wu et al., 2011).

The third approach is whole-cell patch clamping. Nevertheless, this approach is proved inappropriate in IMCD3 and M1 cells due to their fragility. To circumvent this limitation, we

performed whole-cell patch clamping in 293T cells, treated with vehicle as control or aldosterone. Alternatively, 293T cells were transiently transfected with an empty vector or a construct encoding Dot1a, Af9, or Af17. A transcriptional increase in ENaC genes by either aldosterone treatment or overexpressing Af17 resulted in an increase in benzamil-sensitive Na^+ current density. Oppositely, overexpression of Dot1a and Af9 decreased the ENaC-mediated Na^+ current (Reisenauer et al., 2009). Thus, multiple and independent lines of data indicate that transcriptional changes in ENaC genes by modulating Dot1a, Af9, and Af17 expression through aldosterone treatment or transient/stable transfection are translated into changes in ENaC activity in all cell model systems (293T, IMCD3, and M1 cells) examined.

5. Mouse Models with Genetic Defects in ENaC Regulators

5.1 *Sgk1*^{-/-} mice

On a standard NaCl diet, *Sgk1*^{-/-} mice showed apparently normal renal water and electrolyte excretion, which is consistent with no significant changes in αENaC mRNA and protein abundance; despite of a significant lower level of Af9 phosphorylation, compared with WT littermates. Under Na^+ restriction, however, *Sgk1*^{-/-} mice failed to upregulate renal tubular Na^+ reabsorption, leading to Na^+ depletion despite the presence of increased plasma aldosterone levels (Wulff et al., 2002). This phenotype is associated with impaired Af9 phosphorylation and reduced αENaC expression at both mRNA and protein levels. Taken together, the results indicate that Sgk1 plays an important role in the adaptational response to altered physiological or pathological conditions.

5.2 *MR*^{-/-} mice

The biological significance of aldosterone classical action via MR is clearly demonstrated by the generation and characterization of *MR*^{-/-} mice. *MR*^{-/-} mice die in the second week after birth, showing at day 8 PHA-1 phenotype with hyponatremia, hyperkalemia, high renal salt wasting, and a strongly activated RAAS. Unexpectedly RNase protection analysis of the 8-day-old *MR*^{-/-} mice did not reveal a significant decrease in the mRNA abundance of the subunits of ENaC and Na^+, K^+ ATPase (Berger et al., 1998). Our recent study revealed that the transcriptional defect in ENaC occurs at an earlier developmental stage and is masked by a compensatory mechanism. *MR*^{-/-} mice have decreased mRNA levels of ENaC genes at day 5 after birth (Zhang, Zhou, et al., 2013), possibly due to less activated RAAS. The plasma aldosterone concentration in these mutant neonates may be insufficient to attenuate Dot1a–Af9-mediated repression and to activate substantial amount of GR. The impaired ENaC expression may be at least partially responsible for the progressively developed PHA-1 phenotype.

5.3 *AF17*^{-/-} mice

Af17^{-/-} versus *Af17*^{+/+} mice fed a normal Na^+ diet have impaired renal water and electrolyte retention and decreased BP (Chen et al., 2011). This phenotype is associated with increased H3 K79 methylation in whole-kidney lysate and at the αENaC promoter; impaired ENaC expression at both mRNA and protein levels, and significantly lower active channel number, open probability, and effective activity of ENaC. In contrast, inducing high levels of plasma aldosterone by dietary Na^+ restriction, high dietary potassium loading, or aldosterone

infusion completely compensated for *Af17* deficiency with respect to sodium handling and blood pressure (BP) (Chen et al., 2011).

5.4 *Dot1^{AC}* mice

Despite having 20% fewer principal cells in which ENaC is expressed (Wu, Chen, Zhou, et al., 2013), CNT/CD-specific *Dot1l* conditional knockout mice (*Dot1l^{AC}*) expressed 36% greater levels of *αENaC* mRNA compared with *Dot1l^{f/f}* mice as measured by real-time RT-qPCR (Zhang, Yu, et al., 2013). The effect of CNT/CD-specific *Dot1l* deletion on *αENaC* mRNA expression in the *Dot1l^{AC}* mice would likely have been even greater if the mice expressed normal numbers of principal cells. Taken together, these data support the conclusion that genetic inactivation of *Dot1l* removes Dot1a-mediated repression of *αENaC* transcription and leads to upregulation of *αENaC* expression, and they reinforce the notion that Dot1a functions as a negative regulator of basal *αENaC* transcription.

6. Regulation of ENaC Activity by Other Regulatory Proteins

Other aldosterone-stimulated ENaC regulatory factors include the *S*-adenosyl-L-homocysteine hydrolase (SAHHase) (Stockand, Al-Baldawi, Al-Khalili, Worrell, & Eaton, 1999; Stockand et al., 2001), glucocorticoid-induced leucine zipper protein (GILZ) (Bhalla, Soundararajan, Pao, Li, & Pearce, 2006; Muller et al., 2003; Soundararajan, Wang, Melters, & Pearce, 2007; Soundararajan, Zhang, Wang, Vandewalle, & Pearce, 2005), the small G protein K-Ras2A (Brennan & Fuller, 2006; Mastroberardino et al., 1998; Verrey, 1999; Verrey et al., 2000), and the channel-activating protease CAP1 (Narikiyo et al., 2002). SAHHase activity, but not expression was enhanced by aldosterone, which in part, increases ENaC open probability (P_o). GILZ markedly increased ENaC membrane expression and ENaC-mediated Na^+ transport, possibly by inhibiting extracellular signal-regulated kinase (ERK) signaling. K-Ras2A also increased the mean activity of ENaC at the cell surface. The complexity of ENaC regulation is also supported by the recent findings that maturation and activation of ENaC subunits involve α - and γ ENaC cleavage mediated by CAP1 (Caldwell, Boucher, & Stutts, 2004; Vallet, Chraïbi, Gaeggeler, Horisberger, & Rossier, 1997; Vuagniaux, Vallet, Jaeger, Hummler, & Rossier, 2002; Vuagniaux et al., 2000) or furin (Bruns et al., 2007; Harris, Garcia-Caballero, Stutts, Firsov, & Rossier, 2008; Hughey et al., 2004; Sheng, Carattino, Bruns, Hughey, & Kleymann, 2006). CAP1 stimulates ENaC activity probably by cleaving γ ENaC (Bruns et al., 2007; Thomas & Itani, 2004) and/or by increasing ENaC P_o (Caldwell et al., 2004; Vallet et al., 1997; Vuagniaux et al., 2000, 2002). Furin-mediated cleavage of α - and γ ENaC activates the channels by relieving Na^+ self-inhibition (Sheng et al., 2006). The vesicle traffic regulatory protein syntaxin 1A contains distinct inhibitory and stimulatory domains that interact with ENaC subunits and determine the overall ENaC functionality/regulation under distinct physiological conditions (Condliffe, Zhang, & Frizzell, 2004; Qi et al., 1999; Saxena, Singh, Kaur, & George, 2007). However, the aldosterone effect on furin or syntaxin 1A expression has not been documented yet.

7. Conclusion and Future Directions

We propose a model summarizing our understanding how Dot1a–Af9-mediated repression of *αENaC* transcription is achieved or relieved in the absence or presence of aldosterone

(Fig. 4). It can be speculated that deregulation of the Dot1a–Af9 complex may result in defects in renal Na⁺ excretion, and, thus, potentially abnormal blood pressure in humans. Phenotypically, such deregulation may be reminiscent of the monogenic forms of Na⁺ retention and hypertension (Liddle's syndrome) or renal Na⁺ wasting and hypotension (pseudohypoaldosteronism type 1), two types of disorders due to gain-of-function and loss-of-function mutations in ENaC genes, respectively. The evidence showing the physiological relevance of Dot1a–Af9 in regulating blood pressure is emerging. In a recent clinical study, a polymorphism in *hDOTIL* (rs2269879) is associated with blood pressure response to hydrochlorothiazide in Caucasians, and a polymorphism in *hAF9* (rs12350051) is associated with untreated blood pressure in African-Americans (Duarte et al., 2012).

In addition to regulation of Na⁺ balance, aldosterone may contribute to glomerulosclerosis and interstitial fibrosis (Hostetter & Ibrahim, 2003). Inactivation of *Dot1l* in CNT/CD results in upregulation of many genes including *endothelin 1* and *Lcn2* (Wu, Chen, Zhang, et al., 2013). These genes have profibrotic properties. Endothelin 1 has been previously shown as a target of aldosterone action (Zhang, Xia, Reisenauer, et al., 2006; Zhou et al., 2012). It is directly regulated by Dot1a–Af9 complex (Zhou et al., 2012). Hence, future research to identify if some patients with chronic kidney disease have genetic defects in Dot1a–Af9 pathways and to determine if *Dot1l*^{AC} mice develop kidney fibrosis with aging may extend our knowledge of Dot1–Af9-mediated repression far beyond ENaC and may lead to new diagnostic, prognostic, and therapeutic strategy for management of kidney fibrosis.

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References

- Alangari AA. Genomic and non-genomic actions of glucocorticoids in asthma. *Annals of Thoracic Medicine*. 2010; 5(3):133–139. <http://dx.doi.org/10.4103/1817-1737.65040>. [PubMed: 20835306]
- Alvarez de la Rosa D, Zhang P, Naray-Fejes-Toth A, Fejes-Toth G, Canessa CM. The serum and glucocorticoid kinase sgk increases the abundance of epithelial sodium channels in the plasma membrane of *Xenopus* oocytes. *The Journal of Biological Chemistry*. 1999; 274(53):37834–37839. [PubMed: 10608847]
- Asher C, Wald H, Rossier BC, Garty H. Aldosterone-induced increase in the abundance of Na⁺ channel subunits. *The American Journal of Physiology*. 1996; 271(2 Pt 1):C605–C611. [PubMed: 8770001]
- Berger S, Bleich M, Schmid W, Cole TJ, Peters J, Watanabe H, et al. Mineralocorticoid receptor knockout mice: Pathophysiology of Na⁺ metabolism. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95(16):9424–9429. [PubMed: 9689096]
- Bhalla V, Soundararajan R, Pao AC, Li H, Pearce D. Disinhibitory pathways for control of sodium transport: Regulation of ENaC by SGK1 and GILZ. *American Journal of Physiology Renal Physiology*. 2006; 291(4):F714–F721. [PubMed: 16720863]
- Biswas D, Milne TA, Basrur V, Kim J, Elenitoba-Johnson KS, Allis CD, et al. Function of leukemogenic mixed lineage leukemia 1 (MLL) fusion proteins through distinct partner protein complexes. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(38):15751–15756. <http://dx.doi.org/10.1073/pnas.1111498108>. [PubMed: 21896721]
- Boyd C, Naray-Fejes-Toth A. Gene regulation of ENaC subunits by serum- and glucocorticoid-inducible kinase-1. *American Journal of Physiology Renal Physiology*. 2005; 288(3):F505–F512. [PubMed: 15536167]

- Brennan FE, Fuller PJ. Mammalian K-ras2 is a corticosteroid-induced gene in vivo. *Endocrinology*. 2006; 147(6):2809–2816. [PubMed: 16543373]
- Bruns JB, Carattino MD, Sheng S, Maarouf AB, Weisz OA, Pilewski JM, et al. Epithelial Na⁺ channels are fully activated by furin- and prostaticin-dependent release of an inhibitory peptide from the gamma-subunit. *The Journal of Biological Chemistry*. 2007; 282(9):6153–6160. [PubMed: 17199078]
- Caldwell RA, Boucher RC, Stutts MJ. Serine protease activation of near-silent epithelial Na⁺ channels. *American Journal of Physiology Cell Physiology*. 2004; 286(1):C190–C194. [PubMed: 12967915]
- Chen SY, Bhargava A, Mastroberardino L, Meijer OC, Wang J, Buse P, et al. Epithelial sodium channel regulated by aldosterone-induced protein sgk. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96(5):2514–2519. [PubMed: 10051674]
- Chen L, Wu H, Pochynyuk OM, Reissenauer MR, Zhang Z, Huang L, et al. Afl17 deficiency increases sodium excretion and decreases blood pressure. *Journal of the American Society of Nephrology*. 2011; 22(6):1076–1086. <http://dx.doi.org/10.1681/ASN.2010121270> (selected by Faculty of 1000. Invited author interview by Hemodialysis.com, pii: ASN.2010121270). [PubMed: 21546577]
- Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Allis CD. Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Molecular Cell*. 2000; 5(6):905–915. [PubMed: 10911985]
- Condliffe SB, Zhang H, Frizzell RA. Syntaxin 1A regulates ENaC channel activity. *The Journal of Biological Chemistry*. 2004; 279(11):10085–10092. [PubMed: 14703519]
- Cooper MS, Bujalska I, Rabbitt E, Walker EA, Bland R, Sheppard MC, et al. Modulation of 11beta-hydroxysteroid dehydrogenase isozymes by proinflammatory cytokines in osteoblasts: An autocrine switch from glucocorticoid inactivation to activation. *Journal of Bone and Mineral Research*. 2001; 16(6):1037–1044. [PubMed: 11393780]
- Cowling RT, Birnboim HC. Expression of serum- and glucocorticoid-regulated kinase (sgk) mRNA is up-regulated by GM-CSF and other proinflammatory mediators in human granulocytes. *Journal of Leukocyte Biology*. 2000; 67(2):240–248. [PubMed: 10670586]
- Dahlmann A, Pradervand S, Hummler E, Rossier BC, Frindt G, Palmer LG. Mineralocorticoid regulation of epithelial Na⁺ channels is maintained in a mouse model of Liddle's syndrome. *American Journal of Physiology Renal Physiology*. 2003; 285(2):F310–F318. [PubMed: 12684224]
- Debonneville C, Flores SY, Kamynina E, Plant PJ, Tauxe C, Thomas MA, et al. Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na⁺ channel cell surface expression. *The EMBO Journal*. 2001; 20(24):7052–7059. [PubMed: 11742982]
- Dooley R, Angibaud E, Yusef YR, Thomas W, Harvey BJ. Aldosterone-induced ENaC and basal Na⁺/K⁺-ATPase trafficking via protein kinase D1-phosphatidylinositol 4-kinaseIIIbeta trans Golgi signalling in M1 cortical collecting duct cells. *Molecular and Cellular Endocrinology*. 2013; 372(1–2):86–95. <http://dx.doi.org/10.1016/j.mce.2013.03.011>. [PubMed: 23541637]
- Duarte JD, Zineh I, Burkley B, Gong Y, Langae TY, Turner ST, et al. Effects of genetic variation in H3K79 methylation regulatory genes on clinical blood pressure and blood pressure response to hydrochlorothiazide. *Journal of Translational Medicine*. 2012; 10:56. <http://dx.doi.org/10.1186/1479-5876-10-56>. [PubMed: 22440088]
- Erfurth F, Hemenway CS, De Erkenez AC, Domer PH. MLL fusion partners AF4 and AF9 interact at subnuclear foci. *Leukemia*. 2004; 18(1):92–102. [PubMed: 14603337]
- Fakitsas P, Adam G, Daidie D, van Bemmelen MX, Fouladkou F, Patrignani A, et al. Early aldosterone-induced gene product regulates the epithelial sodium channel by deubiquitylation. *Journal of the American Society of Nephrology*. 2007; 18(4):1084–1092. [PubMed: 17344426]
- Faresse N, Debonneville A, Staub O. USP2-45 represses aldosterone mediated responses by decreasing mineralocorticoid receptor availability. *Cellular Physiology and Biochemistry*. 2013; 31(2–3):462–472. <http://dx.doi.org/10.1159/000343382>. [PubMed: 23548632]
- Faresse N, Ruffieux-Daidie D, Salamin M, Gomez-Sanchez CE, Staub O. Mineralocorticoid receptor degradation is promoted by Hsp90 inhibition and the ubiquitin-protein ligase CHIP. *American Journal of Physiology Renal Physiology*. 2010; 299(6):F1462–F1472. <http://dx.doi.org/10.1152/ajprenal.00285.2010>. [PubMed: 20861078]

- Feng Q, Wang H, Ng HH, Erdjument-Bromage H, Tempst P, Struhl K, et al. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Current Biology*. 2002; 12(12):1052–1058. [PubMed: 12123582]
- Firestone GL, Giampaolo JR, O'Keeffe BA. Stimulus-dependent regulation of serum and glucocorticoid inducible protein kinase (SGK) transcription, subcellular localization and enzymatic activity. *Cellular Physiology and Biochemistry*. 2003; 13(1):1–12. [PubMed: 12649597]
- Gumz ML, Popp MP, Wingo CS, Cain BD. Early transcriptional effects of aldosterone in a mouse inner medullary collecting duct cell line. *American Journal of Physiology Renal Physiology*. 2003; 285(4):F664–F673. [PubMed: 12770840]
- Harris M, Garcia-Caballero A, Stutts MJ, Firsov D, Rossier BC. Preferential assembly of epithelial sodium channel (ENaC) subunits in *Xenopus* oocytes: Role of furin-mediated endogenous proteolysis. *The Journal of Biological Chemistry*. 2008; 283(12):7455–7463. <http://dx.doi.org/10.1074/jbc.M707399200>. [PubMed: 18195015]
- Helms MN, Yu L, Malik B, Kleinhenz DJ, Hart CM, Eaton DC. Role of SGK1 in nitric oxide inhibition of ENaC in Na⁺-transporting epithelia. *American Journal of Physiology Cell Physiology*. 2005; 289(3):C717–C726. [PubMed: 15843443]
- Hemenway CS, de Erkenez AC, Gould GC. The polycomb protein MPC3 interacts with AF9, an MLL fusion partner in t(9;11)(p22;q23) acute leukemias. *Oncogene*. 2001; 20(29):3798–3805. [PubMed: 11439343]
- Hostetter TH, Ibrahim HN. Aldosterone in chronic kidney and cardiac disease. *Journal of the American Society of Nephrology*. 2003; 14(9):2395–2401. [PubMed: 12937319]
- Hou J, Speirs HJ, Seckl JR, Brown RW. Sgk1 gene expression in kidney and its regulation by aldosterone: Spatio-temporal heterogeneity and quantitative analysis. *Journal of the American Society of Nephrology*. 2002; 13(5):1190–1198. [PubMed: 11961006]
- Huang DY, Boini KM, Friedrich B, Metzger M, Just L, Osswald H, et al. Blunted hypertensive effect of combined fructose and high-salt diet in gene-targeted mice lacking functional serum- and glucocorticoid-inducible kinase SGK1. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*. 2006; 290(4):R935–R944.
- Hughey RP, Bruns JB, Kinlough CL, Harkleroad KL, Tong Q, Carattino MD, et al. Epithelial sodium channels are activated by furin-dependent proteolysis. *The Journal of Biological Chemistry*. 2004; 279(18):18111–18114. [PubMed: 15007080]
- Hummler E, Vallon V. Lessons from mouse mutants of epithelial sodium channel and its regulatory proteins. *Journal of the American Society of Nephrology*. 2005; 16(11):3160–3166. [PubMed: 16192419]
- Ichimura T, Yamamura H, Sasamoto K, Tominaga Y, Taoka M, Kakiuchi K, et al. 14-3-3 Proteins modulate the expression of epithelial Na⁺ channels by phosphorylation-dependent interaction with Nedd4-2 ubiquitin ligase. *The Journal of Biological Chemistry*. 2005; 280(13):13187–13194. [PubMed: 15677482]
- Iida S, Seto M, Yamamoto K, Komatsu H, Tojo A, Asano S, et al. MLLT3 gene on 9p22 involved in t(9;11) leukemia encodes a serine/proline rich protein homologous to MLLT1 on 19p13. *Oncogene*. 1993; 8(11):3085–3092. [PubMed: 8414510]
- Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001; 293(5532):1074–1080. [PubMed: 11498575]
- Kamynina E, Staub O. Concerted action of ENaC, Nedd4-2, and Sgk1 in trans-epithelial Na⁺ transport. *American Journal of Physiology Renal Physiology*. 2002; 283(3):F377–F387. [PubMed: 12167587]
- Kellner M, Peiter A, Hafner M, Feuring M, Christ M, Wehling M, et al. Early aldosterone up-regulated genes: New pathways for renal disease? *Kidney International*. 2003; 64(4):1199–1207. [PubMed: 12969137]
- Kitagawa H, Yanagisawa J, Fuse H, Ogawa S, Yogiashi Y, Okuno A, et al. Ligand-selective potentiation of rat mineralocorticoid receptor activation function 1 by a CBP-containing histone acetyltransferase complex. *Molecular and Cellular Biology*. 2002; 22(11):3698–3706. [PubMed: 11997506]

- Krogan NJ, Dover J, Wood A, Schneider J, Heidt J, Boateng MA, et al. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: Linking transcriptional elongation to histone methylation. *Molecular Cell*. 2003; 11(3):721–729. [PubMed: 12667454]
- Ku NO, Liao J, Omary MB. Phosphorylation of human keratin 18 serine 33 regulates binding to 14-3-3 proteins. *The EMBO Journal*. 1998; 17(7):1892–1906. [PubMed: 9524113]
- Lee IH, Campbell CR, Cook DI, Dinudom A. Regulation of epithelial Na⁺ channels by aldosterone: Role of Sgk1. *Clinical and Experimental Pharmacology & Physiology*. 2008; 35(2):235–241. [PubMed: 18197893]
- Lin JJ, Hemenway CS. Hsp90 directly modulates the spatial distribution of AF9/MLLT3 and affects target gene expression. *The Journal of Biological Chemistry*. 2010; 285(16):11966–11973. <http://dx.doi.org/10.1074/jbc.M110.101642>. [PubMed: 20159978]
- Lin YM, Ono K, Satoh S, Ishiguro H, Fujita M, Miwa N, et al. Identification of AF17 as a downstream gene of the beta-catenin/T-cell factor pathway and its involvement in colorectal carcinogenesis. *Cancer Research*. 2001; 61(17):6345–6349. [PubMed: 11522623]
- Liu W, Wang J, Sauter NK, Pearce D. Steroid receptor heterodimerization demonstrated in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 1995; 92(26):12480–12484. [PubMed: 8618925]
- Loffing J, Pietri L, Aregger F, Bloch-Faure M, Ziegler U, Meneton P, et al. Differential subcellular localization of ENaC subunits in mouse kidney in response to high- and low-Na diets. *American Journal of Physiology Renal Physiology*. 2000; 279(2):F252–F258. [PubMed: 10919843]
- Loffing J, Zecevic M, Feraille E, Kaissling B, Asher C, Rossier BC, et al. Aldosterone induces rapid apical translocation of ENaC in early portion of renal collecting system: Possible role of SGK. *American Journal of Physiology Renal Physiology*. 2001; 280(4):F675–F682. [PubMed: 11249859]
- Losel R, Feuring M, Wehling M. Non-genomic aldosterone action: From the cell membrane to human physiology. *The Journal of Steroid Biochemistry and Molecular Biology*. 2002; 83(1–5):167–171. [PubMed: 12650713]
- Mastroberardino L, Spindler B, Forster I, Loffing J, Assandri R, May A, et al. Ras pathway activates epithelial Na⁺ channel and decreases its surface expression in *Xenopus* oocytes. *Molecular Biology of the Cell*. 1998; 9(12):3417–3427. [PubMed: 9843578]
- McAvoy T, Allen PB, Obaishi H, Nakanishi H, Takai Y, Greengard P, et al. Regulation of neurabin I interaction with protein phosphatase 1 by phosphorylation. *Biochemistry*. 1999; 38(39):12943–12949. [PubMed: 10504266]
- Min J, Feng Q, Li Z, Zhang Y, Xu RM. Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell*. 2003; 112(5):711–723. [PubMed: 12628190]
- Muller OG, Parnova RG, Centeno G, Rossier BC, Firsov D, Horisberger JD. Mineralocorticoid effects in the kidney: Correlation between alphaENaC, GILZ, and Sgk-1 mRNA expression and urinary excretion of Na⁺ and K⁺ *Journal of the American Society of Nephrology*. 2003; 14(5):1107–1115. [PubMed: 12707381]
- Narikiyo T, Kitamura K, Adachi M, Miyoshi T, Iwashita K, Shiraishi N, et al. Regulation of prostasin by aldosterone in the kidney. *The Journal of Clinical Investigation*. 2002; 109(3):401–408. [PubMed: 11828000]
- Narlikar GJ, Fan HY, Kingston RE. Cooperation between complexes that regulate chromatin structure and transcription. *Cell*. 2002; 108(4):475–487. [PubMed: 11909519]
- Ng HH, Ciccone DN, Morshead KB, Oettinger MA, Struhl K. Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: A potential mechanism for position-effect variegation. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(4):1820–1825. [PubMed: 12574507]
- Ng HH, Feng Q, Wang H, Erdjument-Bromage H, Tempst P, Zhang Y, et al. Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes & Development*. 2002; 16(12):1518–1527. <http://dx.doi.org/10.1101/gad.1001502>. [PubMed: 12080090]
- Nguyen AT, Zhang Y. The diverse functions of Dot1 and H3K79 methylation. *Genes & Development*. 2011; 25(13):1345–1358. <http://dx.doi.org/10.1101/gad.2057811>. [PubMed: 21724828]

- Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM, et al. hDOT1L links histone methylation to leukemogenesis. *Cell*. 2005; 121(2):167–178. [PubMed: 15851025]
- Ono S, Kusano E, Muto S, Ando Y, Asano Y. A low-Na⁺ diet enhances expression of mRNA for epithelial Na⁺ channel in rat renal inner medulla. *Pflügers Archiv*. 1997; 434(6):756–763. [PubMed: 9306009]
- Park J, Leong ML, Buse P, Maiyar AC, Firestone GL, Hemmings BA. Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. *The EMBO Journal*. 1999; 18(11):3024–3033. [PubMed: 10357815]
- Pietras RJ, Nemere I, Szego CM. Steroid hormone receptors in target cell membranes. *Endocrine*. 2001; 14(3):417–427. <http://dx.doi.org/10.1385/ENDO.14:3:417>. [PubMed: 11444440]
- Pradervand S, Vandewalle A, Bens M, Gautschi I, Loffing J, Hummler E, et al. Dysfunction of the epithelial sodium channel expressed in the kidney of a mouse model for Liddle syndrome. *Journal of the American Society of Nephrology*. 2003; 14(9):2219–2228. [PubMed: 12937297]
- Prasad R, Leshkowitz D, Gu Y, Alder H, Nakamura T, Saito H, et al. Leucine-zipper dimerization motif encoded by the AF17 gene fused to ALL-1 (MLL) in acute leukemia. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91(17):8107–8111. [PubMed: 8058765]
- Qi J, Peters KW, Liu C, Wang JM, Edinger RS, Johnson JP, et al. Regulation of the amiloride-sensitive epithelial sodium channel by syntaxin 1A. *The Journal of Biological Chemistry*. 1999; 274(43):30345–30348. [PubMed: 10521407]
- Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature*. 2000; 406(6796):593–599. [PubMed: 10949293]
- Reisenauer MR, Anderson M, Huang L, Zhang Z, Zhou Q, Kone BC, et al. AF17 competes with AF9 for binding to Dot1a to up-regulate transcription of epithelial Na⁺ channel alpha. *The Journal of Biological Chemistry*. 2009; 284(51):35659–35669. <http://dx.doi.org/10.1074/jbc.M109.038448>. [PubMed: 19864429]
- Reisenauer MR, Wang SW, Xia Y, Zhang W. Dot1a contains three nuclear localization signals and regulates the epithelial Na⁺ channel (ENaC) at multiple levels. *American Journal of Physiology Renal Physiology*. 2010; 299(1):F63–F76. <http://dx.doi.org/10.1152/ajprenal.00105.2010>. [PubMed: 20427473]
- Robert-Nicoud M, Flahaut M, Elalouf JM, Nicod M, Salinas M, Bens M, et al. Transcriptome of a mouse kidney cortical collecting duct cell line: Effects of aldosterone and vasopressin. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98(5):2712–2716. [PubMed: 11226305]
- Rong R, Surace EI, Haipok CA, Gutmann DH, Ye K. Serine 518 phosphorylation modulates merlin intramolecular association and binding to critical effectors important for NF2 growth suppression. *Oncogene*. 2004; 23(52):8447–8454. [PubMed: 15378014]
- Sahin P, McCaig C, Jeevahan J, Murray JT, Hainsworth AH. The cell survival kinase SGK1 and its targets FOXO3a and NDRG1 in aged human brain. *Neuropathology and Applied Neurobiology*. 2013; 39(6):623–633. <http://dx.doi.org/10.1111/nan.12023>. [PubMed: 23363009]
- Savory JG, Prefontaine GG, Lamprecht C, Liao M, Walther RF, Lefebvre YA, et al. Glucocorticoid receptor homodimers and glucocorticoid-mineralocorticoid receptor heterodimers form in the cytoplasm through alternative dimerization interfaces. *Molecular and Cellular Biology*. 2001; 21(3):781–793. <http://dx.doi.org/10.1128/MCB.21.3.781-793.2001>. [PubMed: 11154266]
- Saxena SK, Singh M, Kaur S, George C. Distinct domain-dependent effect of syntaxin1A on amiloride-sensitive sodium channel (ENaC) currents in HT-29 colonic epithelial cells. *International Journal of Biological Sciences*. 2007; 3(1):47–56. [PubMed: 17200691]
- Sayegh R, Auerbach SD, Li X, Loftus RW, Husted RF, Stokes JB, et al. Glucocorticoid induction of epithelial sodium channel expression in lung and renal epithelia occurs via trans-activation of a hormone response element in the 5'-flanking region of the human epithelial sodium channel alpha subunit gene. *The Journal of Biological Chemistry*. 1999; 274(18):12431–12437. [PubMed: 10212217]

- Seeböhm G, Strutz-Seeböhm N, Baltaev R, Korniyuchuk G, Knirsch M, Engel J, et al. Regulation of KCNQ4 potassium channel prepulse dependence and current amplitude by SGK1 in *Xenopus* oocytes. *Cellular Physiology and Biochemistry*. 2005; 16(4–6):255–262. [PubMed: 16301825]
- Setiawan I, Henke G, Feng Y, Bohmer C, Vasilets LA, Schwarz W, et al. Stimulation of *Xenopus* oocyte Na(+), K(+)ATPase by the serum and glucocorticoid-dependent kinase sgk1. *Pflügers Archiv*. 2002; 444(3):426–431. [PubMed: 12111252]
- Sheng S, Carattino MD, Bruns JB, Hughey RP, Kleyman TR. Furin cleavage activates the epithelial Na⁺ channel by relieving Na⁺ self-inhibition. *American Journal of Physiology Renal Physiology*. 2006; 290(6):F1488–F1496. [PubMed: 16449353]
- Singer MS, Kahana A, Wolf AJ, Meisinger LL, Peterson SE, Goggin C, et al. Identification of high-copy disruptors of telomeric silencing in *Saccharomyces cerevisiae*. *Genetics*. 1998; 150(2):613–632. [PubMed: 9755194]
- Snyder PM, Olson DR, Thomas BC. Serum and glucocorticoid-regulated kinase modulates Nedd4-2-mediated inhibition of the epithelial Na⁺ channel. *The Journal of Biological Chemistry*. 2002; 277(1):5–8. [PubMed: 11696533]
- Soodvilai S, Jia Z, Fongsupa S, Chatsudhipong V, Yang T. Liver X receptor agonists decrease ENaC-mediated sodium transport in collecting duct cells. *American Journal of Physiology Renal Physiology*. 2012; 303(12):F1610–F1616. <http://dx.doi.org/10.1152/ajprenal.00283.2012>. [PubMed: 23077096]
- Soundararajan R, Wang J, Melters D, Pearce D. Differential activities of glucocorticoid-induced leucine zipper protein isoforms. *The Journal of Biological Chemistry*. 2007; 282(50):36303–36313. [PubMed: 17956870]
- Soundararajan R, Zhang TT, Wang J, Vandewalle A, Pearce D. A novel role for glucocorticoid-induced leucine zipper protein in epithelial sodium channel-mediated sodium transport. *The Journal of Biological Chemistry*. 2005; 280(48):39970–39981. [PubMed: 16216878]
- Spindler B, Verrey F. Aldosterone action: Induction of p21(ras) and fra-2 and transcription-independent decrease in myc, jun, and fos. *The American Journal of Physiology*. 1999; 276(5 Pt 1):C1154–C1161. [PubMed: 10329965]
- Srinivasan RS, de Erkenez AC, Hemenway CS. The mixed lineage leukemia fusion partner AF9 binds specific isoforms of the BCL-6 corepressor. *Oncogene*. 2003; 22(22):3395–3406. [PubMed: 12776190]
- Stockand JD. New ideas about aldosterone signaling in epithelia. *American Journal of Physiology Renal Physiology*. 2002; 282(4):F559–F576. [PubMed: 11880316]
- Stockand JD, Al-Baldawi NF, Al-Khalili OK, Worrell RT, Eaton DC. S-Adenosyl-L-homocysteine hydrolase regulates aldosterone-induced Na⁺ transport. *The Journal of Biological Chemistry*. 1999; 274(6):3842–3850. [PubMed: 9920939]
- Stockand JD, Zeltwanger S, Bao HF, Becchetti A, Worrell RT, Eaton DC. S-Adenosyl-L-homocysteine hydrolase is necessary for aldosterone-induced activity of epithelial Na(+) channels. *American Journal of Physiology Cell Physiology*. 2001; 281(3):C773–C785. [PubMed: 11502554]
- Strahl BD, Allis CD. The language of covalent histone modifications. *Nature*. 2000; 403(6765):41–45. [PubMed: 10638745]
- Suzukawa K, Shimizu S, Nemoto N, Takei N, Taki T, Nagasawa T. Identification of a chromosomal breakpoint and detection of a novel form of an MLL-AF17 fusion transcript in acute monocytic leukemia with t(11;17)(q23;q21). *International Journal of Hematology*. 2005; 82(1):38–41. [PubMed: 16105757]
- Thomas CP, Itani OA. New insights into epithelial sodium channel function in the kidney: Site of action, regulation by ubiquitin ligases, serum- and glucocorticoid-inducible kinase and proteolysis. *Current Opinion in Nephrology and Hypertension*. 2004; 13(5):541–548. [PubMed: 15300161]
- Vallet V, Chraïbi A, Gaeggeler HP, Horisberger JD, Rossier BC. An epithelial serine protease activates the amiloride-sensitive sodium channel. *Nature*. 1997; 389(6651):607–610. [PubMed: 9335501]
- Vallon V, Huang DY, Grahmmer F, Wyatt AW, Osswald H, Wulff P, et al. SGK1 as a determinant of kidney function and salt intake in response to mineralocorticoid excess. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*. 2005; 289(2):R395–R401.

- Vallon V, Wulff P, Huang DY, Loffing J, Volkl H, Kuhl D, et al. Role of Sgk1 in salt and potassium homeostasis. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*. 2005; 288(1):R4–R10.
- Vallon V, Wyatt AW, Klingel K, Huang DY, Hussain A, Berchtold S, et al. SGK1-dependent cardiac CTGF formation and fibrosis following DOCA treatment. *Journal of Molecular Medicine*. 2006; 84(5):396–404. [PubMed: 16604333]
- van Leeuwen F, Gafken PR, Gottschling DE. Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell*. 2002; 109(6):745–756. [PubMed: 12086673]
- Verrey F. Early aldosterone action: Toward filling the gap between transcription and transport. *The American Journal of Physiology*. 1999; 277(3 Pt 2):F319–F327. [PubMed: 10484514]
- Verrey F, Fakitsas P, Adam G, Staub O. Early transcriptional control of ENaC (de)ubiquitylation by aldosterone. *Kidney International*. 2008; 73(6):691–696. <http://dx.doi.org/10.1038/sj.ki.5002737>. [PubMed: 18094676]
- Verrey F, Pearce D, Pfeiffer R, Spindler B, Mastroberardino L, Summa V, et al. Pleiotropic action of aldosterone in epithelia mediated by transcription and post-transcription mechanisms. *Kidney International*. 2000; 57(4):1277–1282. [PubMed: 10760054]
- Vuagniaux G, Vallet V, Jaeger NF, Hummler E, Rossier BC. Synergistic activation of ENaC by three membrane-bound channel-activating serine proteases (mCAP1, mCAP2, and mCAP3) and serum- and glucocorticoid-regulated kinase (Sgk1) in *Xenopus* oocytes. *The Journal of General Physiology*. 2002; 120(2):191–201. [PubMed: 12149280]
- Vuagniaux G, Vallet V, Jaeger NF, Pfister C, Bens M, Farman N, et al. Activation of the amiloride-sensitive epithelial sodium channel by the serine protease mCAP1 expressed in a mouse cortical collecting duct cell line. *Journal of the American Society of Nephrology*. 2000; 11(5):828–834. [PubMed: 10770960]
- Waldegger S, Barth P, Raber G, Lang F. Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94(9):4440–4445. [PubMed: 9114008]
- Waldegger S, Gabrysch S, Barth P, Fillon S, Lang F. h-sgk serine-threonine protein kinase as transcriptional target of p38/MAP kinase pathway in HepG2 human hepatoma cells. *Cellular Physiology and Biochemistry*. 2000; 10(4):203–208. [PubMed: 11093030]
- Wang H, Huang ZQ, Xia L, Feng Q, Erdjument-Bromage H, Strahl BD, et al. Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science*. 2001; 293(5531):853–857. [PubMed: 11387442]
- Weger S, Wendland M, Kleinschmidt JA, Heilbronn R. The adeno-associated virus type 2 regulatory proteins rep78 and rep68 interact with the transcriptional coactivator PC4. *Journal of Virology*. 1999; 73(1):260–269. [PubMed: 9847329]
- Westin S, Rosenfeld MG, Glass CK. Nuclear receptor coactivators. *Advances in Pharmacology*. 2000; 47:89–112. [PubMed: 10582085]
- Wu H, Chen L, Zhang X, Zhou Q, Li JM, Berger S, et al. Aqp5 is a new transcriptional target of dot1a and a regulator of aqp2. *PLoS One*. 2013; 8(1):e53342. <http://dx.doi.org/10.1371/journal.pone.0053342>. [PubMed: 23326416]
- Wu H, Chen L, Zhou Q, Zhang W. AF17 facilitates Dot1a nuclear export and upregulates ENaC-mediated Na⁺ transport in renal collecting duct cells. *PLoS One*. 2011; 6(11):e27429. <http://dx.doi.org/10.1371/journal.pone.0027429>. [PubMed: 22087315]
- Wu H, Chen L, Zhou Q, Zhang X, Berger S, Bi J, et al. Aqp2-expressing cells give rise to renal intercalated cells. *Journal of the American Society of Nephrology*. 2013; 24(2):243–252. <http://dx.doi.org/10.1681/ASN.2012080866> (Editorial in the same issue of *J Am Soc Nephrol* 163–165, Selection by Faculty of 1000). [PubMed: 23308014]
- Wulff P, Vallon V, Huang DY, Volkl H, Yu F, Richter K, et al. Impaired renal Na⁽⁺⁾ retention in the sgk1-knockout mouse. *The Journal of Clinical Investigation*. 2002; 110(9):1263–1268. [PubMed: 12417564]

- Yang J, Young MJ. The mineralocorticoid receptor and its coregulators. *Journal of Molecular Endocrinology*. 2009; 43(2):53–64. <http://dx.doi.org/10.1677/JME-09-0031>. [PubMed: 19617444]
- Yoo D, Kim BY, Campo C, Nance L, King A, Maouyo D, et al. Cell surface expression of the ROMK (Kir 1.1) channel is regulated by the aldosterone-induced kinase, SGK-1, and protein kinase A. *The Journal of Biological Chemistry*. 2003; 278(25):23066–23075. [PubMed: 12684516]
- Yun CC, Palmada M, Embark HM, Fedorenko O, Feng Y, Henke G, et al. The serum and glucocorticoid-inducible kinase SGK1 and the Na⁺/H⁺ exchange regulating factor NHERF2 synergize to stimulate the renal outer medullary K⁺ channel ROMK1. *Journal of the American Society of Nephrology*. 2002; 13(12):2823–2830. [PubMed: 12444200]
- Zhang W, Hayashizaki Y, Kone BC. Structure and regulation of the mDot1 gene, a mouse histone H3 methyltransferase. *The Biochemical Journal*. 2004; 377(Pt 3):641–651. [PubMed: 14572310]
- Zhang D, Li S, Cruz P, Kone BC. Sirtuin 1 functionally and physically interacts with disruptor of telomeric silencing-1 to regulate alpha-ENaC transcription in collecting duct. *The Journal of Biological Chemistry*. 2009; 284(31):20917–20926. <http://dx.doi.org/10.1074/jbc.M109.020073>. [PubMed: 19491102]
- Zhang W, Xia X, Jalal DI, Kunciewicz T, Xu W, Lesage GD, et al. Aldosterone-sensitive repression of ENaCalpha transcription by a histone H3 lysine-79 methyltransferase. *American Journal of Physiology Cell Physiology*. 2006; 290(3):C936–C946. [PubMed: 16236820]
- Zhang W, Xia X, Reisenauer MR, Hemenway CS, Kone BC. Dot1a-AF9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENaC {alpha} in an aldosterone-sensitive manner. *The Journal of Biological Chemistry*. 2006; 281(26):18059–18068. [PubMed: 16636056]
- Zhang W, Xia X, Reisenauer MR, Rieg T, Lang F, Kuhl D, et al. Aldosterone-induced Sgk1 relieves Dot1a-Af9-mediated transcriptional repression of epithelial Na⁺ channel alpha. *The Journal of Clinical Investigation*. 2007; 117(3):773–783. Comment in the same issue of *J Clin Invest* 592–595, Selection by Faculty of 1000. [PubMed: 17332896]
- Zhang W, Yu Z, Wu H, Chen L, Kong Q, Kone BC. An Af9 cis-element directly targets Dot1a to mediate transcriptional repression of the alphaENaC gene. *American Journal of Physiology Renal Physiology*. 2013; 304(4):F367–F375. <http://dx.doi.org/10.1152/ajprenal.00537.2011>. [PubMed: 23152297]
- Zhang X, Zhou Q, Chen L, Berger S, Wu H, Xiao Z, et al. Mineralocorticoid receptor antagonizes Dot1a-Af9 complex to increase alphaENaC transcription. *American Journal of Physiology Renal Physiology*. 2013; 305(10):F1436–F1444. <http://dx.doi.org/10.1152/ajprenal.00202.2013>. [PubMed: 24026182]
- Zhou Q, Liu K, Wu H, Chen L, Pouranan V, Yuan M, et al. Spironolactone rescues Dot1a-Af9-mediated repression of endothelin-1 and improves kidney injury in streptozotocin-induced diabetic rats. *PLoS One*. 2012; 7(10):e47360. <http://dx.doi.org/10.1371/journal.pone.0047360>. [PubMed: 23077601]

Figure 1.

Diagrams of Dot1a, Af9, and Af17. Structural features in mouse Dot1a (NP_955354.1) (A), mouse Af9 (AAH21420.1) (B), and mouse Af17 (NP_647472.2) (C). MD, methyltransferase domain (Min et al., 2003); NSL, nuclear localization signal (Reisenauer, Wang, Xia, & Zhang, 2010); LZ, leucine zipper (Reisenauer et al., 2009; Zhang, Xia, Reisenauer, Hemenway, & Kone, 2006); Af9/Af17, Af9/Af17-interacting domain (Reisenauer et al., 2009; Zhang, Xia, Reisenauer, et al., 2006); YEATS, YEATS domain; Dot1a/MR/Hsp90, Dot1a/MR/Hsp90-interacting domain; PHD-ZF, PHD-zinc-finger-like domain. *Domain search:* <http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>

Figure 2.

Diagram of the 5'-flanking region of α ENaC. Fragments designated Ra–R3 are shown along with their relative positions to the major transcription start site (+1) of α ENaC. ● and ■ represent the putative GRE site (-811) and GRE half sites (-983, -416, -325, -241, and -234), respectively. ◇ indicates the Af9-binding site (+78) (Zhang, Xia, Jalal, et al., 2006; Zhang, Xia, Reisenauer, et al., 2006; Zhang, Yu, et al., 2013).

Figure 3.

Representative images showing that AF17 facilitates Dot1a cytoplasmic distribution. 293T cells were transiently transfected with GFP-Dot1a and RFP-AF17, and examined by epifluorescence microscopy (20×). Arrow: a cell showing typical nuclear localization pattern of GFP-Dot1a in the absence of detectable RFP-AF17. *This figure was originally published in Reisenauer et al. (2009). © The American Society for Biochemistry and Molecular Biology*

Figure 4.

Model for Dot1a–Af9-mediated repression of α ENaC transcription. Under basal conditions (–Aldo), Af9 recruits Dot1a to form a nuclear complex, which indirectly or directly through Af9 DNA-binding activity binds specific sites of the α ENaC promoter, leading to hypermethylation of histone H3 K79 and repression of α ENaC transcription (Zhang, Xia, Jalal, et al., 2006; Zhang, Xia, Reisenauer, et al., 2006; Zhang, Yu, et al., 2013). Af17 relieves the repression by competing with Af9 for binding Dot1a and promoting Dot1a redistribution from the nucleus to cytoplasm (Reisenauer et al., 2009; Wu et al., 2011). Hsp90 also interacts with Af9 and apparently promotes its cytoplasmic distribution to derepress the α ENaC promoter (Lin & Hemenway, 2010). Aldosterone (+Aldo) stimulates α ENaC transcription by multiple mechanisms. Through the classical action, aldosterone binds and activates the nuclear hormone receptors (NR) that are either glucocorticoid receptor or mineralocorticoid receptor homo- or heterodimers to bind the glucocorticoid response element in the α ENaC promoter and transactivate α ENaC. In parallel, aldosterone releases Dot1a–Af9-mediated repression by limiting the formation of the complex through (A) downregulating Dot1a (Zhang, Xia, Jalal, et al., 2006) and Af9 expression (Zhang, Xia, Reisenauer, et al., 2006), presumably via nuclear receptor-dependent or -independent (not shown) mechanisms, (B) decreasing the Dot1a–Af9 interaction via Sgk1-mediated phosphorylation of Af9 at S435 (Zhang et al., 2007), and (C) counterbalancing Dot1a–Af9 complex by activating MR to compete for binding Af9 (Zhang, Zhou, et al., 2013). These concerted actions lead to histone H3 K79 hypomethylation at specific subregions of the α ENaC promoter. Sgk1 might join and downregulate the Dot1a–Af9 complex associated with the α ENaC promoter (not shown for simplicity). In all cases, Af9-free Dot1a binds DNA nonspecifically and catalyzes histone H3 K79 methylation throughout the genome under basal conditions (not shown). Meth, methylation.